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Problems of Drug Dependence 1983

Proceedings of the 45th Annual Scientific Meeting

The Committee on Problems of Drug Dependence, Inc.

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES
Public Health Service • Alcohol, Drug Abuse, and Mental Health Administration

Problems of Drug Dependence, 1983

Proceedings of the 45th Annual Scientific Meeting, The Committee on Problems of Drug Dependence, Inc.

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Foreword

The Committee on Problems of Drug Dependence, Inc. (CPDD) is a uniquely valuable independent organization of internationally recognized experts in a variety of disciplines. From its early years, it has fulfilled two functions that are not duplicated by any other group: organizing and conducting the most prestigious annual scientific meeting in the drug dependence field, and coordinating a national system for assessment of the pharmacologic efficacy and abuse potential of opiate drugs. For the current year, the results of both can be seen in this volume.

The proceedings in this monograph include in condensed form the papers presented at the 45th Annual Scientific Meeting of the CPDD, held in Lexington, Kentucky, June 12-15, 1983, and the annual reports of the CPDD drug testing program. Progress reports of dependence liability studies supported by the National Institute on Drug Abuse and summaries of NIDA's intramural research programs are included as well.

Members of the scientific community and other interested readers will find they can use this volume as a "state of-the-art" summary of the latest developments in biological and chemical drug abuse research. For the fifth year, the National Institute on Drug Abuse is pleased to publish the proceedings of the CPDD Annual Scientific Meeting as a NIDA Research Monograph.

William Pollin, M.D.
Director
National Institute on Drug Abuse

The papers in this monograph were presented or read by title at the 45th Annual Scientific Meeting of the Committee on Problems of Drug Dependence, Inc., in Lexington, Kentucky, June 12-15, 1983. Louis S. Harris, Ph.D., the editor, is chairman of the Department of Pharmacology, Medical College of Virginia, Richmond, Virginia. Opinions expressed in the papers are those of the authors and do not necessarily reflect the opinions or official policy of the National Institute on Drug Abuse or any other part of the U.S. Department of Health and Human Services.

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In Memoriam: H. Frank Fraser, M.D.

Dr. Frank Fraser, Former Asssociate Director of the Addiction Research Center in lexington, Kentucky, died in Louisville on November 6, 1982. He is survived by his wife, Dorothy, two sons,Robert and Thomas, and one daughter, Jane Fraser Carpenter.

Frank had a distinguished career in medical research ranging over many fields. He graduated from Cornell University in 1932 and immediately entered the U.S Public Health Service. After a tour of duty at the U.S. Penitentiary at Atlanta,he was assigned to the Laboratory of Nutrition in the National Institutes of Health. While in that laboratory, Dr. Fraser discovered that cozymase (coenzyme 1) would cure blacktongue in dogs, the canine analog of human pellegra. This discovery provided the clue to nicotonic acid being the pellegra preventive vitamin as was soon proved at the University of Wisconsin. He then was

assigned to the Tennessee valley Authority where he studied the bioavailability of phosphate in fertilizer made from TVA rocks. During the war he worked in the Laboratory of Industrial Hygeine at NIH where he studied the ability of different types of clothing to maintain body temperature under conditions of extreme cold.

After a tour of duty in Germany, he came to the Addiction Research Center in Lexington in 1949 and remained in that laboratory until his retirement from the Public health Service in 1963, when he became a research advisor to the Eli Lilly Company until 1971. In 1971, he returned to the ARC and to NIDA *as a* consultant. Even after many illnesses had taken a toll, he continued to work and at the time of his death he was writing a book setting forth his theories of drug dependence.

Frank's contributions to the study of drug dependence were many. He evaluated many opioid analgesics for dependence liability and he was especially interested in the addictiveness of the active optical isomers of opioids and in the dependence liability of the demethylated derivatives of opioids. He also made many contributions to the evaluation of opioid antagonists, particularly pentazocine and cyclazocine. Frank was equally at home in research in the clinical wards and in the animal laboratory. He had a great interest in studying the subjective responses to opioids and other drugs of dependence. He developed quantitative methodology for this purpose which is still in use. Thus, he was a pioneer in psychopharmacology.

frank was a veritable idea mill, producing new hypotheses as such a fantastic rate that the experimental resources he had available could not keep up. Many of his ideas provided bases for important and productive collaborations. He contributed over 40 papers to the scientific literature. His enthusiasm for science, pharmacology and drug dependence was infectious and provided inspiration to his colleagues.

Frank served on the Expert Panel on Drug Dependence of the World Health Organization and also served for a time as Chairman of the Committee on Problems of Drug Dependence.

Frank was a very outgoing person who loved jokes. Often he could not finish a story he was trying to tell because he would start to laugh so hard as he approached the "punch line" that he could not continue but had to hold his sides and roll on the floor."

Frank was also an ardent fisherman and for years maintained a trailer and a boat on Lake Cumberland. He was an enthusiastic bridge player, regularly participating in duplicate bridge tournaments in which he earned many master points.

He will be sorely missed by his family, his many colleagues and friends.

Harris Isbell, M.D.
Department of Pharmacology
University of Kentucky Medical Center
Lexington, Kentucky

Introduction of Nathan B. Eddy Memorial Award Recipient

Sydney Archer

I was pleased and honored to have been asked by the winner of the Nathan B. Eddy Memorial Award to introduce him to this audience. Eric J. Simon, who was born in Wiesbaden, Germany, received his early education in that city, but after emigrating to the United States he received his B.S. in Chemistry at Case Institute of Technology in 1944. After a stint in the U.S. Army from 1944-46, he returned to the University of Chicago where he received his Ph.D. in organic chemistry with Professor Kharasch in 1951. Shortly thereafter, he deserted his field for biochemistry. After a short interlude as a postdoctoral fellow with Professor Shemin at Columbia College of Physicians and Surgeons, Eric accepted a position as a Research Associate in Biochemistry at Cornell from 1952 to 1959. He became an Assistant Professor of Medicine at NYU School of Medicine in 1959 and has been associated with that institution ever since, where he is now a Professor of Psychiatry and Pharmacology.

His early research activities did not deal with opiates, but in 1963 he published a paper in Nature entitled "Inhibition of RNA synthesis of E. coli by the narcotic drug levorphanol." As far as I can determine, this was his first exposure to opiates. If one continues to read his bibliography, it is easy to discern early signs of dependence and, as we all know, he is now a confirmed addict. He is the current Secretary of the INRC or, as Eddy Way so quaintly calls it, the Junkie Club. His investigations in this field culminated with the discovery of the opiate receptor in 1973, which was also found independently in the laboratories of Snyder in Baltimore and Terenius in Uppsala. Since that time, Professor Simon has made numerous contributions in this field and he is clearly recognized as one of the leaders in the field of opiate research. He was the recipient of the Research Pacesetter Award presented by the National Institute on Drug Abuse in 1977. In 1980, he was the recipient of the Louis and Bert Freedman Award of the New York Academy of Sciences and, most recently, received an Honorary Doctorate degree from the University Rene Descartes in Paris. He has served on several

Study Sections and is the current Secretary of the International Narcotics Research Conference. He has served on the editorial board of several important journals in the biomedical field.

Yet, the most important thing about Eric is not his accomplishments in science and the honors that have followed, but the fact that he is a warm, modest, considerate and gentle human being who is loved by his family, friends and colleagues. I know of no more deserving recipient of the Nathan B. Eddy Memorial Award than Eric J. Simon, and I want to take this opportunity to thank the anonymous Awards Committee for selecting him to be the 1983 recipient of this honor.

Recent Studies on Opioid Receptors: Heterogeneity and Isolation (The Nathan B. Eddy Memorial Award Lecture)

Eric J. Simon

INTRODUCTION

I want to thank Dr. Sydney Archer for his very kind and flattering presentation. I am deeply honored and very grateful to CPDD for having chosen me to receive the 1983 Nathan B. Eddy Memorial Award. It is a great honor to receive an award in the memory of Dr. Eddy. He was one of the founders of CPDD and for 50 years he was a leader of this organization and a strong force in drug abuse research. Though trained as a physician he made his most important contributions in the chemistry and pharmacology of the opiates. His laboratory contributed many important papers on the structure-activity relationship of natural and synthetic analgesics. His account of the first 42 years of the Committee is outstanding for its accuracy and completeness. Nathan B. Eddy was a great man to whose memory it is a privilege to contribute.

This award is also a great honor because of the outstanding recipients who preceded me and because of the many excellent and deserving scientists who have not yet been so honored.

I would not be here today without the outstanding work done by my collaborators and students. I am deeply grateful to them and I wish to single out for special mention Prof. Jacob M. Hiller who has been my coworker and friend throughout all of our work on opioid receptors.

I also want to thank my wife, Irene, for her understanding, patience and love throughout many years. Her willingness to take much responsibility off my shoulders made possible whatever success I have been able to achieve in my profession.

This paper will present a summary of our recent studies on opioid receptor multiplicity and our efforts to solubilize and purify the opioid binding sites. At the end, I will make some comments regarding the relationship of these studies to drug dependence.

OPIOID RECEPTOR MULTIPLICITY

Three types of opioid receptors were suggested by Martin (Martin et al. 1978, Gilbert and Martin 1978) based on his pharmacological studies in chronic spinal dogs. He named them after their prototypic ligands, mu for morphine, kappa for ketocyclazocine and sigma for SKF 10,047. These 3 receptor types have stood the test of time extremely well and they have also been postulated based on in vitro bioassay and binding studies. Work in Hans Kosterlitz's laboratory (Lord et al. 1977) has suggested the existence of yet another receptor type, which binds enkephalins with high affinity and has been designated delta.

Recent work in our laboratory (Bonnet et al. 1981) has revealed that heterogeneity similar to that found in rat or guinea pig can also be seen in human brain. Competition binding data for several regions of human brain are presented in Table 1.

TABLE 1

Binding competition experiments in human brain regions

n = no. of experiments. Results are expressed as the mean IC_{50} ± standard error of the mean. From Bonnet et al. 1981.

Brain region	n	^{3}H-naloxone		^{3}H-DADL	
		Naloxone	DADL	Naloxone	DADL
Thalamus	5	2.7±0.3	49±4.9	1.4±0.1	13±2.2
Amygdala	3	2.8±0.2	63±24	14 ±2.9	3.3±0.7
Frontal cortex	4	2.7±0.3	57±4.9	12 ±5.4	2.2±0.5
Striatum	5	2.6±0.4	58±15	31 ±5.2	3.8±0.6

Naloxone generally competes more effectively against labeled naloxone than against ^{3}H-enkephalin, while the opposite is true for Dala^2-Dleu^{5}enkephalin. An exception to this can be seen in the thalamus where naloxone competes somewhat better-against ^{3}H-DADL than against itself. This finding, similar to results in rat brain (Chang et al. 1979) is best explained by the presence in the thalamus of a large preponderance of mu sites. The presence of mu and delta sites is suggested by the above study. More recent results from our laboratory, suggest the presence of kappa and sigma sites in human brain also (Itzhak et al. 1982).

One problem that plagued studies on opiate receptor heterogeneity for some years was the inability to inhibit or inactivate one or another class selectively by agents that are not ligands of the receptors. This led Robson and Kosterlitz (1979) and J. Smith in my laboratory (Smith and Simon 1972) to do selective protection experiments. In these studies it was shown that a given type of binding site could be selectively protected while all others were inactivated by irreversible agents (N-ethylmaleimide was used by us, phenoxybenzamine by Robson and Kosterlitz). This approach provided strong evidence for the existence of

different receptor types and provided a method for enriching a tissue in a given type of opioid binding site.

More recently our laboratory (Hiller et al. 1981) has reported that ethanol and other aliphatic alcohols inhibit binding to delta sites selectively (Fig. 1) This inhibition is reversible and much evidence was accumulated to demonstrate that the effect of the alcohol is on the binding site and not on the ligand.

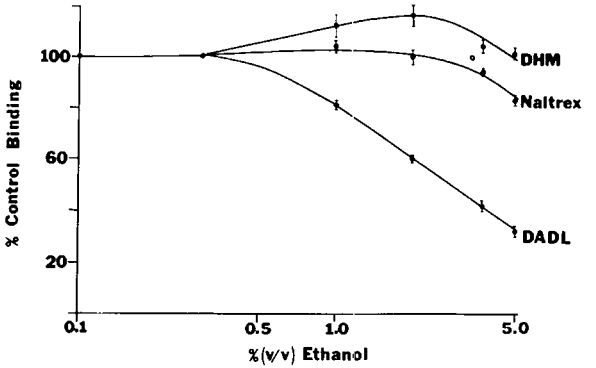

Fig. 1. Effects of ethanol on the binding of ^{3}H-labeled opioids to opioid receptors in rat brain membrane preparations. Duplicate 2 ml samples (0.9 to 1.1 mg of protein per milliliter) in 0.05M Tris HCl (pH 7.4) containing 1 mM dipotassium EDTA were incubated with 1 nM ^{3}H-dihydromorphine (specific activity, 73.2 Ci/mmole), ^{3}H-naltrexone (8.5 Ci/mmole), and ^{3}H-DADL (31.0 Ci/mmole) . To assess specific binding, samples were incubated in the presence or absence of 1 µM unlabeled ligand. From Hiller et al. 1981. (Copyright 1981 by AAAS).

The mechanism of the alcohol effect is not understood. We have suggested that the alcohol increases membrane fluidity (a well known action of alcohols) and that the delta sites are more sensitive than mu and kappa sites to this change in their membrane environment. Further work is required to prove or disprove this hypothesis. The intriguing possibility that there may be a relationship between alcohol and the endogenous opioid system is also worthy of further investigation.

In order to indicate that our laboratory also carried out research which does not involve "grinding and binding." I will present one in vivo study which bears on the heterogeneity of opioid receptors. Dr. Kenneth Carr in my laboratory has been producing aversive reactions in rats by stimulation of the pain-related

nucleus reticularis gigantocellularis. (NGC) with indwelling electrodes. The rats are trained to press a lever to escape from the stimulation. In a recent study (Carr et al. 1982) he showed that both systemic morphine and ethylketazocine (EKC) will effectively reduce escape attempts, which is interpreted to represent analgesia. However, the effect of morphine is readily blocked by low doses of naloxone while the effect of EKC is only partially blocked even at 10 times higher doses of naloxone. This result suggests that the two drugs act via different opioid receptors. It is also the first instance in which a supraspinally elicited aversion is reduced by a kappa analgesic.

The molecular basis of receptor heterogeneity is not known. There are a number of possible explanations. For example, there could be a single receptor which can exist in several different conformations. There could also be separate receptor molecules for many or all of the types described. These could represent different gene products (polypeptide chains) or different post-translational modifications of the same polypeptide chain (different carbohydrates, lipids, disulfide bridges, etc.). Which of these (and other) possibilities are the correct ones will only be known when it becomes possible to isolate and try to separate different opioid receptor types. My laboratory is working actively in this direction and I will describe some of our recent findings.

ISOLATION OF OPIOID BINDING SITES

I have already indicated one reason for undertaking the difficult work of isolating and purifying opioid receptors. In addition to trying to establish the molecular basis of receptor heterogeneity there are other important possibilities. Antibodies against the receptor can be produced and aid in the study of receptor function and distribution. The receptors can be reconstituted into membranes to study their effects on ion fluxes. The chemical composition and subunit structure of the binding site and of coupled enzymes can be elucidated. Finally it will become possible to study the amino acid sequence of the receptor proteins and to learn about the structure of genes coding for them.

Our attempts to isolate opioid receptors began early after their discovery. It was quickly found that they are tightly attached to cell membranes and that the first step, their removal from the membrane and solubilization, may prove to be a difficult one.

In 1975 we reported that we were able to solubilize a macromolecular complex of ^{3}H-etorphine from rat brain (Simon et al. 1975). This complex had all the properties of an etorphine linked to an opioid binding site. The molecular weight was about 400,000.

The solubilization of a receptor molecule that retained its ability to bind opiates in solution did not meet with success until 1980. That year we found in collaboration with Dr. Urs Ruegg at the University of Geneva that we could solubilize active

opioid receptors from the brain of toads (Bufo marinus) using digitonin (Ruegg et al. 1980).

The reason why we tried toad was our realization that it had been possible to. solubilize active ß-adrenergic receptors from non-mammalian tissues, while only prebound ligand-receptor complex could be solubilized from mammals. This did work for us and appears to be due to a decreased sensitivity of receptors from non-mammals to the deleterious effects of detergents.

At about the time we reported successful extraction of opioid receptors from toad brain, success in receptor solubilization was also reported by others. Bidlack and Abood (1980) reported that they were able to solubilize opioid binding sites from rat brain using the detergent Triton X-100. Simonds et al. (1980) had success extracting receptors from neuroblastoma x glioma hybrid cells in culture and also from rat brain using a new detergent called CHAPS.

More recently we (Howells et al. 1982) have learned how to solubilize active opioid binding sites in good yield (20-40%) from mammalian brain (rat, cow, human). The detergents digitonin and glycodeoxycholate worked very well, provided a high concentration (0.5-1 M) of sodium chloride was present during the extraction process. The soluble binding sites had very similar properties to those of membrane-bound sites. An example of this similarity is shown in Fig. 2. Correlation of affinities of widely varying ligands is excellent.

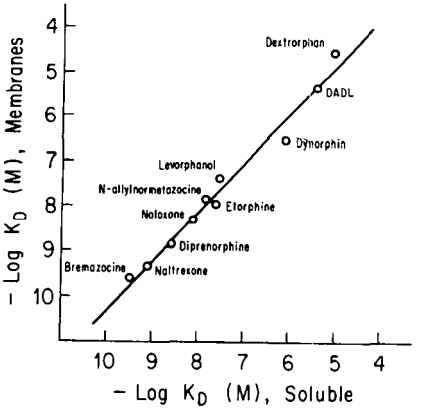

Fig. 2. Correlation of the affinities of various opioid ligands for membrane-bound and soluble binding sites from bovine striatum. Affinities were determined by Scatchard analysis or by competition studies. The line of best fit was determined by linear regression analysis (r=0.99). From Howells et al. 1982. Copyright 1982, The American Society for Pharmacology and Experimental Therapeutics.

We have already been able to obtain results with solubilized binding sites that were not possible with membrane-bound receptors. Thus, we have studied the retention of solubilized receptors on columns of agarose-bound lectins (Gioannini et al. 1982). Lectins are plant (and sometimes animal) proteins that bind sugars specifically. Out of eight lectins used, only one was found to bind opioid receptors. This was wheat germ agglutinin (WGA), a lectin that specifically binds N-acetylglucosamine. The receptors were specifically eluted from the WGA column by solutions containing N-acetylglucosamine. This result was found for soluble opioid receptors isolated from the brains of five species. It was the first evidence that opioid receptors contain sugar moieties and are, therefore, glycoproteins. In addition to this important information a single pass through a WGA column gave 30-50 fold purification of solubilized opioid binding sites.

Dr. T. Gioannini in our laboratory has recently constructed a number of ligand affinity chromatography columns (unpublished results). One column, in particular, in which naltrexone is linked via its 6 position to a side chain attached to agarose, has proved very useful for receptor purification. A combination of lectin and ligand affinity chromatography results in a purification of several hundred fold for soluble opioid receptors. Partial purification has also been achieved recently in the laboratories of Abood, Klee, Loh, and Zukin.

In a very exciting recent study by Dr. Y. Itzhak (Itzhak et al. 1983) in our laboratory we have obtained evidence for a physical separation of kappa sites from mu and delta sites. This was done by sedimentation of digitonin-solubilized receptors from guinea pig brain on a sucrose density gradient. A typical result of such an experiment is shown in Fig. 3, The kappa sites sediment with

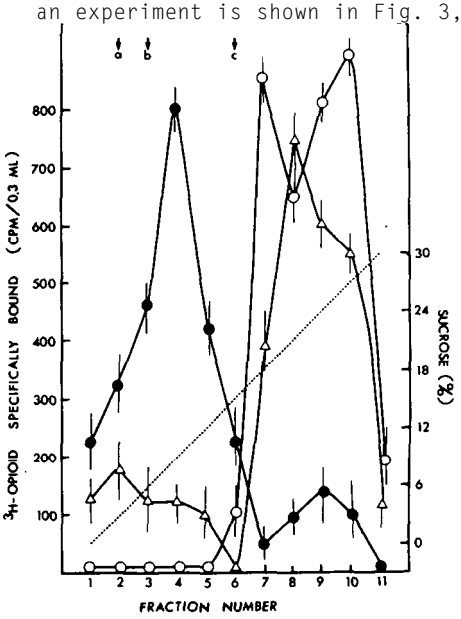

Fig. 3. Sucrose density gradient centrifugation of solubilized opioid binding sites from guinea pig brain membranes. Fractions were assayed for binding of ^{3}H-bremazocine (3.5 nM) in the presence of DAGO and DADL (100 nM each) (●), and for the binding of 3H-DAGO (7 nM) (△) and ^{3}H-DADL (7 nMI (○). Protein markers for the estimation of molecular weight are as follows: a) catalase, b)thyroglobulin (monomer), c) thyroglubulin(dimer). Each point represents the mean ± SEM of at least three determinations. From Itzhak et al. 1983. Copyright 1983. Pergamon Press, Ltd.

a velocity equivalent to a molecular weight of ca. 400,000 (assuming a globular protein), whereas mu and delta sites seem to have a molecular weight of about 7110,000. This suggests that some slight difference in the structure of the latter causes them to dimerize in the low digitonin concentration present in the sucrose. Whatever the reason for the separation it represents the first direct evidence for a molecular difference between receptor types.

RELATIONSHIP OF THE ENDOGENOUS OPIOID SYSTEM TO DRUG DEPENDENCE

The fact that laboratories involved in this type of research have received several Nathan B. Eddy Awards from CPPD and many grants from NIDA reflects the conviction of these distinguished organizations that knowledge of the endogenous opioid system will aid us in our understanding of drug dependence. Nevertheless, it is appropriate for me to address the question why 8-10 years after the major discoveries we do not have definitive answers concerning the biochemical mechanism of drug dependence.

There are basically two questions one can ask. During the development of tolerance and dependence 1) are there changes in opioid receptors? 2) are there changes in endogenous opioid peptides?

Rather careful studies on opioid receptors have led to negative results. Neither the number of binding sites nor the binding affinity seems to be altered during chronic treatment of rats with morphine. These studies were done in whole brain by Klee and Streaty (1974) and in three brain regions by our laboratory (Bonnet et al. 1976). It is therefore still possible that the appropriate brain regions have not yet been examined.

Even more important is the fact that these studies were done prior to our knowledge of receptor heterogeneity. Are there changes in one of the types of opioid receptors? Recent work in several laboratories has shown that selective tolerance can develop to a particular opioid receptor type (for example, Schulz et al. 1980). It is therefore essential to determine whether reduction of a receptor type due to tolerance leads to decreased binding to that type of receptor. The answer to this question is not yet known. If it turns out to be negative, which is entirely possible, the effect of tolerance must be on a step subsequent to binding. Such a mechanism is suggested by the cell culture model of Klee and collaborators in which chronic morphine affects adenylate cyclase and not the binding sites.

A number of reports on changes in levels of opioid peptides have also appeared. However, these results are still controversial and sometimes contradictory. In one report the plasma ß-endorphin levels of normal subjectswere 5-10 times higher than reported by other laboratories, suggesting non-specificity of the antisera used. In another report the effects were observed only after four weeks of morphine pellet implantation, when three days sufficed to render the animal fully tolerant and dependent.

There are, in my view, a number of reasons why these previous studies have been inconclusive. One is the fact that many of the assays were done on opioid peptides in the blood. It is very unlikely that blood levels in any way reflect the situation in the central nervous system. Recent studies done in cerebrospinal fluid from lumbar punctures would seem more promising in this regard. Another reason is the fact that to date people have only measured the levels of ß-endorphin and the enkephalins. We now know that there are several other opioid peptides that may play a role in chronic opiate treatment. Finally, it may become necessary to study the biosynthesis and turnover of the peptides rather than their levels in order to detect significant differences.

My feeling is, therefore, one of considerable optimism. Once we learn more about the fundamental nature and function of the endogenous opioid system it is very probable that we will understand the biochemical basis of the physiological aspects of drug dependence such as tolerance and physical dependence and, perhaps somewhat later, of such behavioral aspects as euphoria and drug-seeking behavior. It is for me impossible to imagine that an endogenous opioid system, already implicated in many acute effects of opiates, would not have an important role in the chronic actions of exogenous opiate drugs. I am even willing to stick my neck out to suggest that once we understand opiate abuse we will also begin to understand other forms of drug abuse and compulsive behavior.

REFERENCES

Bidlack, J.M., and Abood, L.G. Solubilization of the opiate receptor. Life Sci 27:331-340, 1980.

Bonnet, K.A., Groth, J., Gioannini, T., Cortes, M., and Simon, E.J. Opiate receptor heterogeneity in human brain regions. Brain Res, 221:437-440, 1981.

Bonnet, K.A., Hiller, J.M., and Simon, E.J., The effects of chronic opiate treatment and social isolation on opiate receptors in rodent brain. In: Opiates and Endogenous Opioid Peptides, Proceedings of the International Narcotic Research Club Meeting, Aberdeen, U.K. Amsterdam, North Holland Publ. co. 1976. pp. 335-343.

Chang, K-J., Cooper, B.R., Hazum, E., and Cuatrecasas, P. Multiple opiate receptors: different regional distribution in the brain and differential binding of opiates and opioid peptides. Mol Pharmacol, 16:91-104, 1979.

Gilbert, P.E., and Martin, W.R. The effects of morphine- and nalorphine-like druas in the nondependent. morphine-dependent and cyclazocine-dependent chronic spinal dog. J Pharmacol Exp Therap, 198:66-82, 1976.

Gioannini, T., Foucaud, B., Hiller, J.M., Hatten, M.E., and Simon, E.J. Lectin binding of solubilized opiate receptors: Evidence for their glycoprotein nature. Biochem Biophys Res Commun, 105:1128-1134, 1982.

Hiller, J.M., Angel, L.M., and Simon, E.J. Multiple opiate receptors: alcohol selectively -inhibits binding to delta receptors. Science, 214:468-469, 1981.

Howells, R.D., Gioannini, T.L., Hiller, J.M., and Simon, E.J. Solubilization and characterization of active opiate binding sites from mammalian brain. J Pharmacol Exp Ther, 222:629-634, 1982.

Itzhak, Y., Bonnet, K.A., Groth, K., Hiller, J.M., and Simon, E.J. Multiple opiate binding sites in human brain regions: evidence for κ and o sites. Life Sci, 31:1363-1366, 1982.

Itzhak, Y., Hiller, J.M., Gioannini, T.L., and Simon, E.J. Evidence for the solubilization of multiple, opioid binding sites from mammalian brain. Life Sci, 33:191-194, 1983.

Klee, W.A., and Streaty, R.A. Narcotic receptor sites in morphine-dependent rats. Nature, 248:61-63, 1974.

Lord, J.A.H., Waterfield, A.A., Hughes, J., and Kosterlitz, H.W. Endogenous opioid peptides: multiple agonists and receptors. Nature, 267:495-499, 1977.

Martin W.R., Eades, C.G., Thompson, J.A., Huppler, R.E., and Gilbert, P.E. The effects of morphine- and nalorphine-like drugs in the nondependent and morphine-dependent chronic spinal dog. J Pharmacol Exptl Therap, 197:517-532, 1976.

Robson, L.E., and Kosterlitz, H.W. Specific protection of the binding sites of D-Ala2-D-Leu5-enkephalin (-receptors) and dihydromorphine p-receptors. Proc R Soc Lond (Biol) 205:425-432, 1979.

RUegg, U.T., Hiller, J.M., and Simon, E.J. Solubilization of an active opiate receptor from Bufo marinus. Eur J Pharmacol, 69:367-368, 1980.

Schulz, R., Wuster, M., Krenss, H., and Herz, A. Selective development of tolerance without dependence in multiple opiate receptors of mouse vas deferens. Nature (London) 285: 242-243, 1980.

Simon, E.J., Hiller, J.M., and Edelman, I. Solubilization of a stereospecific opiate-macromolecular complex from rat brain. Science, 190:389-390, 1975.

Simonds, W.F., Koski, G., Streaty, R.A., Hjelmeland, L.M., and Klee, W.A. Solubilization of active opiate receptors. Proc Natl Acad Sci USA, 77:4623-4627, 1980.

ABBREVIATIONS

DAGO = DAla^2-MePhe^4-Gly-ol^5-enkephalin - the most selective mu ligand known.

DADL = DAla^2-DLeu^5-enkephalin - a relatively selective delta ligand.

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In Memoriam: Abraham Wikler and H. Frank Fraser--Introductory Remarks

Jerome H. Jaffe

This plenary session of the 45th Annual Meeting of the CPDD is dedicated to the memory of two pioneers in the field of research on addictive disorders, Abraham Wikler and H. Frank Fraser. It is altogether proper that we make such a dedication at this meeting and in this place- Lexington, Kentucky. For it was in Lexington, Kentucky that they lived for most of their professional lives and in Lexington where for 15 years they were colleagues and key figures on a remarkable research team. To appreciate their accomplishments, one must put these accomplishments in the context of the times: The time was 1940.

Himmelsbach had begun an effort to measure the intensity of opiate withdrawal, but apart from this step, little was known of the addictive processes. The way opiates relieved pain was unknown. It was still not certain whether opiate withdrawal was physical or largely psychological. Dalirium tremers was widely believed to be a toxic reaction to the actions of alcohol. There were no narcotic antagonists. Most of the opioids in use today were unknown; the notion of quantifying the subjective effects of drugs was in its perinatal period and the word psychopharmacology had not yet been invented.

Today, when young medical scientists wish to enter a field of research, they seek positions at an established laboratory, already doing work on a problem. Today, 30 years into the golden age of federal funding, there are usually several well-equipped laboratories from which to choose.

In 1940, when Abraham Wikler began his residency in psychiatry at the USPHS Hospital here in Lexington, there was no laboratory for the study of addictions. There was only the barest beginning; the concept was still a gleam in the eye of Harris Isbell. The men whose memories we honor today built the very tools with which they worked and with which we continue to work. Every time we use a questionnaire to measure the effects of an opioid or a euphorigenic drug, we are building on a foundation laid down by Frank Fraser. Every time we invoke notions of conditioning and

reinfor cement to account for drug-seeking behavior, we are using conceptual tools handed to us by Abe Wikler.

Today's plenary session includes four papers that build on work by Abe Wikler or Frank Fraser. Dr. William Martin will speak about opioid agonists and antagonists - the Lexington team was the first to show that nalorphine could precipitate opioid withdrawal giving impetus to the notion of the opioid receptor. Dr. conan Kornetshy will talk about environmental factors influencing pain. The paper by Hill, Kornetsky and Wikler, showing that at the usual dosage, morphine affects anticipatory anxiety associated with painful shock is still a classic. The next two papers touch cm the issue of learning factors - both classic conditioning and operant conditioning - in the addictive process. The first is on conditioning factors in opioid dependence by Charles O'Brien and Joseph Ternes. The second is on conditioned responses to alcohol cues by Ronald Kadden, Ovide Pamerleau, and Roger Meyer. This emphasis cm learning in the development of the addictive process may emerge as Abe's most important contribution.

Dr. Eric Simon, this year's Eddy Award, is honored for his role in the discovery of opioid receptors which, in turn, has helped to open a major frontier in neurobiology. We in the field of drug dependence have much to be proud of. This session dedicated to the memory of Abe Wikler and Frank Fraser should prompt us to recall the words of Robert Burton who said, "a dwarf standing on the shoulders of a giant may see further than a giant himself." Let us then do honor to two of the Lexington giants who have left us.

A Steric Theory of Opioid Agonists, Antagonists, Agonist-Antagonists, and Partial Agonists

William R. Martin

I am pleased and honored to participate in this symposium honoring Abraham Wikler and Frank Fraser. When I first joined the staff of the Addiction Research Center in 1957, Harris Isbell and Frank Fraser were among the great pioneers in clinical psychopharmacology. Frank invited me to collaborate with him in a comparative study of the subjective and physiologic effects of morphine and heroin. I entered these experiments skeptical of the methods that had been developed for measuring subjective effects and states through the cooperative efforts of Harris Hill, Richard Belleville, Harris Isbell, Frank Fraser, and Abe Wikler. I learned that these methods were valid, yielded reproducible experimental results, and were remarkably sensitive and discriminating instruments for measuring drug effects. Despite Abe's involvement in the development of these instruments, he had little faith in them. He didn't trust what people said, only what they did. Of all the wonderful associations at the Addiction Research Center, my interactions with Abe were the longest and most deep. Abe had a profound and tenacious intellect. He had just finished writing his arbeit "The Relation of Psychiatry to Pharmacology" and his interests encompassed all of neuropsychopharmacology. He was only then starting his experiment dealing with conditioned abstinence and drug-seeking behavior, phenomena which came to occupy his interest. Abe, however, retained his interests in opioid antagonists. He and George Acheson invited me to prepare a review of the narcotic antagonists for Pharmacological Reviews. Abe, with Chuck Gorodetzky and Don Jasinski, were the first to recognize the significance of the concepts of multiple opioid receptors and receptor dualism first formulated in this review.

Those who have been involved in the development of strong analgesics have recognized the complexity of the opioid receptor (Beckett et al. 1956; Bentley and Lewis 1972; Archer and Michne 1976). Over the last several years I have been extending these models. The method employed has been to construct clay models of receptors whose surfaces are complementary to Framework Molecular Models of prototypic opioids. These clay models are then used to form plaster shells. These plaster shells which consists of hills and valleys are surveyed and the reactive moieties are placed in rectangular coordinates (Figure 1). The reactive sites are

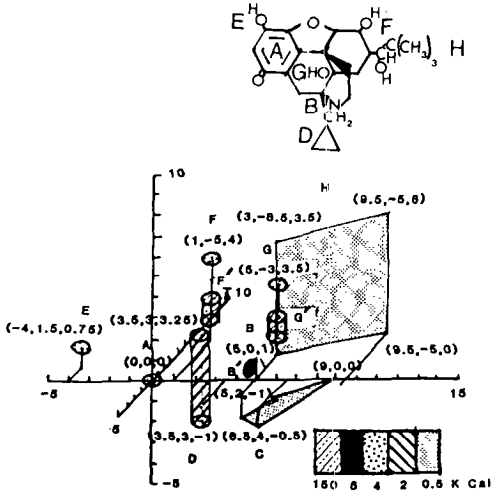

FIGURE 1

The schematic three dimensional oblique representation of the opioid receptor and the structural formula of a general opioid molecule.

then coded to indicate the approximate bond strength between the reactive sites on the receptor and the complementary sites of the opioid molecule. A more detailed description of this model has been presented (Martin, in preparation). The structure of the molecule used to construct the model and the three dimensional receptor map is illustrated in Figure 1. The X-Y plane of the coordinate system is the flat site (Beckett et al. 1956). This is labelled site A. The X axis goes through the center of the flat site to a point which is the vertical projection of site B to the X-Y plane. Site B is the anionic site of the Beckett et al. (1956) model. Site B′ is located approximately l Angstrom counterclockwise from site B and is the anionic site of the K receptor. Site C is closely associated with site B and is one of the sites with which substitutions on the nitrogen can interact. Site D interacts with the ketonic oxygen of ethylketocyclazocine and with certain substitutions on nitrogen. The E site interacts with the 3-OH moiety. The F site has two parts: The F site which interacts with the 14-OH group and the F′ site which can interact with highly reactive substitutions on the 14 position to form covalent bonds. The E site interacts with the 3-OH group. The G site interacts with the 6-OH group. The closely related G′ site interacts with highly reactive substitution on the 6 position. The H position interacts with large hydrophobic substitution on the 7 position (Bentley and Lewis 1972).

μ AND κ RECEPTORS

Sites A and B of the μ receptor (Figure 2) are called nuclear

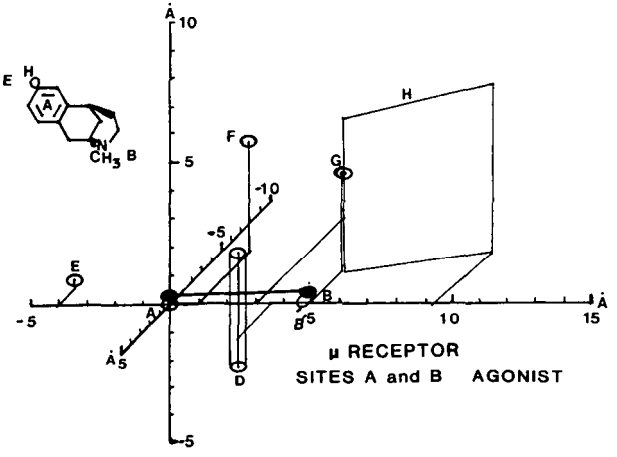

FIGURE 2

Figure 2 illustrates the A and B sites on the structural formula and opioid receptor. The positioning of the μ agonist is schematically illustrated with the filled circles and heavy connecting line.

sites for their occupation is necessary for μ type agonistic effects. Sites C, D, E, possibly F, G, and H are called satellite sites and serve two roles: (1) To enhance the binding of the drug to the receptor and (2) to orient the drug on the receptor. Sites B', A and possibly D are nuclear sites for the κ receptor while sites E, F, G, and H are satellite sites.

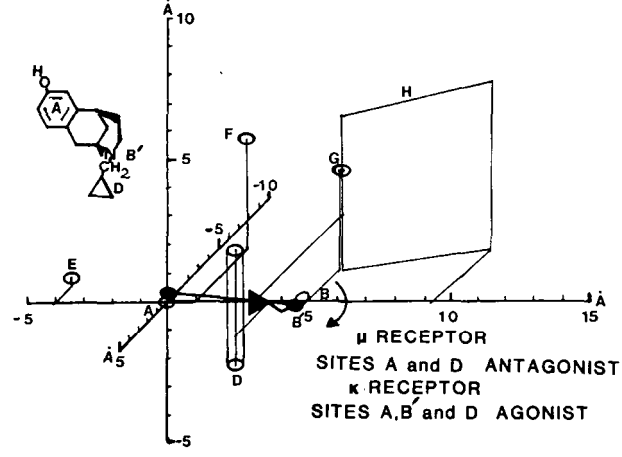

FIGURE 3

The orienting effect of the N-methylcyclopropyl substitution to put it on A, D position at the μ receptor and in the A, B', D position on the κ receptor. The drug on the receptor is schematized with the filled figures.

AGONIST-ANTAGONISTS

Figure 3 illustrates a specific type of pharmacologic redundancy in which the drug acts as a competitive antagonist at the μ receptor and as an agonist at the K receptor. At the μ receptor the N-methylcyclopropyl group interacts with the D site which rotates the drug around the Z-axis off of the B site. Since it no longer occupies the B site it does not exert an agonistic effect; however it still covers the receptor and will compete with a μ agonist. At the κ receptor the interaction of the methyl-cyclopropyl substitution on the nitrogen at the D site will position the nitrogen over the B′ site giving it κ activity.

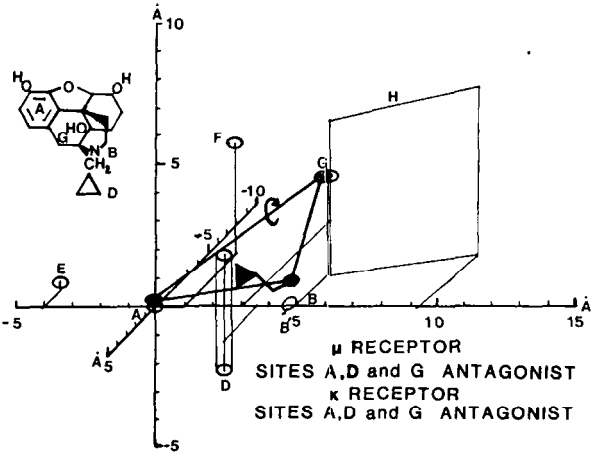

FIGURE 4

The orienting effect of the 14-OH group in conjunction with an N-methylcyclopropyl group which places the drug in the A, high D, G position.

PURE ANTAGONISTS

In the context of this paper, a pure opioid antagonist is defined as a drug which is a competitive antagonist at both the μ and κ receptors. The steric formulation of the pure antagonist is presented in Figure 4. The 14-OH group which conveys antagonistic activity to both N-allyl and methylcyclopropyl-noroxymorphone is postulated to interact with the G site which in turn constrains the rotation of the drug around the Z-axis. For the substitution on nitrogen (allyl or methylcyclopropyl) to interact with the D site the drug must rotate around the A-G axis allowing the substitution on nitrogen to interact at a higher location on the D site, thus shortening the D-G distance. This, however, lifts the nitrogen above both the B and B′ sites, thus precluding both μ and κ agonistic activity. Since the drug still covers both the μ and κ receptors, it acts as a competitive antagonist at both receptors.

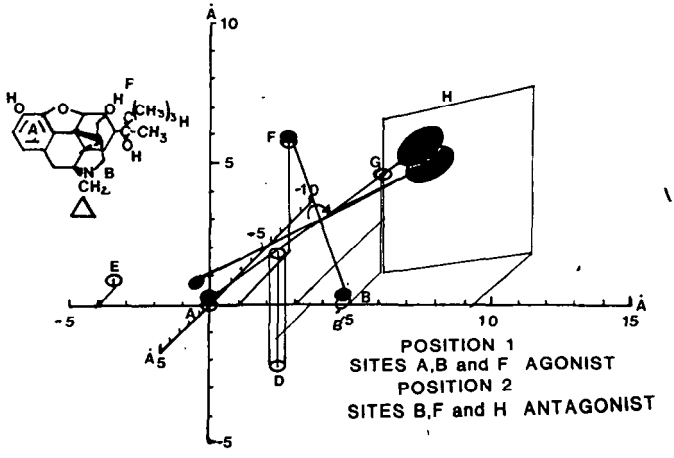

FIGURE 5

The orienting effect of 7 position substitutions interacting with the H site. Buprenorphine is shown occupying the receptor in an agonist and antagonist position.

PARTIAL AGONISTS

Three partial μ opioid agonists have been identified: Buprenorphine in the chronic spinal dog; profadol and propiram in man. The basic idea underlying the steric theory of partial agonism is that because of the complexity of the receptor, the drug can not only occupy the receptor's nuclear positions but can also occupy it in satellite positions. This principle is illustrated in Figures 5, 6, and 7. Buprenorphine (Figure 5) is postulated to occupy the μ receptor at the A, B, and F sites and thus exert an agonistic effect. However, it can also occupy the receptor in the B, F, and H positions which would not exert an agonistic effect but would cover the receptor and thus act as a competitive antagonist in this position. It is suggested that profadol (Figure 6) can also occupy the receptor in two positions: In the A-B position it would have a μ agonistic property while an interaction between the phenolic OH group and the E site would rotate the propiram molecule around the Z-axis lifting it off of the B site. Thus, it would not exert an agonistic action but would cover part of the receptor and act as an antagonist in this position. Propiram (Figure 7) could also occupy the μ receptor in two positions: The A-B agonist position and the A-E antagonist position. In position 1 (antagonist) the E site is presumed to be occupied by the ethereal oxygen which orients the propiram molecule such that the nitrogen is oriented in a position slightly counterclockwise to the E site and away from the B site.

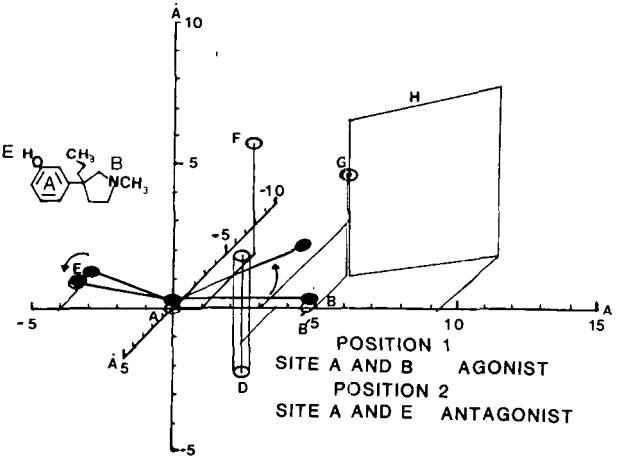

FIGURE 6

Profadol occupies the μ receptor in two position as a consequence of the phenolic hydroxyl group interacting with the E position.

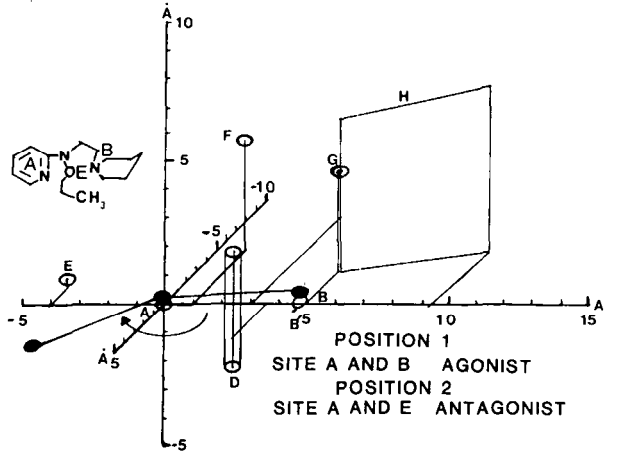

Figure 7

The postulated orienting effect of the ethereal oxygen of propiram.

According to this formulation, the intrinsic activity of an agonist is directly related to its affinity in the agonist position and inversely to its affinity in the antagonist position. This relationship can also be expressed as follows. The intrinsic activity (a) of the partial agonist is defined by equation 4, where K' is the dissociation constant for the drug occupying the receptor in the agonist configuration and K" for the antagonist configuration.

[DR]' Active Agonist Receptor Complex

[DR]" Inactive Agonist Receptor Complex

$$\frac{[D][R]}{[DR]'} = K' \qquad \frac{[D][R]}{[DR]''} = K''$$

$$4. \quad \alpha = \frac{[DR]'}{[DR]' + [DR]''} = \frac{K''}{K' + K''}$$

CONCLUSION

The unique virtue of this steric theory of the opioid receptor is that it provides an explanation for the diverse properties of opioids acting as agonists at different receptors, as agonists at one receptor and antagonists at others (agonist-antagonists), as antagonists and as partial agonists. The postulated principles that are presumed to confer these diverse properties to opioids are three in number: (1) The large number of reactive sites. There is evidence of eleven at this time. The reactive sites play two roles: (2) Their occupancy initiates pharmacologic actions and (3) They orient the drug in the receptor. This model does not necessitate either the postulation of alternative configurations of a receptor (agonist or antagonist) (Belleau 1964; Feinberg et al. 1976) or that the receptor undergoes allosteric changes (Archer and Michne 1976; Portoghese and Takemori, personal communication).

REFERENCES

Archer, S. and Michne, W.F. Recent progress in research on narcotic antagonists. In: Jucker, E., ed. Progress in Drug Research. Vol. 20, 1976.

Beckett, A.H., Casy, A.F. and Harper, N.J. Analgesics and their antagonists: Some steric and chemical considerations. Part III. The influence of the basic group on the biological response. J. Pharm. Pharmacol. 8, 874-883, 1956.

Belleau, B. A molecular theory of drug action based on induced conformational perturbations of receptors. J. Med. Chem. 7, 776-784, 1964.

Bentley, K.W. and Lewis, J.W. The relationship between structure and activity in the 6, 14-endoethenotetra hydrothebaine series of analgesics. In: Kosterlitz, H.W.., Collier, H.O.J. and Villa-rreal, J.E., ed. Agonist and Antagonist Actions of Narcotic Analgesic Drugs. London, MacMillan, 1972. pp. 7-16.

Feinberg, A.P., Creese, I. and Snyder, S.H. The opiate receptor: A model explaining structure-activity relationships of opiate agonists and antagonists. Proc. Natl. Acad. Sci. 73(11):4215-4219, 1976.

Martin, W.R. Pharmacology of opioids. Pharmacological Reviews (in preparation).

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Environmental Influences in the Perception of Pain

Conan Kornetsky

INTRODUCTION

My presentation will describe some of the early experiments by Abraham Wikler, Harris Hill, Harold Flanary, and myself on the effects of environmental variables on the perception of pain. I will try to relate these early experiments to current research findings and place them in the Zeitgeist of the period, circa 1950. An appropriate sub-title for my topic would be "Old wine in new bottles and new wine in old bottles."

I will not elaborate how Abraham Wikler, Harris Hill, Harold Flanary and I got together except to say that for the most part our roles were complementary. Each of us brought something unique to the collaboration. Wikler brought a sophistication of thought, ideas and experience from many fields. Hill, who had just received his Ph.D. from the University of Indiana, brought a broad base of psychological methodology, the ability to design an idea into an experiment and also a quiet confidence. Flanary brought to the group the ability of designing and putting together the apparatus to fit any crazy idea. As a second year graduate student I brought to the group an attribute not present in any of the staff, a highly developed degree of naivete.

The topic, environmental influences on the perception of pain, with specific reference to stress-induced analgesia (SIA) has received a great deal of attention in the last few years. However, the issue was not lost in the work of the research group at the USPHS Hospital in Lexington, Kentucky over 30 years ago. Wikler, in a review paper published in 1950 wrote,

> It appears reasonable to postulate that the recognition of the pain threshold in man is a discriminative function for which an optimum cortical excitatory state (CES) is required. Emotional disturbances, pain, etc. may raise pain threshold by elevating CES while analgesics may raise threshold by lowering CES, in both cases away

> from the optimum level required for most precise discrimination. Therefore the net effects of analgesics or other drugs on pain threshold in any individual under a given set of circumstances (e.g., tranquil, in pain, emotionally disturbed, etc.) may be in the direction of increase or decrease in discriminative ability and hence the pain threshold may be elevated or lowered by the drug or drug combinations used. (Wikler 1950)

Wikler then downgraded the importance of his own statement,

> Such a concept, while only a restatement of the fact that mental factors modify the effects of drugs on 'pain threshold,' may be useful in planning further investigations. (Wikler 1950)

The concept that pain or other competing factors could alter the perception of pain was, as Wikler indicated not an entirely new idea, but was expressed very early in the history of medicine. In the 5th century BC one of the Aphorisms of Hippocrates (Chadwick and Mann 1950, Section II, #46, page 154) stated, "If a patient be subject to two pains arising in different parts of the body simultaneously, the stronger blunts the other."

In 1940 Hardy, Wolff, and Goodell, using thermal radiation to produce pain, reported that pain in one part of the body raises the threshold to nociceptive stimulation to another part of the body. Many examples of such phenomena are given in the later comprehensive review by Beecher in his 1959 book, Measurement of Subjective Responses.

Kelly, more recently (1982), argues that if such phenomena are not simply distraction, then the analgesia must last beyond the duration of the induced pain experience. A 1945 experiment by Parsons and Goetzl clearly met the demands of Kelly. These investigators used electrical stimulation of a tooth pulp to produce pain. The threshold was defined as the voltage at which the subjects first experienced painful sensations. The external painful stimulus used to cause changes in the tooth stimulation threshold was induced by spraying ethylchloride on a small area of the subject's tibiae. Not only was the pain threshold elevated, but it was clearly elevated long after the cessation of the ethylchloride spraying. Unfortunately, these investigators did not have naloxone available to see if it would block the observed threshold-raising effect of the induced pain.

The role of culturally determined environmental factors in perception of pain was also known during the 1940s. Molone (1949) had observed during World War II that Okinawan children showed relatively little response to pain in the presence of major tissue damage. This lack of response was not seen in the Okinawan children living in close proximity to Westerners. Isbell at the Lexington Hospital was very much aware of the differences in response to

clinical pain between the Oriental patient reared in Asia and those reared in the United States. Beecher (1946) had already published his classic description of the wounded soldiers at Anzio during World War II. He observed that badly wounded men, not in shock, with clear sensorium showed little evidence that they were in pain. Even earlier was the report of William McDougall, who, as a member of a Cambridge University Anthropological expedition to British New Guinea (Murray Island), measured the pain threshold in 47 Murray Islanders. He used a weighted pointed object and reported the threshold in terms of kilograms of pressure. He stimulated the thumbnail, forefinger, and forehand. Subjects were asked to cry "stop" when they felt pain. However, he stated "I found that in nearly all cases it was possible to detect the moment of onset of pain by observing the slight flinching which is commonly caused in expectant subjects."

He compared the thresholds of Murray Island boys and men with those of English boys and men. These results are shown in the Table. As can be seen the threshold was higher in the Islanders although the younger subjects had thresholds not different from those of the adult Englishmen.

TABLE

Sensibility to Pain (x)
(in kilograms of pressure)

Subjects (N)	Thumb Nail	Forefinger	Forehand
Murray Island Men (47)	6.7	5.5	6.2
Murray Island Boys (18) (10-14 yrs)	3.8	3.0	
English Men (23)	3.8	3.6	3.8
English Boys (5) (13-14 yrs)	2.9	2.4	

(From McDougall, 1901)

In order to determine the specificity of the finding, McDougall also measured the threshold for two-point discrimination and discrimination of differences between weights. For this type of sensory stimulation the Islanders had lower thresholds than the English subjects. Of interest is that after the study by McDougall there were, as far as I could find, no experimental studies that compared pain threshold in various cultural groups until a 1965 report by Sternbach and Tursky. It is not surprising that investigators were aware that environmental factors could influence the perception of pain, for during this period and even earlier many investigators were studying the influence of past experience on perception in modalities other than pain (Braly 1933; Bruner and Goodman 1947; Bruner and Postman 1948). These investigations were often referred *to* as the "new look in perception."

During the period of the 1940's and 50's many of the studies that experimentally tested the analgesic actions of the narcotic drugs in man yielded conflicting results despite the well accepted analgesic action of these drugs in the clinic. Although many investigators reported consistent rises in the threshold for pain after morphine, most investigators obtained variable results. Wolff, Hardy, and Goodell (1940) found consistent rises in the pain threshold for thermal radiation stimulation following morphine administration, using the same procedure; however, Andrews (1943), Denton and Beecher (1949), Chapman and Jones (1944), and Isbell (unpublished data) found that the pain threshold might be elevated, lowered, or unchanged following morphine. Wikler believed that these discrepancies were a function of the large variability in emotional response by subjects to experimental pain and that much of this could be attributed to a class of responses which he called "anticipatory."

THE LEXINGTON EXPERIMENTS

In order to study these anticipatory responses we embarked on a series of experiments specifically designed to manipulate the degree of anticipatory fear or anxiety in the subjects. In the first of these experiments (Hill et al. 1952a), former narcotic addicts (patients at the USPHS Hospital, Lexington, KY, with a history of narcotic addiction) were used as subjects. In this experiment simple reaction time (RT) was tested under four conditions. 1) Electric shock penalties delivered to the hand for "slow" RT's, 2) shock penalties for slow RT's after morphine, 3) knowledge of RT no shock control, and 4) morphine-control. Subjects were seated in front of an opaque screen in which the response key and neon bulbs were mounted. The subjects were told that the bulbs would light in an "on your mark," "get set?" "go" sequence. The lowest light indicated that a trial was beginning ("on your mark"). The second bulb from the top flashed two sec later and indicated that a trial was beginning ("get set"). The other two lights were stimulus lights; one was above and one below the "get set" light. Only one of these was lit for any one trial, the subject responding to either by quickly moving the key to the left. The preparatory interval between the warning and the "go" light was variable, a pre-determined random order of 1, 2, or 3 seconds. For all pre-conditions the two "go" lights had identical meaning for the subjects and these appeared an equal number of times. After the pre period each of the two "go" lights had different meanings to the subject. If the first was lit, it would indicate that the previous response was fast; if the second was lit, it would indicate that the previous response was slow. RT was determined with know-ledge of performance with and without morphine.

The same procedure was also used with the subject receiving elec-tric shock for slow RT. Thus, if in the previous trial the response was slow, the top light came on and at the completion of the response the subject received the electric shock. The dose of morphine was 15 mg delivered intramuscularly. Figure 1 shows the results of this experiment. Whether or not the subject's RT was considered

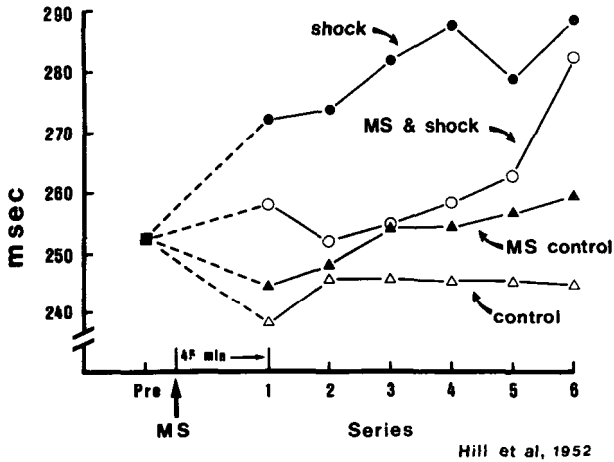

Figure 1. Effects of morphine [MS] on RT as a function of aversive electric shock. (Adapted from hill et al. 1952a)

slow or fast was based on the median of the series. What is clear is that anticipation of the shock certainly slowed RT and morphine clearly ameliorated this disruption in RT. It is not clear from the experiment whether or not the anticipation of the shock made the shock feel worse than it really was. The verbal report of the subjects suggest that the shock itself was not the disturbing factor but it was the anticipation of the shock. This experiment led to two additional experiments, one using electrical shock and one using thermal radiation as the nociceptive stimulus.

In order to test the hypothesis that the stress or anxiety level of the subject could alter the threshold for pain, the following experiment was carried out (Hill et al. 1952b). Volunteer subjects were randomly assigned to one of two groups and prior to the determination of the threshold they were treated quite differently. In one group everything was done to decrease any fear the subject might have regarding the anticipated painful stimuli. This was called the "Informal" group. Subjects in the other group were given no reassurance and nothing was done to decrease their putative apprehension. This group was called the "Formal" group. Both groups were studied with and without 15 mg of morphine. Except for the difference in the formality of the experiment the procedure was identical for all subjects.

The subjects were first trained in the procedure and then received two suprathreshold stimuli that were called the "standard." Subsequent stimuli would be rated by the subject as either "stronger"

or "weaker" than the standard. If drug were to be given, it was then administered. One hour later the threshold was determined by means of the psychophysical method of limits. Figure 2 shows the percent of "stronger" responses as a function of shock intensity in watts. The upper graph shows the subjects in the "Formal" group and the lower graph the subjects in the "Informal" group. Of most interest is that morphine significantly raised the threshold only in the "Formal" group of subjects and that the informal procedure alone had almost as much effect as morphine in raising the threshold. Note that the threshold after morphine was almost

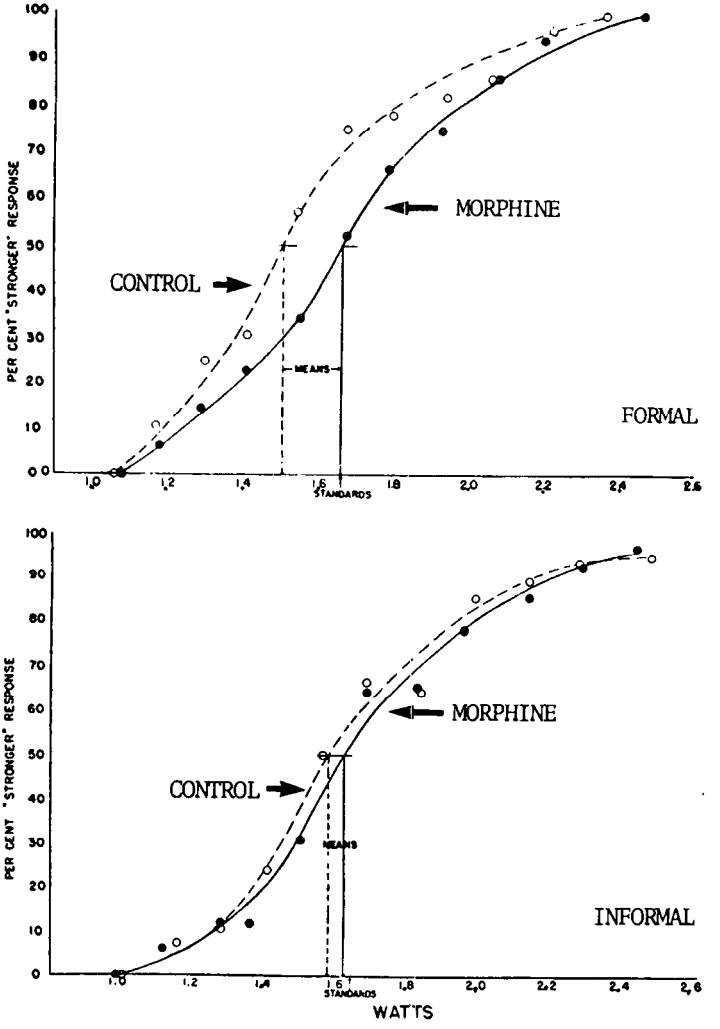

Figure 2. The percent "stronger" responses of subjects with and without morphine as a function of intensity of electrical stimulation under "Formal" and "Informal" experimental conditions. (Adapted from Hill et al. 1952b)

identical in the "Formal" and "Informal" conditions. Further, the more anxiety-provoking formal treatment of the subjects resulted in an overestimation of the intensity of the previously delivered "standard" stimulus, and morphine resulted in a more accurate estimate of the intensity of the "standard."

This experiment was repeated using thermal radiation to the forehead of the subjects as the nociceptive stimulus (Kornetsky, 1954). In this experiment a number of additional variables were added in order to determine the role of the anticipation of the stimulus in the pain experience. These were the amplitude of the galvanic skin response (GSR) expressed as conductance, latency of the GSR, anticipatory GSR's and time from onset of the stimulus to when the subject thought he felt pain. The dose of morphine was as in the previous study 15 mg, administered intramuscularly 60 min before the test. The results of this study are summarized in figure 3. The verbal report results completely replicated the previous study in which electric shock was the stimulus. In this study the mean number of times the subjects stated that the stimulus was "stronger" than the previously delivered "standard" is shown in the lower right chart. As can be seen morphine only caused a significant decrease in the number of reported "stronger" stimulations in the "Formal" group of subjects.

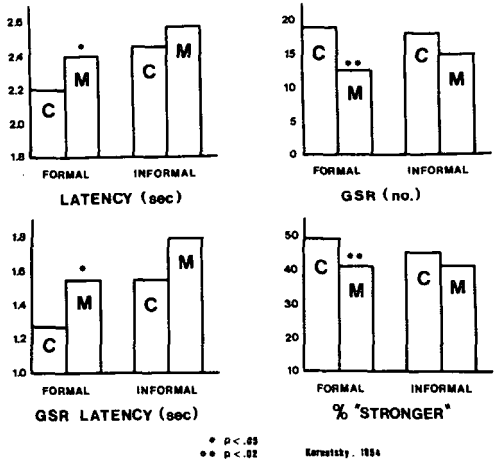

Figure 3 The effects of morphine (M) compared to control (C) on latency of response (motor response), GSR latency, amplitude of GSR to thermal radiation stimuli, and percent stimuli judged as "stronger" than a pre-treatment standard under "Formal" and "Informal" experimental conditions. Based on data from Kornetsky, 1954)

In each case illustrated morphine was only effective in the more anxiety-provoking paradigm. Although latency of GSR and number of anticipatory conditioned GSR's were significantly altered by morphine, the actual amplitude of the GSR was not affected, indicating the importance of the anticipatory state in the perception of the painful stimulus.

DISCUSSION

These studies raise some important questions regarding current experiments in which stress or pain are used to induce analgesia. These early Lexington experiments suggest that pain threshold can also be raised by decreasing the stress of the painful encounter, certainly a maneuver not foreign to the clinician. Stress or pain or simply decreasing the anticipation of a painful experience are probably not the only maneuvers that will produce analgesia. Pleasurable experiences such as sex (Beecher 1959, p. 153), listening to music, watching the Superbowl, etc., will also decrease pain and often the pain does *not* return immediately after the cessation of the behavior.

Experimental evidence that it need not be a painful stimulus that induces analgesia is given by the 1965 report of Cox and Valenstein. These investigators demonstrated that rewarding electrical stimulation to the hypothalamus attenuated the aversive properties of foot shock in the rat.

Of interest is that Mayer et al. (1971) in their paper on brain stimulation-induced analgesia reported that there was a point-biserial correlation of 0.83 between rewarding self-stimulation rates of response and whether or-not these sites produced analgesia. Although analgesia produced by intracranial stimulation is considered a discovery of the 1970's, the Cox and Valenstein experiment was done in 1965 and as early as 1954 Heath reported that stimulation of the septal area resulted in immediate relief from intractable pain. Lilly in 1960 reported that rewarding brain stimulation raises the threshold for aversive central stimulation.

In the 1970's numerous studies were published demonstrating that various stressors would raise the threshold to pain (e.g., Akil et al. 1976). An important question is the specificity of this stress-induced analgesia. Kelly stated in a 1982 publication, "It has never been proven that the sensory deficit induced by stress is limited to the modality of pain." Although some investigators have stated that responses to other sensory modalities are not affected, there are no published experiments in which the threshold for stimulation to other modalities was done with the same care *that* these investigators used *in* measuring the threshold for pain stimulation.

Evidence, however, that other aspects of behavior can be altered by the stress of inescapable shock is given by the learned helplessness model (Ovennier and Seligman 1967; Seligman and Maier 1967) in which animals given inescapable electric shock

showed subsequent performance deficits. The argument often given for stress-induced effects being specific for perception of pain stimulation alone is based on the reports that the induced analgesia can be blocked by naloxone. Since naloxone itself causes increases in sensitivity to nociceptive stimulation (e.g., Buchsbaum et al. 1977 in man; Carmody et al. 1979 in rats and mice; and Sasson and Kornetsky 1983 in rats), simply increasing the animal's sensitivity to the pain stimulus under test may result in apparent block of the induced analgesia.

The discrepancy between earlier work by Wikler and his colleagues in which stress seemed to lower the threshold for pain and current work in which stress raises the threshold may be a function of where one's attention is directed. In the Lexington studies the stress (the "Formal" procedure) directed the subject's attention toward the stimulus. In the stress-induced analgesia studies the stress is directing the subjects attention away from the nociceptive stimuli.

In conclusion alteration in pain thresholds by stress or pain is not a new phenomenon and the attempts to relate the phenomonon to an analgesic endorphin-like action may result in losing sight of a more general role of the endorphins in stress and their possible role in modulating the attentional components of perceptions.

REFERENCES

Akil, H., Madden, J., Patrick, R.L., and Barchas; J.D. Stress-induced increase in endogenous opiate peptides: concurrent analgesia and its partial reversal by naloxone. In: Kosterlitz, H.W., ed. Opiates and Endogenous Opiate Peptides. Amsterdam: Elsevier/North-Holland, 1976. pp. 63-70.

Andrews, H.L. The effect of opiates on pain threshold in post-addicts. J. Clin. Invest, 22:511-516, 1943.

Beecher, H.K. Pain in men wounded in battle. Ann. Surg, 123:96-105, 1946.

Beecher, H.K. Measurement of Subjective Responses. New York: Oxford University Press, 1959. 494 pp.

Braly, K.W. The influence of past experience on visual perception. J. Exp. Psychol, 16:613-643, 1933.

Bruner, J.S., and Goodman, C.C. Values and need as organizing factors, in perception. J. Abnorm. Soc. Psychol, 42:33-44, 1947.

Bruner, J.S., and Postman, L. Symbolic value as an organizing factor in perception. J. Abnorm. Soc. Psychol, 43:195-201, 1948.

Buchsbaum, M.S., Davis, G.C., and Bunney, W.E., Jr. Naloxone alters pain perception and somatosensory evoked potentials in normal subjects. Nature, 270:620-622, 1977.

Carmody, J.J., Carroll, P.R., and Morgans, D. Naloxone increases pain perception in rats and mice. Life Sci, 24:1149-1152, 1979.

Chadwick, J., and Mann, W.N. The Medical Works of Hippocrates. Oxford: Blackwell Scientific Publications, 1950. 301 pp.

Chapman, W.P., and Jones, C.M. Variations in cutaneous and visceral pain sensitivity in normal subjects. J Clin Invest, 23:81-91, 1944.

cox, V.C., and Valenstein, E.S. Attenuation of aversive properties of peripheral shock by hypothalamic stimulation. Science, 149: 323-325, 1965.

Denton, J.E., and Beecher, H.K. New Analgesics I. Methods in the clinical evaluation of new analgesics. J Amer Med Assoc, 141: 1051-1057, 1949.

Hardy, J.D., Wolff, H.G., and Goodell, H. Studies on pain. A new method for measuring pain threshold: observations on spacial summation of pain. J Clin Invest, 19:649-657, 1940.

Heath, R.G. Studies in Schizophrenia. Cambridge: Harvard University Press, 1954.

Hill, H.E., Kornetsky, C., Flanary, H.G., and Wikler, A. Studies on anxiety associated with anticipation of pain. AMA Arch Neurol Psychiat, 67:612-619, 1952a.

Hill, H.E., Kornetsky, C., Flanary, H.G., and Wikler, A. Effects of anxiety and morphine on discrimination of intensities of painful stimuli. J Clin Invest, 31:473-480, 1952b.

. Kelly, D.D. The role of endorphins in stress-induced analgesia. In: Verebey, K., ed. Opioids in Mental Illness: Theories, Clinical Observations, and Treatment Possibilities. Annals of the New York Academy of Sciences, 398:260-271, 1982.

Kornetsky, C. Effects of anxiety of morphine on the anticipation and perception of painful radiant thermal stimuli. J Comp Physiol Psychol, 47:130-132, 1954.

Lilly, J.C. Learning motivated by subcortical stimulation. In: Ramey, E.R. and O'Doherty, D.S. eds. Electrical Studies on Unanesthetized Brain. New York: Paul B. Hoeber, Inc, 1960. pp. 78-105.

Mayer, D.J., Wolfe, T.L., Akil, H., Carder, B., and Liebskind, J.C. Analgesia from electrical stimulation in the brainstem of the rat. Science, 174:1351-1354, 1971.

McDougall, W. Cutaneous sensations. In: Hadden, A.C., ed. Reports of the Cambridge Anthropological Expedition to Torres Straits, Vol. 2. Cambridge: Cambridge University Press, 1901. pp. 189-195.

Moloney, J.C. The Magic Cloak. Wakefield, MA: Montrose Press, 1949. 299 pp.

Overmier, J.B., and Seligman, M.E.P. Effects of inescapable shock upon subsequent escape and avoidance responding. J Comp Physiol Psychol, 63:28-33, 1967.

Parsons, C.M., and Goetzl, F.R. Effects of induced pain on pain threshold. Soc Exp Biol, 60:327-329, 1945.

Sasson, S., and Kornetsky, C. Naloxone lowers brain stimulation escape thresholds. Pharmacol Biochem and Behav, 18:231-233, 1983.

Seligman, M.E., and Maier, S.F. Failure to escape traumatic shock. J Exp Psychol, 74:1-9, 1967.

Sternbach, R.A., and Tursky, B. Ethnic differences among housewives in psychophysical and skin potential responses to electric shock. Psychophysiol, 1:241-246, 1965.

Wikler, A. Sites and mechanisms of action of morphine and related drugs in the central nervous system. Pharmacol Rev, 2:435-506, 1950.

Wolff, H.G., Hardy, J.D., and Goodell, H. Studies on pain. Measurement of the effect of morphine, codeine, and other opiates on the pain threshold and an analysis of their relation to the pain experience. J Clin Invest, 19:659-680, 1940.

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Classical Conditioning in Opiate Dependence

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INTRODUCTION

Opioid use provides an excellent opportunity for classical conditioning. The opiate drug is a Pavlovian unconditioned stimulus (US) which is reliably and consistently followed by an unconditioned response (UCR) which involves several efferent systems. Whether in animals or in humans, drug administration usually involves a set of complex procedures and several sensory systems, which can function as a conditioning stimulus (CS). Dr. Wikler, one of the pioneers we are honoring today, often pointed out that repeated drug use could produce conditioned effects. According to his analysis, the CS should initially acquire the power to elicit a conditional response (CR) which resembles the UCR. In an opiate-dependent individual another possible conditioning situation can occur. As the last dose of drug is metabolized a withdrawal syndrome occurs and in this instance environmental stimuli or rituals may be paired with the withdrawal symptoms. After repeated pairings, the CS alone may acquire the ability to evoke a conditioned response which resembles withdrawal effects. In Wikler's terms, the individual has acquired an additional disease, a disease sui generis as he called it.

Despite vastly improved addiction treatment programs in the 1980's we still have no available treatment modality which directly deals with the conditioning aspects of addiction. Why is this? Part of the problem relates to the confusion about which type of conditioned effects to treat, conditioned opioid-like effects, or conditioned withdrawal-like effects. Additionally there is confusion about which systems to measure: analgesia, temperature, salivation or euphoria. Do these effects occur commonly or do they only occur in a few unusual patients? Are these phenomena lawful and orderly? What about the apparent contradictions in the literature? Definitive answers to these questions are elusive. The goal of this paper is to clarify some of the issues. We will begin by describing three categories of opioid-conditioned phenomena: conditioned

opioid like effects, conditioned withdrawal-like effects and conditioned tolerance. Then we will attempt to put some of the sources of conflict into perspective and discuss their clinical implications.

OPIOID-LIKE CONDITIONING

Historically, the first observed conditioned opioid responses were reported by Pavlov and colleagues. They found that morphine injections caused profuse salivation, vomiting and sleep in dogs. After several trials, the mere sight of the technician who gave the injection was enough to produce the same effects as morphine: salivation, vomiting and sleep. This was such a reliable effect in Pavlov's lab that it was used as a demonstration for students. It was reported by other labs in the early literature and seemed to be a well-established response.

However, more recently Lynch (1976) had difficulty producing a morphine conditioned response in dogs. After several failures, he consulted Dr. W. Horsley Gantt, who had been a student of Pavlov in Leningrad. Gantt pointed out that Lynch was administering four conditioning trials in a one-hour session. Following Gantt's advice, he began limiting the trials to one or two per session with no other protocol Changes. After a few more sessions, Lynch began to observe behavioral, salivary, heart rate and respiratory responses to the conditional stimulus. He found morphine-like conditioned responses to be robust and parts of the response were very resistant to extinction.

Similar results have been reported in humans and in rats. These are summarized in Table I. While these responses can be reliably produced, they require specific conditions as illustrated in the anecdote from Lynch's lab. The human results (Meyer and Mirin 1979) were obtained in selected hospitalized patients who self-injected sterile coded saline solution which might have contained opioid. The more naturalistic the setting, the greater the likelihood of opioid like or "placebo" effects. Injections of saline given by a nurse were less likely to produce euphoria or physiological charges than those self-administered after a "cook--up" ritual in a simulated "shooting gallery' (O'Brien et al. 1975). Most subjects showed mild opioid-like effects which extinguished after one to three unreinforced trials (saline injections). A few, however, persisted in opioid-like responses after many saline self-injections. Such subjects have been clinically described as needle-freaks (Levine 1972).

WITHDRAWAL-LIKE CONDITIONING

A quite different type of opioid conditioned response has also been described. These are conditioned withdrawal-like responses

TABLE 1. Opioid-Like Conditioning[1]

CS	CR	DOSE	SUBJECTS	AUTHORS
Sight of experimenter who previously injected morphine	Salivation, vomiting, sleep	(not specified)	dogs	Pavlov, 1927
Sight of Syringe	Salivation	Morphine, 30-80 mg/kg	dogs	Collins and Tatum 1925
Tone	Hyperthermia	Morphine, 20 mg/kg	rats	Miksie et al. 1975
Distinct injection environment	Hyperthermia	Morphine, 5-200 mg/kg	rats	Eikelboom and Stewart 1979
Tone	Reduction of withdrawal signs ("wet-dog shakes")	Morphine, 15 mg/kg	rats	Numan, Smith, and Lal, 1975
Tone	Resumption of operant behavior suppressed during morphine withdrawal	Morphine, 40 mg/kg	rats	Tye and Iverson, 1975
Buzzer	Increased salivation, gastric secretion and heart rate.	2mg/kg (higher dose in Table II)	dogs	Rush et al., 1970
Self-Injection of Saline when opiate expected; or self-injection of opiate in presence of naltrexone	Subjective reports of "taste" "rush," and euphoria; meiosis and increased skin temperature in some subjects, quickly extinguished in most subjects	History of street opiate; variable dose and duration	humans	O'Brien, 1975
Self-injection of heroin (0.5-4.5 mg/dose) in presence of naltrexone 75 mg	Slight decrease in pupil size and decrease in respiratory rate on first few injections, disappeared with later injections. (CR seen only in the 11 of 22 subjects who voluntarily continue to self-inject heroin while receiving naltrexone.	History of street heroin addiction	humans	Meyer and Mirin, 1979

1. Adapted from Grabowski, J., and O'Brien, C.P. Conditioning factors in opiate use. In: Mello, N.K., ed. Advances in Substance Abuse: Behavioral and Biological Research. Greenwich, CT: JAI Press, Inc., 1981. pp. 69-122.

and they were first described by Dr. Wikler. He noted the appearance of withdrawal symptoms (yawns, tearing, rhinorrhea) in post-addicts in response to verbal stimuli. These former addicts were in the Addiction Research Center at Lexington during the 1940's and 50's. Dr. Wikler noted the appearance of withdrawal symptoms during group therapy sessions when drug-taking was discussed. He also heard fascinating anecdotes from patients who told of feeling well after months or years of incarceration and then returning to their home, usually New York City. As soon as they caught sight of their former drug-using environment, however, withdrawal symptoms occurred and relapse to drug use ensued.

Wikler interpreted these reports as classical. conditioning of withdrawal signs and symptoms. In the laboratory, this interpretation of the anecdotal reports has been supported by experimentally producing conditioned withdrawal in animals and in humans. A summary of the reports is given in Table II, Our group has demonstrated that withdrawal symptoms can be conditioned in human subjects (O'Brien, Testa, O'Brien, Brady, and Wells, 1977). Naloxone, a narcotic antagonist, was used as the US to produce mild dysphoria and physiological and behavioral signs of withdrawal, the UCR. After patients in methadone maintenance were given training injections of small doses of naloxone paired with a compound (tone and odor) CS test trials in which the CSs were paired with placebo injections also produced similar withdrawal signs. It should be noted that the CR which is described in this section is a withdrawal response which resembles the UCR to naloxone. This type of conditioning does not occur until after physical dependence upon the opiate has developed and occurs only in those instances where a CS is correllated with the onset of the withdrawal syndrome. An addict who maintains his habit so as to avoid withdrawal sickness would have little or no opportunity to acquire the conditioned withdrawal reaction. Hence, the directly conditioned withdrawal CR may not provide an adequate explanation of relapse to narcotics use.

CONDITIONED TOLERANCE EFFECTS

There appears to be a third type of conditioning in which compensatory mechanisms become conditioned during the addictive cycle. In such cases, the conditioned stimulus comes to elicit a CR which is opposite or antagonistic to the unconditioned response. Unlike the two previous CRs, the conditioned compensatory response is an atypical result of a Pavlovian conditioning paradigm because the CR and the drug-elicited response tend to cancel each other (see Siegel 1977 for a review of compensatary CRs).

Wikler (1973) first pointed out that reactions to direct drug effects could be conditioned. He described this as conditioning of adaptive responses to drugs and these

TABLE II. Experimentally Conditioned Withdrawal Studies[1]

SUBJECTS	UCS	UCR	CS	CR	AUTHORS
Rhesus monkeys dependent on morphine or other opiates	Nalorphine, 2 mg/kg injection	Salivation, vomiting, tremors	Injection procedure (Saline on Test Trials)	Withdrawal-like syndrome	Irwin and Seevers, 1956
Rats dependent on morphine	Metabolism of morphine	Gradual onset of withdrawal measured as "wet dog shakes"	Distinct withdrawal environment	Increased "wet dog shakes" in distinct environment	Wilker and Pescor, 1967
Rhesus monkeys dependent on morphine	Injection of nalorphine, 0.2 mg/kg	Increased heart rate and respiration, decreased brain temperature, emesis, salivation; suppression of bar-pressing for food	Red light	Suppression of bar-pressing for food, heart rate decrease, vomiting, excessive salivation	Goldberg and Schuster, 1970
Humans dependent on methadone	Naloxone, 0.1 mg/70 kg I.M.	Increased heart rate, blood pressure, respiratory rate, pupil size; decreased skin temperature, nausea, lacrimation, rhinorrhea, characteristic motor behavior	Tone/odor saline - injection, I.M.	Increased heart rate blood pressure, respiratory rate; decreased skin temperature, nausea, lacrimation, rhinorrhea, characteristic motor behavior	O'Brien et al.,1976 O'Brien et at., 1977

1. Adapted from Grabowski and O'Brien, 1981. (See Table I.) Copyright 1981, JAI Press, Inc. Reprinted by permission.

adaptations would be opposite to the direct drug effect. Perhaps the clearest example of this is with atropine. One unconditioned effect is an inhibition of salivary secretions. After several trials, however, both dogs and cats begin to show increased salivation prior to the injection of atropine. This is presumably an adaptive response to the anticipated dry mouth.

Perhaps the most compelling evidence in favor of conditioned compensatory mechanisms has been reported by Shepard Siegel (1975, 1976). He has shown that the stimuli associated with morphine injection come to elicit a reaction which reduces the analgesic effect of morphine, thus demonstrating conditioned tolerance. Siegel assessed morphine effects by measuring analgesia on a hot plate. In one experiment, separate groups of rats were given repeated morphine injections in one of two environments: their home cages or the test situation (the hot plate). When both groups were subsequently tested on the hot plate after morphine injection, only those rats which received morphine in their home cage showed evidence of analgesia to pain. The other rats were tolerant to the effects of morphine, that is, showed no analgesia. In addition, these tolerant rats were hyperalgesic when given a placebo injection of physiological saline instead of their regular morphine. Siegel (1975) also reported that repeated placebo injections following the development of tolerance were effective in extinguishing the conditioned compensatory reaction of hyperalgesia. He argues that morphine tolerance can be accounted for in terms of a conditioning theory which posits the acquisition of timely compensatory responses in anticipation of central drug effects. His data (Siegel 1975) suggest that the compensatory response is elicited by the stimuli which have been paired with drug.

Although Siegel has only measured the analgesic (1975) and pyretic (1976) components of morphine effects, it would be interesting to determine whether Other physiological concomitants of the drug state act in the same manner. Siegel proposed that the hyperalgesia he observed is a conditioned compensatory CR. If Siegel's rat data can be extrapolated to humans, they support our speculation that the incidence of relapse to narcotic use among detoxified addicts is due to the fact that cues associated with the drug come to produce compensatory CRs. However, the procedures which produce compensatory CRs instead of drug-like CRs are uncertain. Even in controlled animal experiments, similar protocols can produce dissimilar results. Thus, Sherman's (1979) attempts at replication of Siegel's experiments were only partially successful (see note added in proof, Sherman 1979).

There is evidence that most drug-associated cues produce compensatory effects in humans (See Table III). When the subject is in a drug-associated environment and performing rituals which reliably predict the onset of drug effect, the subject exhibits behavioral and physiological responses opposite

TABLE III. Conditioned Response Opposite to Opioid Effect[1]

CS	CR	DOSE	SUBJECTS	AUTHORS
Saline injection	Hyperalgesia	Morphine, 5 mg/kg	rats	Siegel, 1975
Saline injection	Hypothermia	Morphine, 5 mg/kg	rats	Siegel, 1978
Distinctive pre-injection environment N	Hypothermia	Morphine, 5-200 mg/kg	rats	Eikelboom and Stewart, 1979
Slides of drug-related and drug-taking stimuli	Negative affects, usually withdrawal	History of heroin use, dose unknown	humans	Teasdale, 1973
Pre-injection rituals	Decreased skin temperature, increased pupil size, tachycardia, subjective reports of craving and withdrawal sickness	History of street opiate use, drug-free or on opiate antagonist	humans	O'Brien, 1975
Self-injection after one or more unreinforced trials (opiate blocked by naltrexone or syringe contains saline)	Increased skin temperature, increased pupil size, tachycardia, increased respiration, subjective reports of craving, sickness, and anger.	Same as above	humans	O'Brien et al., 1975
Drug objects, slides, video tapes of drug-taking behavior	Decreased skin temperature, increased heart rate, increased scores on withdrawal scales	Two groups of former street opiate users, one stabilized on methadone, one drug-free	humans	Ternes et al., 1980
Video tapes of drug-related behavior	Tachycardia, subjective reports	Recently detoxified former opiate addicts	humans	Sideroff and Jarvik, 1980
Buzzer	Tachycardia (UCR at this dose was initial heart rate increase, then decrease)	10 mg/kg (lower dose in Table I)	dogs	Rush et al., 1970

1. Adapted from Grabowski and O'Brien, 1981. (See Table I.) Copyright 1981, JAI Press, Inc. Reprinted by permission.

to opioid effects. These responses would tend to diminish observed opioid effects and thus they may mediate conditioned tolerance. In fact, there is preliminary evidence (Ternes 1981) that recently detoxified addicts receiving unsignalled (unexpected) opioid infusions show relatively non-tolerant drug effects whereas the same dose produces a more tolerant reaction when it is self-injected.

An obvious source of confusion is that conditioned withdrawal and conditioned compensatory responses appear to be similar. Although they can arise by different mechanisms, their typography is identical. In the event that a compensatory CR such as hypothermia is attributed to a lack of narcotic in the system by the addict, the effect may be mislabeled as withdrawal symptoms. Perhaps this is why so many drug-associated stimuli appear to produce withdrawal-like reactions in addicts and why these responses are so difficult to extinguish (O'Brien et al. 1979).

SOURCES OF CONFLICT

While we have not reviewed each type of conditioning in depth, it is evident that there are enormous complexities and numerous sources of conflicts which help to explain the discrepant studies in the literature. First we should ask why sane conditioned effects resemble opioid effects and others oppose them. A major issue is the identity of the drug UCR in a given situation. Recently, Eikelboom and Stewart (1982) in a comprehensive review of this issue proposed that drug-like CR'S occur when the drug acts on the <u>afferent</u> arm of a reflex arc so that the observed effect or UCR is actually a homeostatic response to this afferent stimulation. According to this model, compensatory CR's occur when the drug acts on the <u>efferent</u> limb of a reflex arc. In this case, the drug-elicited response in the efferent arm is the US and the CNS-mediated response in the efferent arm which opposes the drug effect is the UCR. The CR mimics the UCR and in this instance, since it opposes the drug effect, it is a compensatory CR.

The model described by Eikelboom and Stewart is appealing, but there are difficulties in a simple use of the afferent and efferent categories. This is particularly problematic for an opioid because of recent advances in determining the opioids' site of action. In the dorsal horn of the spinal cord, for example, opioids apparently produce analgesia by mimicking the effects of endogenous opiates released from enkephalinergic nerve fibers which synapse onto the afferent pain fiber. The result of the opioids: activity on the pain fiber is a reduction in the release of substance P, a transmitter of pain-related impulses. This is a case of neuromodulation which fits poorly into an afferent-efferent integrator model. Also, there is no postulated mechanism for a compensatory response to this neuromodulation.

We have observed an interesting pattern of behavioral and physiological response when drug addicts are allowed to go through their drug preparation rituals, followed by injection under double blind conditions of either saline or opioid drug. Prior to injection physiological changes occur such as temperature decrease. These may be characterized as signs of withdrawal. After the injection of saline, some warming of the skin temperature and other behavioral high-like symptoms occur.

These can be characterized as opioid-like CR's. However, over repeated injection trials, the opioid-like CR's disappear and the pre-injection withdrawal-like CR's increase in strength and duration. Typically, these withdrawal-like CR'S are particularly resistant to extinction. Although it is easy to characterize these conditioned responses as withdrawal-like or drug-like, the specific conditions controlling their development and the degree of interdependence in establishment of these responses in the human opiate abuser is still obscure.

One study which observed morphine-dependent rats in both pre-injection and post-injection environments (Eikelboom and Stewart 1979) reported findings similar to those observed in opioid-dependent humans: conditioned hypothermia in the pre-injection environment and conditioned hyperthermia in the post-injection environment. In a subsequent study, these investigators (Eikelboom and Stewart 1981) found that temporal cues, not environmental cues, were the salient CRs for the pre-injection hypothermia. But they confirmed the finding that the preinjection CR was withdrawal-like and the post-injection CR was opioid-like. This pattern is similar to what we found in human addicts (O'Brien 1975).

Another source of confusion about the form of the conditioned response may result from deficiencies in the designs of experiments which do not use continuous measurements in the same subjects. Also, complex conditioning environments in laboratory or clinical settings make it difficult to readily identify the appropriate conditioned stimuli.

There is evidence in the animal literature pertaining to the uncertainty about what stimuli associated with drug use will be the most salient and thus most likely to acquire control over conditioned responding. For example, even in the simplified experimental situation where a bell or a distinctive cage is used as the CS, there are multiple stimulus components competing for control. As suggested above, this competition potentially is greater in the clinical situation where "natural" conditioning presumably is taking place. If a subject has drug effects paired with complex environmental stimuli (for example, sounds, smells, sights), conditoning to one CS may overshadow that of another stimulus. If in the test situation a less salient stimulus is evaluated, the elicited CR may be weaker or perhaps different. The phenomenon of "overshadowing" was

recently studied by Walter and Riccio (1983). These authors have also pointed cut that internal stimuli produced by the early effects. of the opioid may themselves serve as CS's eliciting a CR. Internal cues may well be part of a complex CS, but since they require the injection of the day, they are always linked to a UCR and thus a test trial with CS but no UCR is not possible.

Interestingly, at another level, we have evidence that the cognitive expectations of the human subject, that is whether he expects to receive drug or saline drug during a particular self-injection, can affect his subjective evaluation of the experience. For example, if the individual expects to receive drugs and does not, although he may report feeling high, his physiology and behavior do not suggest a drug effect. Such an individual may represent the so-called "needle freak." Alternatively, other individuals may not report getting high on these saline self-injections even though their physiology and behavior support the notion. In addition, the emotional state of an addict at the time of his exposure to drug-related stimuli can markedly affect the incidence and magnitude of a CR to a drug-related CS.

As the above examples indicate, the conditioning phenomena are truly multifaceted and exceedingly complex. We believe that some increased resolution to these problems could be obtained by studies designed to repeatedly and continuously assess these conditioning phenomena in the same subjects. Furthermore, the state of the organism at the time of conditioning: drug free. methadone maintained, or withdrawn should also be studied to determine whether it has any systematic effect on the type of conditioned response observed.

CLINICAL APPLICATIONS

Classically conditioned responses to opioid drugs have been experimentally reproduced in several animal species and in human subjects. Clinical anecdotes and studies of addicts in treatment suggest that conditioning occurs naturally during the development of human opioid dependence. These studies in patients have shown responses to drug-related stimuli or rituals that can be interpreted as conditioned responses. However, it is not yet clear what role if any these conditioned responses play in the maintenance of the opioid-dependent state.

Although there are many aspects of drug-related conditioning which remain unclear, our group is trying to determine whether it is time for direct clinical applications. Elsewhere in the volume, Childress et al. (1984) report the preliminary results of a new controlled study in which systematic extinction is integrated into a comprehensive treatment program for opioid dependence. The data so far indicate that significant conditioned responses are detectable in at least 30-40% of patient volunteers: that the responses do not diminish with the

passage of time and delivery of excellent standard therapy; and that the responses do diminish with many extinction trials although, as previously reported (O'Brien et al. 1979) they are quite resistant to extinction. The results so far also indicate a very important aspect of the experimental treatment: it is acceptable to patients. Many patients spontaneously report the value of systematic exposure to imagery, slides, video tapes and rituals related to drug use. They have been keeping appointments and remaining involved in the treatment. Some have noted the occurrence of apparently conditioned responses when they are exposed to certain situations outside of the clinic. Thus patients are seeing the relevance of this unusual addition to their treatment program.

At this time we don't know whether the broad set of stimuli composing the extinction paradigm will add anything to standard treatments. Only long-term follow-up using objective outcome criteria can determine that. However this is the direction in which we are led by Dr. Wikler and other pioneers who have demonstrated the conditioning effects of opioids. It may be that conditioning plays a role in alcoholism and other forms of drug dependence as well, but it is with opioids that the data are the most comprehensive and clear. We hope that other clinical researchers will not be dissuaded by the complexity of the conditioned responses described here. The addiction syndrome is a complex process, and there is ample evidence that conditioning plays a role, at least with some addicts. We now need new experimental treatment approches to determine whether we can influence this portion of the illness and whether this mskesanydifferencein treatment outcome.

REFERENCES

Eikelboom, R., Stewart, J. Conditioned temperature effects using morphine as the unconditioned stimulus. Psychopharmacology, 61:31-38, 1979.

Eikelboom, R., Stewart, J. Conditioning of drug-induced physiological responses. Psychological Review, 89:597-528, 1982.

Levine, D.G. Needle freaks: Compulsive self-injection by drug abusers. American Journal of Psychiatry, 131:297-300, 1974.

Lynch, J.J., Stein, E.A., Fertsiger, A.P. An analysis of 70 years of morphine classical conditioning: Implication for clinical treatment of narcotic addiction. The Journal of Nervous & Mental Disease. 163:147-158, 1976.

Meyer, R.E. and Mirin, S.M. The Heroin of Stimulus: Implications for a Theory of Addiction. New York: Plenum Medical Book company, 1979.

O'Brien, C.P. Experimental analysis of conditioning factors in human narcotic addiction. Pharmacological Reviews, 27:533-543, 1975.

O'Brien, C.P., Testa, T., O'Brien, T.J., Brady, J.P. and Wells, B. Conditioned narcotic withdrawal of humans. Science, 195:1000-1002, 1977.

Sherman, J.E. The effects of conditioning and novelty on the rat's analgesic and pyretic responses to morphine. learning and Motivation, 10:383-418, 1979.

Siegel, S. Evidence from rats that morphine tolerance is a learned response. Journal of Comparative and Physiological Psychology, 89:498-506, 1972.

Siegel, S. Morphine analgesic tolerance: Its situation specificity supports a Pavlovian conditioning model. Science, 193: 323-325, 1976.

Siegel, S. The role of conditioning in drug tolerance and addiction. In M.E. Jarvik (Ed.) Psychopharmacology in the Practice of Medicine. New York: Appleton-Century Crofts, 1977.

Siegel, S. Classical conditioning, drug tolerance and drug dependence. In Y. Israel, F.B. Glaser, H. Kalant, R.E. Popham, W. Schmidt and R.G. Smart (Eds.) Research Advances in Alcohol and Drug Problems (Vo1.7). New York: Plenum, 1983.

Ternes, J.W. An opponent process view of tolerance in human narcotic addicts. Paper presented in the symposium Social Psychological Applicatiins of Opponent Process Theory: Addiction, attachment, altruism and beating boredom. Annual Convention of the Eastern Psychological Association, New York, 1981.

Walter, T.A., and Riccio, D.C. Overshadowing effects in the stimulus control of morphine analgesic tolerance. Behavioral Neuroscience, 97:658-662, 1983.

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On the Stimulus Control of Drinking in Alcoholics

Ronald M. Kadden, Ovide F. Pomerleau, and Roger E. Meyer

Habitual use of beverage alcohol in such a way as to cause serious health and psychosocial problems has been the subject of considerable investigation in behavioral research laboratories for some time. Our objective in this presentation is to review this work as it relates to the persistence of drink-seeking behavior and high rates of relapse following treatment, phenomena so frequently observed that they have been incorporated as part of the "alcohol dependence syndrome" (Edwards and Gross 1976).

Behavioral analysis of alcohol dependence has viewed drinking as a discriminated operant whose stimulus antecedents and reinforcing consequences maintain the behavior at high strength (Miller and Mastria 1977). Much theoretical and research attention have been devoted to the operant--alcohol drinking--and its consequences, through investigations of the reinforcing potency of alcohol and the role of schedules of reinforcement (see review by Falk et al. 1983). Relatively less attention has been paid to the antecedent stimulus factors which play an important role in the control of drinking, despite widespread recognition of their relevance to the problem (e.g., Keller 1972; Warlatt and Gordon 1980; Mathew et al. 1979; Nathan and Lisman 1976; Stricklet et al. 1979).

The work that has been done to examine the role of stimulus cues for drinking has focused for the most part on Pavlovian conditioning phenomena, with emphasis, on the effect of environmental stimuli in the elicitation of conditioned physiological responses. After sufficient pairings of particular environmental stimuli with alcohol ingestion or withdrawal, these cues become conditional stimuli (CSs) capable of eliciting physiological responses that are experienced by the alcoholic as a distinct subjective state. These elicited responses can in turn serve as discriminative stimuli (S^Ds,) that set the occasion for the operant behavior of drinking (Pomerleau 1981; Pomerleau et al. 1983). This two-factor conditioning model (e.g., Bescorla and Solomon 1967) may help to account for the perseverative nature of alcoholic behavior. by

virtue of its inclusion of involuntary elicited responding, which often occurs without awareness on the part of the individual. The drive state that is thus aroused is thought to be highly compelling.

Adoption of a Pavlovian conditioning model to account for the action of antecedent stimuli raises a number of experimental questions, such as the reactivity of alcoholics to various environmental cues, the relationship of physiological reactions to cognitive factors such as "craving;' and the degree to which reactivity to environmental cues may predict treatment outcome. One of the earliest contributions to this line of investigation was the seminal work of Abraham Wikler who explored the role of conditioned respondents, or what he called "conditioned withdrawal." This work and its implications for opiate addiction have already been described in the preceding paper by O'Brien and Ternes (this volume).

Extension of this approach to the problems of alcohol dependence and relapse was first described in detail by Ludwig and Wikler (1974). Reasoning from a Pavlovian conditioning model, they speculated on possible roles for both exteroceptive (environmental) and interoceptive (arising from "visceral and cerebral neuronal receptors," p. 116) stimuli in reinstituting drinking in abstinent alcoholics. These stimuli, they suggested, become conditioned by repeated association, in close temporal contiguity, with heavy drinking or with the psychophysiological effects of alcohol-withdrawal. When the newly abstinent alcoholic finds himself/herself in the presence of these conditioned stimuli, he/she is likely to experience physiological responses that are preceived and labeled as "craving." The alcoholic is more likely to resume drinking in such a situation (Ludwig and Wikler 1974).

Preliminary data supported this interpretation. In one study, abstinent alcoholic subjects were more likely to agree to perform an operant task that would be reinforced with alcohol if they had received a priming dose of their favorite liquor and mixer, and if a quart of their preferred liquor was placed in view. Alcoholics receiving a non-preferred alcohol mixture, regardless of quantity, and without a liquor bottle in view, refused to perform the operant task (Ludwig et al. 1974). The presence of customary alcohol cues was more effective than the priming dose of alcohol.

In a subsequent study, the alcohol-related cues were enhanced by providing a barroom simulation in the laboratory. The subjects in that study were alcoholics who had been either binge or' steady drinkers. The object was to test the hypothesis that steady drinkers, having presumably had more frequent pairings of alcohol-related cues with heavy drinking and withdrawal, would show stronger conditioned responses. The data confirmed the hypothesis, showing a higher degree of self-reported craving,

higher rates of operant responding for alcohol, and some indications of greater physiological arousal in former steady drinkers when in the presence of alcohol-related cues, as compared with former binge drinkers, or in the absence of drinking cues (Ludwig et al. 1977). Statistical analyses showed that most of the variance in operant responding in the barroom situation could be accounted for by changes in craving, alpha EEG activity, respiratory rate, and blood pressure. Although the results of these studies were not definitive, they are consistent with the hypothesis that elicited interoceptive cues set the occasion for subsequent alcohol consumption.

These findings have been extended by our own work (Kaplan et al. 1983). As part of a larger study, 16 detoxified alcoholics in treatment were given two drinks of either real (alcoholic) or placebo (nonalcoholic) beer, served in a frosted mug on a tray with an empty beer can, while physiological and subjective responses were recorded. There was a significant correlation between skin conductance to the initial presentation of the beverage (before consumption) and increased desire to drink in the alcoholic subjects, but not in the control subjects. After consuming the drinks, the alcoholics who received placebo thought they had received real beer, whereas the control subjects were less likely to make this error. This suggests a significant cueing function, for the alcoholic subjects, by alcohol-related discriminative stimuli (beer can, frosted mug, physical aspects of the "near-beer" beverage), and possibly a conditioned reinforcing effect as well.

Subjects were then asked to select a reward, in the form of a third drink or a lottery ticket, for completion of a subsequent operant task. Among the alcoholic subjects (but not among the controls) there was a significant correlation between subjects' reporting thinking that the preceding drinks had contained real beer and their choice of the drink as the reward. To further examine the variables affecting the choice of the drink as a reward, a stepwise multiple regression was computed. For the alcoholic subjects, increased desire to drink, a high degree of alcohol dependence, and cardiac acceleration at the presentation of the drink all contributed significantly in predicting the choice of the drink as a reward, accounting for 57% of the total variance. The actual alcohol content of the beverage they had consumed was not a significant predictor of reward choice. For the non-alcoholic control subjects, only desire to drink contributed significantly as a predictor of reward choice (Kaplan et al. 1983).

Further evidence for the role of associative processes in the control of alcohol-related behavior has been developed using the laboratory rat. The unconditioned response of rodents to an injection of ethanol is a dose-related hypothermia. If given repeated ethanol injections in a consistent environment, rats develop tolerance to the hypothermic effects of the ethanol.

Le. Poulos, and Cappell (1979) determined that this tolerance could be explained, at least in part, on the basis of Pavlovian conditioning. Ethanol and saline injections were given on alternate days in two distinctive environments. When ethanol was then given in the environment formerly reserved for the saline injections, previously developed tolerance was partially reversed. This result has been replicated by Mansfield and Cunningham (1980).

Siegel has suggested that the development of tolerance in a distinctive stimulus situation may be due to the conditioning of a response which is opposite in direction to the unconditioned effects of the alcohol. When elicited by the conditioned stimuli, the response serves to prepare the organism to modulate the anticipated effects of the alcohol (Hinson and Siegel 1980). The conditioned response (CR) can be observed by providing a placebo injection in the distinctive environment previously reserved for ethanol injections. This procedure does, in fact, result in a hyperthermic response (Crowell et al. 1981; Le et al. 1979; Mansfield and Cunningham 1980), of sufficient magnitude to account for the previously observed tolerance.

Having demonstrated that the thermic response can be conditioned to environmental stimuli, and having clarified the nature of the elicited conditioned response, a further step was to determine whether this response, like a true CR, could be extinguished by repeated exposures to CS (alcohol-related cues) without reinforcement (by ethanol). The hyperthermic response to placebo injections did in fact decline over the course of extinction sessions, and, upon retest with an ethanol injection, the extinction procedure was found to have reversed the previously developed tolerance (Crowell et al. 1981; Mansfield and Cunningham 1980).

These studies are of importance in understanding alcohol dependence, because acquired tolerance is a critical factor in controlling the quantity of alcohol consumed by an alcoholic (Cappell and LeBlanc 1983; Maisto et al. 1978). The role of conditioning that has been demonstrated with respect to the alcohol-induced thermal response may also apply to responses having more obvious motivational significance (Donegan et al. 1983).

Encouraged by research supporting the conditionability of alcohol-related stimuli, and by studies showing conditioned salivation to palatable food cues in people with a history of obesity (Wooley et al. 1975, 1979; Rodin 1978), Pomerleau et al. (1983) initiated a series of systematic investigations on conditioning in alcoholics. An attempt was made to maximize the effectiveness of the eliciting stimuli by having each subject smell his favorite alcoholic beverage in an open, labelled bottle, thus utilizing the alcohol-related cues that constitute

the "final common pathway" for drinking. As a control condition, subjects were asked to sniff cedar chips. Subjects included 8 alcoholics in treatment, 10 control subjects who reported no history of problem drinking nor parental alcoholism, and two recovering alcoholics who claimed 2-3 years of sobriety. Both heart rate and skin conductance responses were elevated during alcohol sniff trials for the alcoholic subjects, consistent with the findings of Kaplan et al. (1983), but the elevations in the present instance were not statistically significant. Swallowing rate (shown to be proportional to the amount of salivation) and craving (selection of a number from 0-10 in response to the question, "How badly do you want to drink alcohol?") showed significant elevations for alcoholic subjects on alcohol-sniffing trials. Control subjects were relatively unresponsive. The two recovering alcoholics resembled those in treatment on the swallowing measure. These data strengthen the idea that conditioned respondents are elicited by alcohol-related cues in people with a history of frequent heavy drinking.

Although both swallowing rate (salivation) and craving were significantly elevated, the changes in the swallowing measure were more pronounced and may have provided a more accurate indicator of conditioning and/or desire to drink. The craving measure, like other subjective/cognitive measures, is a verbal operant subject to the influence of the same complex variables as the statement, "I'll never drink again." The theoretical question of whether conditioned respondents such as those observed here are an essential part of the discriminative stimulus complex for drinking behavior, or whether they are simply an interesting epiphenomenon, remains to be resolved. Even if the conditioned responses to alcohol cues are merely correlated with drinking, determination of their magnitude may provide a useful technique for estimating the probability of subsequent drinking in alcoholics (Pomerleau et al. 1983).

We next questioned whether the salivary response is associated with cognitive processes that might facilitate the decision to drink (Cooney, Baker, Pomerleau, & Josephy, in press). Subjects were 15 inpatients in an alcohol rehabilitation program, tested in the same setting and with a procedure similar to that used in the preceding experiment. They were asked to rate their craving for alcohol and their anticipation of its taste. They also completed a 37-item questionnaire (Southwick et al. 1981) designed to assess expectations about the effects of alcohol (e.g., anticipated pleasant effects or impairment from drinking). These assessments were made following three 5-minute trials in which subjects viewed, held, and sniffed a pint bottle of their favorite alcoholic beverage. Salivation was greater while sniffing alcohol and was significantly correlated with several indicators of positive expectations regarding the effects of alcohol, but not with self-rated desire to drink nor with expectations regarding behavioral impairment. The failure

to find a correlation with desire to drink suggests the role of cognitive distortions (denial) in patients engaged in an abstinence-oriented rehabilitation program. Nevertheless, the significant correlations that were obtained do provide some basis for inferring a relationship between a cue-elicited respondent (salivation) and at least some of the cognitive aspects of craving.

This series of studies supports earlier indications of respondent conditioning to alcohol-related cues, and reaffirms the relationship between elicited responses and the decision to drink. The studies have begun to elucidate some of the response variables involved in the conditioning process, and have so far produced findings that are consistent with a two-factor conditioning model.

If, indeed, the two-factor model is valid, then it should provide some predictive power regarding treatment outcome. Those patients who retain respondent activity in the presence of alcohol-related cues might be expected to have a higher probability of relapse than those who do not. We have put this' question to an experimental test with 50 alcoholic subjects. The basic design uses the sniff-test procedure described previously and seeks to correlate measures of physiological reactivity to alcohol-related cues with a number of indicators of outcome six months after treatment. The treatment outcome data are now being collected. The finding of a significant relationship between physiological reactivity and treatment outcome would be an important step toward providing an objective means of identifying high-risk patients.

Should this study validate the role of conditioned respondents as a factor in treatment outcome, then the logical next step would be to study the effect of extinguishing the respondents. This should reduce the probability of relapse if, as postulated by the two factor model, respondents serve as S^Ds that set the occasion for drinking. Repeated presentations of the sight and smell of an alcoholic beverage without permitting consumption should eventually result in a decrement in the conditioned respondents (Cooney, Baker & Pomerleau, in press) and therefore diminish their ability to precipitate drinking.

If this procedure were adopted clinically, a careful analysis of each patient's drinking history would be necessary to identify the major stimuli requiring extinction (Poulos et al. 19811. This approach to treatment was in fact undertaken in a study of six cases by Blakey and Baker (1980). Patients were given graded exposure to the "triggers" that set off their drinking, but were not allowed to drink. Five of the patients attained abstinence and reported a lack of desire to drink.

FINAL CONSIDERATIONS

This review has examined a number of clinical and experimental findings that implicate conditioned respondents as a factor in the stimulus control of problem drinking. At this writing, the case is far from being definitively established, and additional. controlled experimental studies are called for. As the work proceeds, other issues are sure to come to the fore. It may be possible at this time to anticipate some of them.

One area of further research will undoubtedly focus on the process by which environmental stimuli become conditioned as alcoholism develops, and whether alcohol drinking cues are conditioned to the intoxicating effects of alcohol, the withdrawal effects, or both. Further interest may also be devoted to the question of whether the elicited responses resemble the unconditional response to alcohol, are opposite in direction to the alcohol response, or are in some other way preparatory for ethanol ingestion. The specific answer to this question will undoubtedly vary, depending on the response systems involved (Lynch et al. 1973).

A variable that has received but scant attention to date is the difficulty of extinguishing certain conditioned respondents (Lynch et al. 1973). This phenomenon may prove to be an important element of the treatment-resistance of addictive behavior, and may impose a formidable obstacle to improving the effectiveness of treatment. Other means of behavior change may therefore have to be considered, such as response suppression through the use of aversive contingencies, or the counterconditioning of alternative responses (preferably ones incompatible with the undesirable response). perhaps through the use of biofeedback techniques. Relapse prevention training (Chaney et al. 1978), although emphasizing cognitions, could also be used to teach patients to cope with recurrent conditioned respondents.

Another issue relates to differences in types of alcoholics. It may be found that patient variables, such as severity of dependence, psychiatric diagnosis, or cognitive functioning, interact with conditioning phenomena. For example, patients diagnosed as having anti-social personality disorder have been found to respond in a unique fashion to pre-aversive stimuli, tending to reduce their impact more than “normals” (Hare 1978). If these people were also alcoholic, they might respond differently to alcohol-related cues or prove to be more resistant to extinction procedures, a possibility with far-reaching clinical implications since this subtype constitutes a large, treatment-resistant group of alcoholics (Hesselbrock et al. in press).

The possibility that certain environmental events can prompt substance abuse without prior conditioning will also need to be

considered. For several decades researchers have observed that intermittent presentation of biologically important events such as food or powerful electric shocks can induce a variety of behaviors having no contingent relationship with the scheduled event (see reviews by Falk 1981; Wetherington 1982). These "adjunctive" behaviors are highly persistent and resistant to extinction; examples include schedule-induced water consumption, attack, hoarding, and pica. Translated to the particulars of substance abuse, the suggestion is that various events in daily life occurring on an intermittent basis, in the presence of adequate motivation, can provide "conditions for the initial capture of the stream of behavior" (Falk et al. 1983, p. 72). Drug consummatory behaviors such as cigarette smoking, alcohol drinking, or the self-administration of other psychoactive substances may be sustained past the initial "priming" period because, in the circumstances in which adjunctive behavior is induced, the consequences of substance use may produce neurochemical alterations that affect performance and/or mood (Pomerleau and Pomerleau 1983).

As more data are gathered, modifications of the basic two-factor model may become necessary. Studies of the antecedents to abusive drinking have tended to emphasize the role of Pavlovian conditioning factors. One reason for the appeal of this theoretical approach is that physiological respondents are not as susceptible to voluntary manipulation as are operants. Even if the supposed conditioned respondent turns out to be an epiphenomenon, the physiological reactions may nevertheless accurately reflect the alcoholic's current motivation to drink and, thus, may predict future drinking and relapse. Alternatively, in view of the multiple, concurrent roles that stimuli play, it may be found that greater weight will have to be given to the contribution of environmental events (such as the setting, and substance availability) as discriminative stimuli which set the occasion for abusive drinking. Only systematic experimentation can settle these issues.

REFERENCES

Blakey. R., and Baker, R. An exposure approach to alcohol abuse. Behav Res Ther, 18:319-325, 1980.

Cappell, H., and LeBlanc, A.E. The relationship of tolerance and physical dependence to alcohol abuse and alcohol problems. In: Kissin, B., and Begleiter, H., Eds. The Pathogenesis of Alcoholism: Biological Factors. Volume 7. The Biology of Alcoholism. New York: Plenum Press, 1983, pp. 359-414.

Chaney, E.F., O'Leary, M.R., and Marlatt, G.A. Skill training with alcoholics. J Consult Clin Psychol, 46:1092-1104, 1978.

Cooney, N.L., Baker, L.H., and Pomerleau, O.F. Cue exposure for relapse prevention in alcohol treatment. In: McMahon, R.J., and Craig, K.D., Eds. Advances in Clinical Behavior Therapy. New York: Brunner/Mazel, in press.

Cooney, N.L., Baker, L.H., Pomerleau, O.F., and Josephy, B. Salivation to drinking cues in alcohol abusers: Toward the validation of a physiological measure of craving. Behav, in press.

Crowell, C.R., Hinson, R.E., and Siegel, S. The role of conditional drug responses in tolerance to the hypothermic effects of ethanol. Psychopharmacology, 73:51-54, 1981.

Donegan, N.H., Rodin, J., O'Brien, C.P. and Solomon, R.L. A learning-theory approach to commonalities. In: Levison, P.K., Gerstein, D.R., and Maloff, D.R., Eds. Commonalities in Substance Abuse and Habitual Behavior. Lexington, Bass.: Lexington Books, 1983, pp. 111-156.

Edwards, G., and Gross, M.M. Alcohol dependence: Provisional description of a clinical syndrome. Br Med J, 1(6017):1058-1061, 1976.

Falk, J.L. The environmental generation of excessive behavior. In: Mule, S.J., Ed. Behavior in Excess. New York: Free Press, 1981, pp. 313-337.

Falk, J.L., Dews, P.B., and Schuster, C.R. Commonalities in the environmental control of behavior. In: Levison, P.K., Gerstein, D.R., and Maloff, D.R., Eds. Commonalities in Substance Abuse and Habitual Behavior. Lexington, nass.: Lexington Books, 1983, pp. 47-110.

Hare, R.D. Electrodermal and cardiovascular correlates of psychopathy. In: Hare, R.D., and Schalling, D., Eds. Psychopathic Behaviour: Approaches to Research. Chichester: John Wiley, 1978, pp. 107-143.

Hesselbrock, M.N., Hesselbrock, V.M., Babor, T.F., Stabenau, J.R., Meyer, R.E., and Weidenman, M. Antisocial behavior, psychopathology and problem drinking in the natural history of alcoholism. In: Goodwin, D.W., Van Dusen, K., and Mednick, S., Eds. Longitudinal Studies of Alcoholism. Nijhoff Publishing Co., in press.

Hinson, R.E., and Siegel, S. The contribution of Pavlovian conditioning to ethanol tolerance and dependence. In: Rigter, H., and Crabbe, J.C., Eds. Alcohol Tolerance and Dependence. Amsterdam: Elsevier/North-Holland Biomedical Press, 1980, pp. 181-199.

Kaplan, R.F., Meyer, R.E., and Stroebel, C.F. The symptoms of alcohol withdrawal as predictors of behavioral and physiological responses to an ethanol stimulus. In: Harris, L.S., Ed. Problems of Drug Dependence: 1982. National Institute on Drug Abuse Research Monograph 43. DHEW Pub. No. (ADM) 77-741. Washington, DC: Supt. of Docs., U.S. Govt. Print. Off., 1983, pp. 273-279.

Keller, M. On the loss-of-control phenomenon in alcoholism. Br J Addict, 67:153-166, 1972.

Le, A.D., Poulos. C.X., and Cappell. H. Conditioned tolerance to the hypothermic effect of ethyl alcohol. Science, 206:1109-1110. 1979.

Ludwig, A.M., Cain, R.B., Wikler, A., Taylor, R.M., and Bendfeldt, F. Physiologic and situational determinants of drinking behavior. In: Gross, M.M., Ed. Alcohol Intoxication and Withdrawal Experimental Studies III: Advances in Experimental Medicine and Biology. New York: Plenum Press, 1977, pp. 589-600.

Ludwig, A.M., and Wikler, A. "Craving" and relapse to drink. Quart J Stud Alc, 35:108-130, 1974.

Ludwig, A.M., Wikler, A., and Stark, L.H. The first drink: Psychobiological aspects of craving. Arch Gen Psychiatry, 30:539-547, 1974.

Lynch, J.J., Fertziger, A.P., Teilelbaum, H.A., Cullen, J.W., and Gantt, W.H. Pavlovian conditioning of drug reactions: Some implications for problems of drug addiction. Cond Reflex, 8(4):211-223, 1973.

Maisto, S.A., Henry, R.R.. Sobell, M.B., and Sobell, L.C. Implications of acquired changes in tolerance for the treatment of alcohol problems. Addict Behav 3:51-55. 1978.

Mansfield, J.G.. and Cunningham, C.L. Conditioning and extinction of tolerance to the hypothermic effect of ethanol in rats. J Comp Physiol Psychol, 94(5):962-969, 1980.

Marlatt, G.A.. and Gordon, J.R. Determinants of relapse: Implications for the maintenance of behavior change. In: Davidson, P., and Davidson, S., Eds., Behavioral Medicine: Changing Health Lifestyles. New York: Brunner/Mazel, 1980, pp. 410-452.

Mathew, R.J., Claghorn, J.L., and Largen, J. Craving for alcohol in sober alcoholics. Am J Psychiatry, 136(4B):603-606, 1979.

Miller, P.M., and Mastria, M.A. Alternatives to Alcohol Abuse: A Social Learning Model. Champaign. Illinois: Research Press, 1977.

Nathan, P.E., and Lisman, S.A. Behavioral and motivational patterns of chronic alcoholics. In: Tarter, R.E., and Sugerman, A.A., Eds. Alcoholism: Interdisciplinary Approaches to an Enduring Problem. Reading, Mass.: Addison-Wesley, 1976.

Pomerleau. O.F. Underlying mechanisms in substance abuse: Examples from research on smoking. Addict Behav, 6:187-196, 1981.

Pomerleau, O.F., Fertig, J., Baker, L., and Cooney, N. Reactivity to alcohol cues in alcoholics and non-alcoholics: Implications for a stimulus control analysis of drinking. Addict Behav, 8(1):1-10, 1983.

Pomerleau, O.F., and Pomerleau, C.S. Neuropeptides and the reinforcement of smoking. (under editorial review) 1983.

Poulos, C.X., Hinson, R.E., and Siegel, S. The role of Pavlovian processes in drug tolerance and dependence: Implications for treatment. Addict Behav 6:205-211, 1981.

Rescorla, R.A., and Solomon, R.L. Two-process learning theory: Relationships between Pavlovian conditioning and instrumental learning. Psychol Rev, 74:151-182, 1967.

Rodin, J. Has the internal versus external distinction outlived its usefulness? In: Bray, G., Ed. Advances in Obesity Research, Vol. 2, London: Newman Press, 1978.

Southwick, L., Steele. C., Marlatt, A., and Lindell, M. Alcohol-related expectancies: Defined by phase of intoxication and drinking experience. J Consult Clin Psychol, 49:713-721, 1981.

Strickler, D.P., Dobbs, S.D., and Maxwell, W.A. The influence of setting on drinking behaviors: The laboratory vs the barroom. Addict Behav, 4:339-344, 1979.

Wetherington, C.L. Is adjunctive behavior a third class of behavior? Neurosci Biobehav Rev, 6(3):329-350, 1982.

Wooley, S., Wooley, O., and Dyrenforth, R. Obesity treatment re-examined: The case, for a more tentative and experimental approach. In: Krasnegor, N., Ed. Behavioral Analysis and Treatment of Substance Abuse. National Institute on Drug Abuse Research, Monograph 25. Rockville, ED: NIDA, 1979.

Wooley, S., Wooley, O., and Woods, W. Effect of calories on appetite for palatable food in obese and nonobese humans. J Comp Physiol Psychol, 89:619-625, 1975.

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Progress Report on the Stimulant-Depressant Abuse Liability Evaluation Project

James H. Woods

The Committee on Problems of Drug Dependence has initiated a provisional program for the assessment of abuse liability of compounds with depressant or stimulant profiles. Currently, the program is evaluating a set of methods that may be applied in the future to the open assessment of compounds in animals. The Committee has agreed to proceed with this provisional program to evaluate its feasibility for open evaluation of compounds. The compounds that have been evaluated so far have bean solicited by the stimulant-depressant committee. The initial pair of compounds was selected to provide an assessment of reliability of result and to characterize a new agent with the procedures used in the laboratories.

The laboratories currently engaged in this effort are situated at the Addiction Research Center, Lexington, (Drs. Gorodetzky, Cone, Risner, Shannon, and Vaupel), University of Chicago (Drs. Johanson and Schuster), Johns Hopkins (Drs. Brady, Ator, Griffiths, and Lamb), and Medical College of Virginia (Drs. Harris and Aceto). These laboratories use a set of procedures that may provide a general assessment of these agents in a variety of species. The initial approach was to submit two compounds under blind procedures (designated CPDD 1 and 2); the laboratories would use procedures that they considered relevant to abuse liability assessment of the compounds. The overall objective to be met at some point in the future is the selection of an optimal set of procedures that will complement each other in providing the appropriate preclinical assessment of abuse liability of unknown compounds in animals.

To accomplish blind assessment, compounds were solicited by Dr. Jacobson, and sent to individual laboratories under code labels. Each compound was assessed under blind conditions and reports on the findings described and discussed at two meetings of the groups. The compounds were identified for the laboratories and for the purposes of reporting preliminary results at the CPDD scientific meeting in Lexington.

A brief review of the procedures used by the laboratories and their results with the two compounds will now be presented by individual

laboratories. The compounds will be described as CPDD 1 (diazepam) and CPDD 2 (bromazepam).

Addiction Research Center

Both compounds were examined in the intact rat (1.0-10.0 mg/kg); both decreased body temperature, produced ataxia and sedation. CPDD 2 produced a loss of the righting reflex. It was concluded that both were likely sedative agents. In the sling dog preparation, a set of beagles were used to compare pentobarbital, diazepam, CPDD 1, and CPDD 2. Each of the compounds was given intravenously using a cumulative dose procedure. Pentobarbital was examined in 0.5 log unit increments from 0.3-30.0 mg/kg, diazepam and CPDD 2 in a similar fashion in a dose range of 0.1-10.0 q/kg, and CPDD 1 in a dose range of 0.03-3.0 mg/kg. Observations were made under blind conditions by two observers. Each drug reduced body temperature, had only slight effects upon respiration, pulse, pupil size, and the nictitating membrane. Pentobarbital produced a marked effect on the skin twitch at 30.0 q/kg. Neither diazepam nor CPDD 1 or CPDD 2 produced comparable sedation to 30.0 mg/kg pentobarbital.

In rats trained to discriminate diazepam from saline, CPDD 1 (i.p.) was compared to diazepam (s.c.) and pentobarbital (S.C.). CPDD 1 substituted completely for diazepam and was as potent as diazepam and more potent than pentobarbital. CPDD 2 was not examined, but bromazepam has been evaluated by Dr. Shannon; it too substitutes for diazepam in this procedure. The CGS-8216 antagonist reversed the discriminative effects of CPDD 1 and diazepam, but not pentobarbital.

In the dog, CPDD 1 and 2 were examined and compared to CGS-8216, diazepam, pentobarbital, and methaqualone upon food-reinforced performances in a multiple schedule of fixed-interval and fixed-ratio components. All drugs ware administered orally except CGS-8216 which was given i.v. CPDD 1 and 2 produced 50% increases in fixed-interval responding at some doses (1.0-10.0 mg/kg) as did diazepam over the same range of doses. Pentobarbital increased responding at 10.0 mg/kg. Methaqualone and CGS-8216 failed to increase fixed-interval responding over the range of doses examined. Each of the drugs, except CGS-8216 (0.01-3.0 mg/kg), reduced fixed-ratio rate of responding. CGS8216 antagonized the rate-increasing effects of diazepam (CGS-8216; 1.0 q/kg) but failed to antagonize the actions of pentobarbital on either component of the schedule (CGS-8216; 3.0 mg/kg) or the rate-reducing effects of methaqualone (0.1 mg/kg; CGS-8216).

University of Chicago

CPDD 1 and 2 were examined in groups of pigeons trained to discriminate oxazepam (4.0 mg/kg) from saline or d-amphetamine (2.0 q/kg) from saline. Both CPDD 1 and 2 substituted completely for oxazepam; CPDD 2 suppressed rates of responding to a greater extent than CPDD 1 though they were equipotent in substituting for oxazepam as cues. In the amphetamine-trained pigeons, CPDD 1 and 2

substituted for amphetamine in two of five pigeons. The discriminative effects of CPDD 1 and 2 were antagonized by pretreatments of Ro 15-1788 (0.03 mg/kg).

In modestly food-deprived rhesus monkeys, CPDD 1 and 2 increased food intake. CPDD 2 was more potent than CPDD 1; both effects were antagonized by RO 15-1788 (1.0 q/kg).

In rhesus monkeys trained to self-inject cocaine or pentobarbital on fixed-ratio 10 schedules, CPDD 1 and 2 were studied over a series of doses in a substitution procedure. CPDD 1 failed to maintain rates of self-injection responding above vehicle in a cocaine-trained monkey but did so in a pentobarbital-trained monkey. This is in keeping with the individual differences in self-injection behavior of diazepam in this procedure. Historical controls with diazepam suggest that it will maintain self-injection responding in less than a majority of monkeys whereas most monkeys that self-inject pentobarbital also self-inject diazepam. The range of doses of CPDD 1 studied were 0.02-0.4 q/kg/injection. CPDD 2 has been studied more extensively; it has failed to be self-injected in two cocaine monkeys, but was self-injected at rates above vehicle in 3 of 4 pentobarbital-trained monkeys. The doses of CPDD 2 studied were 0.001-0.03 mg/kg/injection.

Johns Hopkins University

This laboratory has made extensive use of the baboon in both drug self-injection and drug-discrimination studies. They first carried out dose-ranging studies of the effects of CPDD 1 and 2 in baboons performing on a food-reinforced fixed-ratio schedule; observational effects were also noted. CPDD 2 was more potent than CPDD 1 in this procedure; both produced similar signs of ataxia. CPDD 1 was similar in potency to diazepam and much less potent than triazolam.

In their self-injection procedure, cocaine was the maintenance drug and various doses of CPDD 1 (0.01-1.0 mg/kg/injection) and 2 (0.1-1.0 q/kg/injection) were studied. Dose-effect curves were flat and neither compound was self-injected considerably more than saline.

CPDD 2 produced overt behavioral effects not readily' apparent at the doses of CPDD 1 studied. After a single injection of 1.0 mg/kg of CPDD 2, clear ataxia was seen and the baboon almost fell off the bench. Shortly thereafter, the baboon began lever pressing steadily for food pellets (available 24 hr/day under an fixed-ratio 10 schedule of reinforcement). In fact, food pellet intake became unusually high for both baboons studied under CPDD 2.

In the drug discrimination procedure,. the baboons were trained to discriminate lorazepam (1.0 mg/kg) from the no-drug condition in a two-lever procedure similar to those described above. In each of three baboons, CPDD 1 and 2 produced complete drug-appropriate responses at 3.2 mg/kg. Lorazepam was at least three times more potent, and diazepam was equivalent in potency to CPDD 1 and 2. CPDD 2 reduced rates of responding at the largest dose evaluated

(3.2 mg/kg) whereas CPDD 1 failed to reduce rates even at 5.6 mg/kg. Ro 15-1788 (1.0 mg/kg) antagonized completely the discriminative effects of lorazepam, CPDD 1, and CPDD 2.

Medical College of Virginia

The research at this laboratory is to be directed to assessment of physiological dependence development. Their participation in the program was agreed upon later in the history of the project, and they have not yet had the opportunity to complete a set of studies on these compounds.

Summary

The laboratories reported data on CPDD 1 that was indeed very similar to that of their direct or historical controls for diazepam. It is clear that the antagonists used in the program will reverse only a benzodiazepine-induced behavioral effect. The data on bromazepam suggest a very similar pattern and potency for this benzodiazepine compared to diazepam.

Plans

Additional compounds have been submitted to the research groups including compounds with stimulant profiles. When agreement among research groups has been arrived at with respect to the composition of a complete battery of assessment procedures and sufficient background material has been obtained with a variety of known compounds, the group will attempt a thorough evaluation of the effort. If they deem it worthwhile, the group will seek the support of the Committee on Problems of Drug Dependence for the establishment of an open drug evaluation program.

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The author has acted as a scribe for the group of laboratories involved in the project. He takes responsibility for any mistakes in the transcription of these findings for the group. The research described herein was supported by grants by the Committee on Problems of Drug Dependence.

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Progress Report From the NIDA Addiction Research Center (Preclinical Laboratory), Lexington, Kentucky

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As last year, I will begin with a brief administrative update. At this time, all appears progressing on schedule for a total relocation of the ARC from Lexington to Baltimore in the summer of 1984. We are looking forward to the possibility of again co-hosting this meeting in 1985 with Johns Hopkins University in conjunction with an ARC 50th anniversary symposium and the dedication of our new facility.

In my progress report this year, I will briefly highlight three areas of research in the preclinical laboratory particularly related to abuse liability and urine screening.

MARIJUANA VALIDITY STUDY

Several years ago the Syva Company marketed reagents for their EMIT drug abuse urine assay (or EMIT-DAD) for detection of cannabinoids in human urine. More recently similar reagents have become available for the portable EMIT single test system (or EMIT-ST). Most recently the Roche Company has produced a cannabinoid radioimmunoassay in their Abuscreen series. Only scarce data are currently available on the time course of detectability of marijuana cigarette smoking using these assays or for direct comparison of the tests. Most data which do exist are anecdotal or come from outpatient studies, where control of drug intake is less than optimal. Because of the interest in these screening assays, and their increased use (for example, by the Department of Defense), we decided to undertake a controlled clinical validity study under inpatient conditions in collaboration with our colleagues in Baltimore.

Subjects for this study were male volunteers, between the ages of 25 and 55, who gave informed consent for participation in a protocol reviewed and approved by the Institutional Review Board of the Baltimore City Hospitals. All gave a history of light to moderate marijuana use and cigarette smoking. All were judged to be in good health by pretest history, physical examination, laboratory and psychological screens. Prior to initiating the

protocol, each subject was required to have seven consecutive days of urine negative for cannabinoids and other drugs of abuse (by the EMIT-DAU screening procedure). The protocol provided for administration to each subject of four drug conditions as follows: 1) One Δ^{9}-THC cigarette and one placebo Δ^{9}-THC cigarette; 2) two Δ^{9}-THC cigarettes; 3) two placebo Δ^{9}-THC cigarettes; and 4) two regular cigarettes. The Δ^{9}-THC cigarettes were the standard 10 mg. cigarettes provided by the Research Technology Branch of NIDA's Preclinical Research Division. Cigarettes were required to be smoked between 0800 and 0830 of the test day using a puff monitoring system. Drug conditions were administered double-blind in random order; each subject's urine was required to be negative for THC metabolites for not less than three consecutive days prior to administration of the next drug condition. Following drug administration various physiological signs were measured and mood questionnaires administered at timed intervals for 24 hr.; the results of this part of the study will be the subject of a subsequent report. All urine samples were collected individually for each subject from time of admission to the research ward (at least 3 days prior to initiation of the protocol) to completion of the protocol. Each subject urinated ad lib (that is, whenever he felt the need to do so) and, in addition, was asked to urinate at 0800, 1600, and 2400, to complete 8-hr. collection periods. After recording volume, pH, and specific gravity, an aliquot of each sample was put aside for immediate EMIT-DAU analysis and a larger aliquot was frozen and shipped to Lexington for further analysis.

All samples were analyzed in random order under blind conditions by EMIT-DAU, EMIT-ST, and the Abuscreen RIA according to the methods described by the manufacturers. All three immunoassays are designed to detect with greatest sensitivity the major metabolite of Δ^{9}-THC, 11-nor-Δ^{9}-THC-9-carboxylic acid. All are calibrated using as a standard the more readily available synthetic Δ^{8} analog. In each assay an unknown sample is considered positive if its reading is greater than that of a cut-off standard, 20 ng./ml. for EMIT-DAU, and 100 ng./ml. for both EMIT-ST and RIA.

Five subjects have now completed the protocol, producing a total of 742 urine samples for analysis. Data were analyzed as percent of urine samples positive by 8 hr. periods. The RIA and EMIT-ST, both with 100 ng./ml. cut-offs, showed close agreement. There were greater than 50% positive urines for approximately 16 hr. following one marijuana cigarette and 24 to 32 hr. following two cigarettes. The more sensitive EMIT-DAU showed greater than 50% positives for 40 hr. following one cigarette and 64 hr. following two cigarettes. Based on analysis of 282 urine samples from the placebo cigarette, regular tobacco cigarette, and pretest conditions, there were no false positives by EMIT-ST or RIA, and .7% false positive by EMIT-DAU.

In order to examine between-subjects variability, the time to the last positive urine sample was determined for each subject under each drug condition. Time to last positive is defined as the

first positive sample succeeded by at least three consecutive negative samples. Considerable variability between subjects was seen. Subjects A and B showed a relatively short time course of detectability, while Subjects D and E remained positive for considerably longer time periods. Subject C appeared to be intermediate following one cigarette and detectable for a long period following two cigarettes. Mean time of detectability by this analysis was in the same range as the previous analysis. Examining concordance of results by the three screening tests, there was a very high concordance between RIA and EMIT-ST, with only 18 of 742 analyses discordant. Both of these tests compared to the EMIT-DAU showed a greater discordance, reflective of the greater sensitivity of the EMIT-DALI test.

Tentative conclusions after completion of approximately one-half of this study are: 1) Marijuana cigarette smoking is detectable in greater than 50% of urine samples by EMIT-ST and RIA for approximately 16 hr. after one marijuana cigarette and 24 to 32 hr. following two marijuana cigarettes. By EMIT-DAU time course of detectability is approximately 40 hr. after one cigarette and 64 hr. after two cigarettes; 2) EMIT-ST and RIA give quite comparable results; 3) there appears to be a large amount of between-subjects variability in time course of detection.

We currently plan, in addition to completion of the second five subjects, to examine possible correlations of patient history with time course of detection, and to analyze immunoassay positive samples by confirmatory methods, based on HPLC, and possibly, GC.

REINFORCING PROPERTIES OF FENCAMFAMINE

Fencamfamine is a sympathomimetic central stimulant which has recently appeared in the illicit drug market of the U.S., being identified in samples from drug seizures in several states. It is marketed as one ingredient of the multi-vitamin preparation Reactivan, which is sold in the United Kingdom for fatigue. It is not presently under control by the DEA and is available from at least one chemical company catalog in the U. S. The abuse of fencamfamine by athletes was described in 1969; and the influence of alkalinization of its urinary detection has been studied. It was concluded that alkalinization procedures did suppress fencamfamine excretion and might make detection in the urine more difficult. Fencamfamine is N-ethyl-3-phenyl-2-norbornanamine. Although, like cocaine, it contains a bridged ring, the overall molecule bears little relationship to that of cocaine. However, fencamfamine has the beta-phenylethylamine structure characteristic of amphetamine and may elicit pharmacological activity as a rigid phenylethylamine analog. It might be noted that fencamfamine has four asymmetric carbons and can exist as a variety of endo- and exo- isomers.

The subjects for this study were two purebred beagle dogs purchased from a commercial supplier. Between experimental sessions, each dog was individually housed in a pen and given free access to drinking water and dry food.

Following recovery from surgery for implantation of a venous catheter, the dogs were given access to response-contingent drug injections under a fixed-ratio with limited hold schedule of reinforcement. Daily experimental sessions were conducted Monday through Friday in operant conditioning chambers equipped with a response pedal (positioned on the floor) and several stimulus lights. Each session consisted of 11 trials, each trial lasting a maximum of 4 min. During each trial, the 15th pedal-pressing response produced a 15-sec. i.v. injection of drug (i.e., an FR-15 schedule). Following acquisition of stable responding (with either 30 or 100 µg./kg./injection cocaine as the reinforcer), the dogs were tested with five doses of fencamfamine HCl (ranging from 10 to 560 µg./kg./injection) and five doses of cocaine HCl (ranging from 10 to 1000 µg./kg./injection). Each dose was tested for five consecutive sessions, with treatment order arbitrarily determined for each dog. Saline (0.1 ml./kg./injection) was also included in the test series and was tested for five consecutive sessions at least twice during the experiments.

Both fencamfamine and cocaine were self-administered above saline levels. Across several doses of both drugs, the dogs self-administered the maximum number of injections available each session. Generally, there was an inverted U-shaped relationship between the number of injections per session and dose per injection. local rates of responding also varied systematically as a function of injection dose. At least one dose of fencamfamine maintained higher local rates than did any dose of cocaine. Finally, overall rates of responding were systematically related to dose per injection, first increasing, then decreasing with increasing doses. As with local response rates, at least one dose of fencamfamine maintained higher overall rates of responding than did any dose of cocaine,

In summary, response-contingent i.v. injections of fencamfamine, like cocaine, can maintain responding under a fixed-ratio schedule of reinforcement. Dose-effect curves for both fencamfamine and cocaine were similar; both the number of injections self-administered per session and the rates of responding were systematically related to amount of drug delivered per injection. Cocaine appeared to be slightly more potent than fencamfamine, but the differences were not large. Taken together, these data suggest that fencamfamine, like cocaine, is a reinforcing drug and may have a potential for abuse.

OPIOID/ANTIHISTAMINE INTERACTIONS

Although the abuse of tripelennamine with paregoric was described as early as 1964, the use of antihistamines and opioids by drug abusers has been reported with increasing frequency only in recent years. Tripelennamine and pentazocine appears to be the most common combination, being known in the vernacular as “T’s and Blues.” In this report, I will briefly summarize two sets of studies presently underway in our laboratories on the opioid/antihistamine interaction.

One study has been examining the interaction of pentazocine with both tripelennamine and other antihistamines using the rat hot-plate method. Drug was administered subcutaneously or intra-peritoneally, and analgesic activity was measured with a low temperature hot plate maintained at 51.5° C. with cut-off time at 45 sec.

Examining the time course of subcutaneously administered penta-zocine analgesia, at doses of 5 to .30 mg./kg., peak effects were seen between 15 and 30 min., with significant effects often lasting to 75 min. A lesser degree of analgesia was also seen with tripelennamine alone in doses of 5 to 20 mg./kg. using i.p. administration.

When 20 mg of tripelennamine (i.p.) was administered simulta-neously with 5 or 10 mg. of pentazocine (s.c.), tripelennamine appeared to potentiate both doses of‘ pentazocine. Lower doses of tripelennamine did not potentiate these doses of pentazocine. The areas under the time action curve for several other anti-histamines alone and in combination with 5 or 10 mg. of pentazocine showed that pentazocine analgesia appeared to be potentiated by diphenhydramine, chlorpheniramine, promethazine, and cyclizine, but not chlorcyclizine.

These data appear to indicate that tripelennamine can potentiate the analgesic effect of pentazocine in rats under some dose combinations, and this potentiating effect does not appear to be limited to tripelennamine alone, but is also common to some other antihistamines.

Earlier studies in our laboratory with the discriminative stimulus model showed that pentazocine partially generalized to SKF-10047 in rats trained to discriminate the SKF compound from saline. Tripelennamine under some dose conditions was found to antagonize these discriminative stimulus effects of pentazocine. Therefore, it was hypothesized that tripelennamine might antagonize dysphoric sigma opioid receptor effects of pentazocine, thereby enhancing its euphorigenic potential. Following from this study, the present study has examined pentazocin.e and several antihistamines in a sigma opioid receptor binding assay. This assay has been previously described and utilizes specific ^{3}H-SKF-10047 binding to sites in a guinea pig brain homogenate which are inaccessible to 1-etorphine. These binding sites are saturable with respect to the presence of increasing concentrations of labeled SKF-10047. Ligand selectivities of these etorphine-inaccessible sites indi-cate that these sites may represent sigma opioid receptors, since psychotomimetic opioids were among the most potent in the assay.

In the present study, the sigma receptor binding assay was per-formed using a 1% guinea pig brain suspension of the particulate fraction. One nM. of ^{3}H-SKF-10047 was incubated with the suspen-sion in the presence of 100 nM. of etorphine. The specific binding was defined by that portion of ^{3}H-SKF-10047 inhibited by 0.1 mM. of unlabeled SKF-10047. Two ml. of the brain

suspension was incubated with inhibitors in the presence of 100 nM. 1-etorphine for 5 min. Then 1 nM. of tritiated SKF-10047 was added to the reaction mixture and the reaction was allowed to proceed at 22° for another 15 min. After sitting on ice for 10 min. the samples were filtered through GF/C filters which had been presoaked in tertiary amyl alcohol-saturated water before use. Samples were then counted in a liquid scintillation counter.

Pentazocine is one of the most potent inhibitors in this assay, with IC50 of 85 nM. Tripelennamine was also examined in this assay and found to be one-fifth as potent as SKF-10047 as an inhibitor of specific SKF binding. The dose response curves of these two drugs also appear to be parallel to each other. The Hill coefficients of both curves were close to unity.

The affinities of various other antihistamines for the sigma opioid receptors showed that in addition to tripelennamine, many other HI-antihistamines were also potent ligands for the psychotomimetic sigma opioid receptors, The most potent one was promethazone with a KI of about 170 nM. Additionally, chorophenirамine, pyrilamine, and chlorcyclizine appeared to be slightly more potent than tripelennamine.

The results so far appear to be consistent with a possible sigma opioid receptor interaction of pentazocine and tripelennamine. Also, consistent with the results of other investigators, this potential opioid/antihistamine interaction does not appear to be limited only to tripelennamine. Further studies with other opioids and other types of antihistamines are presently being pursued.

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Progress Report From the NIDA Addiction Research Center, Baltimore, Maryland

Donald R. Jasinski, John J. Boren, Jack E. Henningfield, Rolley E. Johnson, W. Robert Lange, and Scott E. Lukas

This progress report summarizes four ARC studies that are of interest to the Committee and its activities. These are: 1) The abuse potential of T's and Blues (pentazocine and tripelennamine) , 2) The abuse potential of THC and nabilone; 3) Studies on the use of buprenorphine in treating opioid dependence; and 4) The effects of mecamylamine and nicotine chewing gum on cigarette smoking and subjective response.

ABUSE POTENTIAL OF T'S AND BLUES

Within recent years, there has been concern over abuse of illicitly obtained mixtures of tripelennamine and pentazocine. In the pattern of abuse reported, usually two tablets of pentazocine (50 mg each) and one of pyribenzamine (50 mg) are crushed and injected intravenously. The resulting effects are described anecdotally as highly euphorigenic. The effects of this mixture had been attributed to pentazocine: however, the contribution of the tripelennamine being uncertain.

The present studies were done 1) to learn if tripelennamine itself is a euphoriant and 2) to learn if tripelennamine enhances the euphorigenic activity of pentazocine or antagonizes the dysphoric effect of higher pentazocine doses. This was conducted as a crossover study in nine (9) volunteer opiate abusers who were non-dependent at the time of the study. Drugs were given in single doses intramuscularly under double-blind conditions. Subjects participated at twice weekly drug intervals with drug administrations according to a latin square design. The nine drug treatments given to each subjects were: 1) placebo, 2) pentazocine 40 mg, 3) pentazocine 80 mg, 4) tripelennamine 50 mg, 5) tripelennamine 100 mg, 6) pentazocine 40 mg and tripelennamine 50 mg, 7) pentazocine 40 mg and tripelennamine 100 mg, 8) pentazocine 80 mg and tripelennamine 50 mg, and 9) pentazocine 80 mg and tripalennamine 100 mg. Standard measures for opioid-like effects including changes in pupillary diameter, blood pressure, pulse, temperature and respiratory rate, the single dose opiate questionnaires and

items from the MGB, PCAG and LSD scales (Jasinski, 1977) were used to evaluate drug effects.

Subjects correctly identified placebo and discriminated the various drug conditions from placebo. They identified pentazocine alone as well as tripelennamine alone predominantly as an opioid. The mixtures were also identified as an opioid but at a greater frequency than either of the drugs alone. The onset, time to peak and duration of pentazocine and tripelennamine were similar across all measures. Pentazocine alone and tripelennamine alone produced significant responses on subjects' liking, MBG, PCAG and LSD scale scores. In addition, both drugs raise systolic and diastolic pressure. Pentazocine, but not tripelennamine, constricted pupils. On all measures except pupils, the effects of tripelennamine and pentazocine were additive . There was no evidence of synergistic activity. The tripelennamine appeared to antagonize the miotic effects of pentazocine.

From this data, it is concluded:

1. Tripellennamine was identified as an opiate and induced euphoria in experienced substance abusers.

2. The combination of tripelennamine and pentazocine produced greater subject liking and euphoria than that seen for either drug administered alone.

3. Both tripelennamine and pentazocine raised the systolic and diastolic blood pressure, the combination produced a greater hypertensive response, and this increase was at least additive.

4. Pentazocine constricted pupils, tripelennamine did not; however, in combination, pupillary constriction was slightly antagonized.

5. Low doses of tripelemamine increased the euphoric effects of pentazocine and decreased the dysphoric effects seen at the higher dose. High doses of tripelennamine did not! appreciably further increase the euphoria and did not attenuate the dysphoria of high doses of pentazocine. The combination producing the greatest liking most closely approached the mixture popular in the street (2:1, pentazocine to tripelennamine).

ABUSE POTENTIAL OF DELTA-9-TETRAHYDROCANNABINOL (THC) AND NABILONE

Delta-9-THC is held to be the active ingredient of cannabis. Oral preparations of THC and nabilone (a chemically related cannabinoid) will be introduced into therapeutics as drugs for eliminating the nausea and vomiting associated with cancer

chemotherapy (Lemberger 1980). Although the abuse potential of cannabis is well recognized, there is less evidence demonstrating an abuse potential for THC. First, THC is generally not a reinforcer in operant paradigms (Harris et al. 1974). Secondly, there is little epidemiologic evidence of abuse of THC since it has not been widely available either illicitly or licitly in pure form. The purpose of the present studies was to develop and validate clinical pharmacologic methods to assess the abuse potential of cannabinoids, THC and nabilone. The methods used in this study were essentially similar to those used in other single dose studies and were derived from the methods for studying opioids. This investigation was conducted in subjects with histories of multiple drug abuse including cannabis smoking. It was conducted as a crossover study under double-blind conditions in ten (10) volunteers. The nine drug conditions were given according to a latin square design. The drugs given at twice weekly intervals were: 1) placebo, 2) morphine 15 and 30 mg subcutaneously, 3) THC 5, 10 and 20 mg orally, and 4) nabilone 2, 4 and 8 mg orally. These drugs were given both double blind and double dummy. Cm one occasion subjects smoked a standard NIDA marijuana cigarette (1.5%) and completed the observations in a manner similar to the other conditions. The non-blinded marijuana smoking condition was administered as one of the ten treatments in the latin square. The physiologic measures included changes in pupil size, standing and supine blood pressure and pulse and respiratory rate. The subjects also completed single dose opiate questionnaires, the MBG, PCAG and LSD scales (Jasinski 1977), and a new questionnaire inquiring of strength in terms of the number of joints which they would have to smoke on the street to provide an equal effect. Finally observers completed the observers' single dose opiate questionnaire.

Abuse Potential of THC

In these studies subjects correctly discriminated THC from placebo and morphine and identified THC as marijuana. THC and the marijuana cigarette both produced tachycardia and similar types of subjective effects as indicated by drug identifications and patterns of signs and symptoms. The onset, time to peak, and duration effects of THC were longer than effects after the marijuana cigarette. Both THC and the marijuana cigarette produced morphine-like euphoria as evidenced by increased liking scores, and MBG scale scores. The peak euphoric effect of THC and the marijuana cigarette were at maximum no greater than that of 15 mg of morphine.

On the basis of these studies it is concluded that THC has an abuse potential; however, the abuse potential may be less than that of the marijuana cigarette because of the slower onset after orally administered THC. Further, it is concluded that valid clinical pharmacologic methods were developed to assess the abuse potential of cannabinoids. This data further sug-

gests that the major effects of smoking marijuana are probably due to the action of THC.

Abuse Potential of Nabilone

The subjects did not discriminate between nabilone and THC. All doses of nabilone produced tachycardia, in contrast to THC in which only the large dose produced tachycardia in the supine position. Nabilone and THC produced similar subjective effects including euphoria. The onset and time to peak effects were similar for nabilone and THC. The duration of effects after nabilone was. significantly longer than after THC. Both nabilone and THC elevated supine, systolic and diastolic blood pressure. Nabilone is seven (7) times more potent than THC in producing subjective effects as determined from valid relative potency calculations; however, valid relative potencies could not be obtained for the physiologic effects. On the basis of these studies it is concluded that nabilone has an abuse potential of the cannabinoid type.

STUDIES ON THE USE OF BUPRENORPHINE IN TREATING OPIOID DEPENDENCE

Studies were conducted to demonstrate the efficacy of sublingually and parenterally administered buprenorphine in substituting for' methadone and heroin and subsequently reducing the intensity of withdrawal. Subjects were admitted to the research ward and the transition from the maintenance methadone or heroin to buprenorphine was done under double-blind, double-dummy conditions. After a period of buprenorphine maintenance, placebo was substituted for buprenorphine again under double blind conditions. Throughout the study subjects were observed for self-reported symptoms and measured signs of opioid abstinence (Jasinski 1977).

Sublingual Buprenorphine in Methadone Dependence

Six volunteer methadone maintenance patients were transferred to a daily 2 mg sublingual dose of buprenorphine. The daily maintenance doses of methadone for these subjects were 45, 45, 40, 30, 25 and 25 mg of methadone daily for a mean dose of 35 mg. Abrupt substitution of buprenorphine for methadone was followed by abstinence signs and symptoms of mild intensity that appeared to peak about the third or fourth day and gradually diminish. When compared to untreated methadone withdrawal, it was clear that suppression of abstinence occurred with the substitution of huprenorphine. Thus, buprenorphine administered sublingually substituted for and partially sup pressed methadone withdrawal. The substituted buprenorphine was administered for 28 days, and abruptly discontinued by substitution of placebo. The abrupt substitution of placebo for buprenorphine was followed by emergence of a mild withdrawal syndrome which reached its peak in the third or

fourth day and gradually receded. The comparison with the control data from untreated methadone withdrawal indicates a significantly attenuated withdrawal syndrome. However, when compared with untreated buprenorphine withdrawal, the withdrawal syndrome from substituted buprenorphine was significantly greater.

Subcutaneously Administered Buprenorphine in Methadone Dependence

Eleven methadone maintenance patients were abruptly transferred to buprenorphine subcutaneously administered in doses of 2 n-g daily. The daily maintenance doses of methadone were 60, 60, 60, 58, 55, 51, 50, 40, 30, 30 and 25 mg daily for a mean daily dose of 47 mg. Abrupt substitution of subcutaneously given buprenorphine for methadone is followed by abstinence signs and symptoms of mild intensity that appeared to peak about the third or fourth day and then gradually diminish and resembles those seen after the substitution of buprenorphine by the sublingual route.

Eight of these eleven subjects completed the protocol of double blind buprenorphine administration. These eight subjects were divided into two groups. Buprenorphine 2 mg subcutaneously daily was administered to Group I and Group II for two and six weeks respectively. Group I included three subjects maintained on 60, 55 and 51 mg of methadone daily for a mean daily dose of 55 mg. Group II included five subjects previously maintained on 50, 60, 58, 30, 30 and 25 mg of methadone daily for a mean daily dose of 42 mg of methadone in these. The purpose of these experiments was to test a hypothesis that the emergence of abstinence in methadone maintenance patients after the discontinuation of substituted buprenorphine was due to the reemergence of methadone abstinence and to learn if a longer period of buprenorphine administration was followed by a lesser abstinence syndrome. The abstinence syndrome with both groups was significantly less than untreated methadone withdrawal; however, no significant difference could be demonstrated between the two groups suggesting that the withdrawal syndrome was not merely the reemergence of methadone withdrawal.

Transition and Withdrawal Studies in Heroin-Dependent Patients

Fifteen (15) heroin-dependent. subjects were admitted to the research ward and stabilized on 60 mg of morphine daily, administered as 15 mg subcutaneously at 6 a.m., 10 a.m., 4 p.m. and 10 p.m. After stabilization they were abruptly transferred to buprenorphine under double dummy, double blind conditions. Nine subjects were given sublingual buprenorphine in doses of 2 mg once daily for two weeks. Six other subjects were given subcutaneous buprenorphine 2 mg daily for two weeks. Following two and six weeks of buprenorphine administration there was abrupt termination by the substitution of placebo. By both

routes of administration, buprenorphine substituted for and suppressed the abstinence during the transition from morphine to buprenorphine although there were mild signs of abstinence which were transitory and were diminished by the first week of abstinence. The abrupt withdrawal of the buprenorphine after two weeks of administration was followed by the onset of a mild abstinence syndrome which emerged on the second day of withdrawal and reached its peak on the third and fourth day of withdrawal. The syndrome diminished such that by 7 to 10 days after withdrawal patients were back to baseline in terms of self-report and subjective measures of abstinence. On the basis of these studies as well as the previous data reported to the Committee (Jasinski et al. 1982) it is concluded that buprenorphine is a safe and effective agent to be used *as* a detoxification and maintenance drug in opioid dependence.

THE EFFECTS OF MECAMYLAMINE AND NICOTINE CHEWING GUM ON CIGARETTE SMOKING AND SUBJECTIVE RESPONSE

As reported previously, studies have revealed that nicotine is a dependence-producing substance and shares characteristics with prototypic substances of abuse. On the basis of these observations, it was decided to pursue studies to determine the feasibility of chemotherapy for treatment of tobacco dependence using the paradigm for treating opioid dependence as a model. In addition, we reported that mecamylamine attenuates the responses to intravenous nicotine. This observation suggests that mecamylamine can be used as a form of antagonist therapy similar to naltrexone in opioid dependence. In these same studies we found the intravenous self-administration of nicotine is dose-related, suggesting that pretreatment with nicotine suppresses subsequent nicotine self-injections in a manner similar to methadone's suppression of opioid self-injections in maintained individuals. As a result, the current experiments were conducted to assess the effects of mecamylamine and the effects of nicotine-containing chewing gum (Nicorette) on cigarette smoking behavior.

Effects of Mecamylamine

In accord with its action to attenuate the effects of nicotine, it was hypothesized that acute mecamylamine pretreatment would increase the rate of smoking. This was a collaborative study with Maxine Stitzer, Johns Hopkins University. The subjects were five female hospital employees without substance abuse histories who participated five days weekly. Under double blind conditions subjects were given mecamylamine 25, 5 and 10 mg or placebo at the start of each workshift. Each dose of mecamylamine and placebo was administered four, times according to a latin square sequence. Several measures of effects were taken prior to the administration of mecamylamine or eight hours after administration of mecamylamine; otherwise, the sub-

jects performed their normal duties and were allowed to smoke ad lib.

In accord with the hypothesis, the doses of mecamylamine increased the number of cigarettes smoked, increased carbon monoxide levels and increased verbal reports of less satisfaction with the cigarettes. The conclusion of this study is that mecamylamine pretreatment increased smoking behavior and that mecamylamine in doses to 10 mg once daily is safe and tolerable with minimal side effects.

Effects of Nicotine Chewing Gum

The experimental paradigm was similar to that for the mecamylamine studies; however, these studies were conducted with post-addict volunteers admitted to the research unit. A total of six subjects were studied; however, data are presented from only three of the six subjects. The Nicorette gum contained 0, 2 or 4 mg nicotine per piece of gum. The hypothesis was that nicotine administered by this route should substitute for and decrease cigarette smoking behavior. The nicotine chewing gum in the two doses and placebo were administered throughout the day according to a latin square design across days. Within each day there were seven doses administered at two-hour intervals. Several measures of cigarette smoking behavior and subjective response were taken from 8:30 a.m. to bedtime, which was approximately 16 hours. Data from these three subjects clearly shows that the nicotine gum decreased the number of cigarettes smoked, lowered expired air CO levels, decreased the number of puffs and lessened verbal reports of desire to smoke. On the basis of these studies it is concluded that Nicorette chewing gum is an efficacious and safe means of suppressing cigarette smoking behavior.

EEG Correlates of Nicotine-Induced Euphoria

We previously have reported that acute intravenous injections of nicotine increased liking scores and produce euphoria. Further, these effects have a rapid onset and short duration (one to three minutes) to which acute tolerance develops. A series of studies have been done in which EEG recordings have been made from subjects during the i.v. injection of various doses of nicotine (0.5, 1.5 and 3 mg) and placebo. During the experiment the subject moved a lever with his finger in one of two directions to indicate when he felt euphoric or dysphoric, respectively. Movement of the pen produced a deflection of an event pen directly beneath the EEG tracings. The EEG activity was quantified using computerized power spectral analysis. The results of this study indicate that there is a dose-related decrease in the EEG power and a shift to a faster alpha frequency during euphoric episodes. It is suggested that this phenonema may provide a basis for the development and assessment of animal models of euphoria.

REFERENCES

Harris R.T., Waters, W., McLendon, D. Evaluation of reinforcing capability of delta-9-tetrahydrocannabinol in rhesus monkeys. Psychopharmacologia (Berlin) 37:23-29, 1974.

Jasinski, D.R. Assessment of the abuse potentiality of morphinelike drugs (methods used in man). In: W.R. Martin, (ed.): Drug Addiction I. Morphine, sedative-hypnotic and alcohol dependence. Handbook of Experimental Pharmacology, Vol. 45. Springer-Verlag: Heidelberg, 1977. pp. 197-258.

Jasinski, D.R., Henningfield, Hickey, J.E., and J.E., Johnson. Progress report of the Addiction Research Center, Baltimore, Maryland, 1982. In: Harris, L.S. ed. Problems of Drug Dependence: 1982. National Institute on Drug Abuse Research Monograph 43. DHHS Pub. No. (ADM) 83-1264. Washington, D.C.: supt. of Docs., U.S. Govt. Print. Off., 1983. pp. 92-98.

Lemberger, L.: Potential therapeutic usefulness of marijuana. Ann Rev Pharmacol Toxicol 20:151-172, 1980.

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Antitussive Potencies of l- and d-Opiates and Their Inhibition of Codeine Binding

Thuy T. Chau and Louis S. Harris

Introduction

We have previously reported that l-codeine was 6 times more potent than d-codeine in suppressing the cough reflex in the anesthetized cat (Chau and Harris, 1980). At the doses tested, the 2 isomers did not cause any significant respiratory effect. The blood pressure and heart rate were not affected by l-codeine but were decreased by the d-isomer. The antitussive effects of d- and l-isomers of codeine were not antagonized by doses of naloxone ranging from 1 to 10 mg/kg i.v. Naloxone by itself did not affect the cough reflex. The lack of naloxone antagonism was also shown in the guinea pig. l-Codeine given s.c. (20-60 mg/kg) produced a dose-related antitussive effect. A very high dose of naloxone (10 mg/kg s.c.) was required to partially block the effect of l-codeine in this series of preliminary studies.

We also showed the antinociceptive effect of l-codeine in mice in the tail-flick and hot-plate tests. d-codeine on the contrary caused hyperalgesia, convulsions and death at high doses (100 mg/kg s.c. or p.o.). The analgesic effect of l-codeine was antagonized by naloxone. l-Codeine also inhibited The stereospecific binding of dihydromorphine although its inhibitory potency was rather weak. It is known that codeine has only a weak affinity for the mu receptors (Chaw-Pham et al., 1978). The d-isomer was completely inactive up to 10^{-4} M.

Based upon those preliminary studies, we hypothesized that the antitussive effect of opiates might be mediated by a subtype of receptor which appeared to be non-stereoselective and less naloxone sensitive than the already established opiate receptors. We are now reporting the antitussive effects of the optical isomers of some mu, kappa and sigma opiates and their in vitro inhibitory potencies of ^{3}H-codeine binding in the guinea pig medulla.

Methods

1. Effects on cough reflex, blood pressure, and heart rate

Cats weighing 3 to 4 kg were anesthetized with sodium pentobarbital 35 mg/kg i.p. The cough reflex was initiated by manual stimulation of the pharynx or lower part of the trachea with a probe through a small slit made in the trachea. The respiration rate, normal amplitude and the amplitude produced by the cough reflex were measured by a pneumograph and recorded on a polygraph. The control cough amplitude was initiated several times prior to the i.v. injection of the drug into the femoral vein. A decrease of the cough amplitude upon drug administration at different time points was defined as percent inhibition of the cough reflex. Different doses of each isomer were given and 3 cats were used for each dose. The Litchfield-Wilcoxon method gave the ED_{50} expressed as base and the 95% confidence limits.

In other experiments 1 mg/kg of naloxone HCl was given i.v. to the cat 3-5 min prior to an ED_{84} dose of each drug. Two or three cats were used to investigate the antagonistic effect of naloxone upon each antitussive compound. The possible antagonism of l-codeine hydrochloride (1 mg/kg) or l-morphine sulfate (1mg/kg) by l-SKF 10,047 (3 mg/kg), d-SKF 10,047 (3 mg/kg), by the ED_{16} dose of l- or d-cyclazocine, and by the ED_{16} dose of (±) ketocyclazocine in the-anesthetized cats was also investigated using 2 cats per drug combination.

2. Inhibition of the saturable binding of (-)-^{3}H-codeine in the guinea pig medulla

The method was essentially that previously described (Chau et al., 1982). Several final concentrations of l- or d-opiates ranging from 10^{-10} to 10^{-4} M were tested for their inhibitory effect on (-)-^{3}H-codeine saturable binding. The saturable binding is defined as the difference between (-)-^{3}H-codeine binding in the presence or absence of cold (-)-codeine. The protein content in the homogenate was determined by the method of Lowry et al. (1951) and the results of (-)-^{3}H-codeine saturable binding expressed as pmoles/mg protein. The IC_{so} of each drug, concentration which inhibited the saturable binding of (-)-^{3}H-codeine by 50%, was determined by log probit analysis. Each IC_{50} was repeated 3 times for reproducibility.

Results and Discussion

1. Antitussive effects of the optical isomers of mu, kappa and sigma agonists

The results are presented in Table 1.

Table I

Antitussive Potencies of l- and d-Opiates in the Cat-

Drugs	ED_{50} (95% C.L.) mg/kg i.v., base
l-Codeine	0.27 (0.14 - 0.47)
d-Codeine	1.61 (0.98 - 2.65)
l-Morphine	0.24 (0.13 - 0.43)
d-Morphine	0.18 (0.08 - 0.40)
l-Methadone	0.058 (0.035 - 0.098)
d-Methadone	0.84 (0.40 - 1.73)
Levomethorphan	0.51 (0.21 - 1.25)
Dextromethorphan	1.21 (0.61 - 2.4)
l-Cyclazocine	0.32 (0.20 - 0.50)
d-Cyclazocine	1.57 (0.79 - 3.12)
(±)-Ketocyclazocine	0.30 (0.16 - 0.50)
l-SKF 10,047	Inactive up to lethal dose
d-SKF 10,047	Inactive up to 5 mg/kg

a) N + 3 to 4 cats per dose

b) The cats were anesthetized with pentobarbital 35 mg/kg i.p

c) The cough reflex was induced by manual stimulation of the trachea with a blunt probe.

In our study, we found that, in general, except for morphine, the l-opiates were more potent than the d-isomers but that these differences were much smaller than those found for the other opiate-like pharmacological actions. The optical isomers of the mu and kappa type of opiates exhibit strong cough suppressant effect, whereas the isomers of the purported sigma opiate SKF 10-047 are inactive up to high doses for the d-isomer

and up to lethal doses for the l-isomer. This suggests, at first, that the antitussive effects of opiates may involve both the mu and kappa types of opiate receptors. The fact that both optical isomers have cough suppressant effects suggests that another type of receptor yet may be involved in this effect and exhibit a lesser degree of stereoselectivity than the analgesic receptors as evidenced by the low potency ratio between the l- and the d-isomers. Furthermore, the differences in naloxone sensitivity of the opiates with respect to their antitussive effects as shown in Table II also suggest that the antitussive receptor(s) may be distinct from the analgesic receptors. It is indeed puzzling that naloxone (1 mg/kg i.v.), in our studies, antagonizes the cough suppressant effect of the optical isomers of all the mu agonists except d- and l-codeine. Smaller doses of naloxone also completely antagonize l-morphine while much larger doses of the antagonist fail to block d- or l-codeine. It is very conceivable that there is more than one mechanism by which opiates exert their antitussive effects. The different degrees of naloxone sensitivity are also evidenced by the optical isomers of cyclazocine and the racemic mixture of keto-cyclazocine which are only partially antagonized by naloxone.

2. Interaction between mu, kappa, and sigma agonists in the suppression of the cough reflex in the cat.

(±)-Ketocyclazocine has been postulated to be a kappa agonist (Martin et al., 1976) and cyclazocine, a mu antagonist and mixed kappa and sigma agonist (Gilbert and Martin, 1976). In our studies, l-cyclazocine does not antagonize the anti-tussive effects of l-codeine but blocks that of morphine, be-having like naloxone (Table III). (±)-Ketocycazocine on the contrary partially blocks codeine but not morphine. The in-volvement of the kappa and mu types of opiate receptors needs further investigation. The sigma receptors do not appear to be involved in the cough suppressant effect of opiates as evidenced by the inactivity of the optical isomers of SKF 10,047. It is interesting, however, that l-SKF 10,047 behaves like naloxone in our antitussive model in agreement with suggestion from other investigators that l-SKF 10,047 may be the mu antagonist whereas d-SKF 10,047 may be the sigma agonist (Brady et al., 1982).

The hypothesis that opiates or at least codeine exert their antitussive effects via non-stereoselective and less naloxone-sensitive receptor(s) is verified by our in vitro studies. Both opitical isomers of the mu opiates tested in these studies inhibit the saturable binding of (-)-codeine in crude guinea pig medulla homogenates. A good correlation exists between their in vivo antitussive potencies and their in vitro inhibitory effect on codeine binding. This suggests that the codeine binding sites in our studies may be one of the antitussive sites but a solid evidence of the latter remains to be established. The lack of stereoselectivity of the hypothetical antitussive sites is

also demonstrated by the isomers of kappa opiates, in both *in vivo* and *in vitro* studies. It is, however, interesting to note that the kappa opiates are more potent than the mu opiates in displacing (-)-codeine from its binding sites, suggesting a strong involvement of the kappa sites for which ketocyclazocine and cyclazocine have a greater affinity.

Table II

Effects of Naloxone HCl on the Antitussive Potencies of *d*- and *l*-Opiates in the Anesthetized Cat[a]

Drug	Dose ED_{84}, mg/kg i.v. base	% Inhibition of the Cough WD/Naloxone	% Inhibition of the Cough W/Naloxone[b]
l-Codeine	0.67	84	84 (d)
d-Codeine	2.5	84	80 (d)
l-Morphine	0.43	90	0 0 (c)
d-Morphine	0.43	89	0 (c)
l-Methadone	0.18	94	0 (c)
d-Methadone	1.8	74	0 (c)
Levomethorphan	1.0	78	13
Dextromethorphan	2.1	82	7
Ketocyclazocine	0.5	80	48 (c)
l-Cyclazocine	0.5	82	54 (c)
d-Cyclazocine	3.0	92	44

a) N = 2 to 3

b) Naloxone HCl 1 mg/kg i.v. given to 3.6 min prior to the antitussive opiate.

c) With Naloxone HCl 0.2 mg/kg.

d) Naloxone 10 and 20 mg/kg i.v. did not antagonize *l*- or d-codeine.

Table III

Interaction Between Mu, Kappa, and Sigma Agonists in the Inhibition of the Cough Reflex in the Anesthetized Cats N=2

First Drug (mg/kg i.v. base),	Second Drug (mg/kg i.v. base)	% Inhibition of the Cough Reflex
l-SKF 10,047 (3.0)	l-Codeine (0.75)	80
l-SKF 10,047 (3.0)	l-Morphine (0.43)	10
d-SKF 10,047 (3.0)	l-Codeine (0.75)	80
d-SKF 10,047 (3.0)	l-Morphine (0.43)	100
Ketocyclazocine (0.1)	l-Codeine (0.75)	47
Ketocyclazocine (0.1)	l-Morphine (0.43)	88
l-Cyclazocine (0.2)	l-Codeine (0.75)	86
l-Cyclazocine (0.2)	l-Morphine (0.43)	34
d-Cyclazocine (1.0)	l-Codeine (0.75)	94
d-Cyclazocine (1.0)	l-Morphine (0.43)	88

The two drugs in each combination were given 3-6 min apart.

Table IV

In Vivo and *In Vitro* Studies

Drugs	ED_{50}(a) (mg/kg i.v. base)	IC_{50}(b) (-)Codeine (nM)
l-Methadone	0.06	1.2
d-Morphine	0.18	6.7
l-Morphine	0.24	8.4
l-Codeine	0.27	5.3
Ketocyclazocine	0.30	1.17
l-Cyclazocine	0.32	1.08
Levomethorphan	0.51	5.4
d-Methadone	0.84	24.0
Dextromethorphan	1.21	25.0
d-Cyclazocine	1.57	6.7
d-Codeine	1.61	40.5
Naloxone	----	8% at 10^{-6} M

a) Antitussive effects in the anesthetized cats.

b) Inhibition of (-)-^{3}H-Codeine binding in the crude homogenate of the guinea pig lower brain stem (1×10^{-8} M).

r1 = 0.65 at 5% level (all opiates included).

r2 = 0.87 at 1% level (kappa opiates not included).

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References

Brady, K.T., Balster, R.L., and May, E.L. Stereoisomers of N-Allylnormetazocine: phenyclidine-like behavioral effects in squirrel monkeys and rats. Science, 215:178-180, 1982.

Chau, T.T., and Harris, L.S. Comparative studies of the pharmacological effects of the d- and l-isomers of codeine. J. Pharmacol. Exp. Ther., 215:668-672, 1980.

Chau, T.T., Carter, F.E., and Harris, L.S. ^{3}H-Codeine binding in the guinea pig medulla. Pharmacology, 25:12-17, 1982.

Chau-Pham, T.T., King, G.W., and Dewey, W.L. Sodium-induced alterations of opiate effects on the binding of ^{3}H-dihydromorphine to mouse brain homogenates. Life Sci., 23:1293-1300, 1978.

Gilbert, P.E., and Martin, W.R. The effects of morphine- and nalorphine-like drugs in the non-dependent, morphine-dependent and cyclazocine-dependent chronic spinal dog. J. Pharmacol. Exp. Ther., 198:66-82, 1976.

Lowry, O.H., Rosebrough, N.J., Farr, A.L., and Randall, R.J. Protein measurement with the Folin phenol reagent. J. Biol. Chem., 193:265-275, 1951.

Martin, W.R., Eades, C.G., Thompson, J.A., Huppler, R.E:, and Gilbert, P.E. The effects of morphine and nalorphine-like drugs in the non-dependent and morphine-dependent chronic spinal dog. J. Pharmacol. Exp. Ther., 197:517-532, 1976.

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Inhibition by Adenosine Analogs of Opiate Withdrawal Effects

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SUMMARY

The stable derivatives of adenosine, 2-chloroadenosine and N^6-cyclohexyladenosine, with high affinity for the A, (Ri) adenosine receptor, suppress the naloxone-precipitated withdrawal contracture of the opiate-dependent guinea-pig ileum *in vitro.* These adenosine derivatives also inhibit naloxone-precipitated jumping, diarrhea and weight-loss in morphine-dependent mice. This effect was not due to sedation, since (i) 2-chloroadenosine was effective at a non-sedative dose and (ii) sedative doses of chlordiazepoxide were ineffective.

INTRODUCTION

The final cholinergic motoneurone of the myenteric plexus of guinea-pig ileum possesses specific receptors for adenosine (Paton, 1981; Moody and Burnstock, 1982), whose activation inhibits the release of acetylcholine from the terminal (Vizi and Knoll, 1976; Gustafsson et al. 1978; Hayashi et al. 1978). Since adenosine thus resembles'opiates and alpha-adrenoceptor agonists, it may be expected in the ileal model also (i) to induce dependence, as do normorphine (Collier et al. 1981b) and clonidine (Collier et al. 1981a) and (ii) to inhibit the opiate withdrawal effect, as does clonidine (Collier et al. 1981a). We have recently reported that adenosine and 2-chloroadenosine (2-CA) induce dependence in the isolated ileum (Collier and Tucker, 1983). We report below that 2-CA and N^6-cyclohexyladenosine (CHA), which resist the rapid enzymatic destruction to which adenosine is subject, readily inhibit opiate withdrawal effects, both *in vitro* and *in vivo.*

METHODS

Guinea-pig ileum *in vitro*

Dependence was induced by incubating pieces of ileum with 10^{-6}M normorphine in Krebs solution containing 7 x 10^{-5}M hexamethonium for 18-24h at 4°C, as previously described (Collier et al, 1981b; Collier & Tucker, 1983). Test and control preparations from the same animal were then set up in pairs at 37°C in a solution of the same composition as used for incubation at 4°C and aerated with 5% CO, in O_2, for isometric recording of contraction of the longitudinal muscle. Withdrawal was precipitated 25-30 min after setting up at 37°C by challenge with 3 x 10^{-7}M naloxone. Test preparations were treated with 10^{-7}M 2-CA, 1 min before challenge or with 10^{-7}M CHA, 2-3 min after challenge. After the response to naloxone had been recorded, responses to electrical stimulation (supramaximal voltage; 0.5 ms pulse width; 0.1 Hz) and to acetylcholine were recorded. The withdrawal contracture was expressed as a percentage of the maximal response to acetylcholine.

Mouse in vivo

Using male LACA mice, one 35 mg pellet of morphine was implanted subcutaneously into each mouse, anesthetized with ether. Three days later, mice were treated i.p. with 2-CA, CHA, chlordiazepoxide (CO) or saline, and 25 min later, they were challenged with 1 mg/kg i.p. naloxone. The number of jumps was recorded for 15 min after challenge and the presence or absence of diarrhea recorded at 30 min. Before challenge, and 20 min later, the mice were weighed. In other experiments, 30 min after treatment with 2-CA or CD, opiate-naive mice were run on a conical rota-rod with spiral walls, and their performance measured by the lateral distance that they, travelled before they fell. Treatments were coded; and the animals' responses were recorded by an observer who did not know what treatment each mouse had received.

Materials

The drugs used were: acethylcholine chloride (BDA), adenosine (Sigma), chlordiazepoxide (Roche), 2-chloroadenosine (Sigma), N^6-cyclohexyladenosine (Calbiochem), hexamethonium bromide (Koch-Light), morphine (May & Baker), naloxone hydrochloride (Endo) and normorphine (Wellcome).

RESULTS

Ileum in vitro

Using preparations not previously incubated at 4°C, we obtained the following mean values ± s.e.m. for the concentration required to inhibit by 50% the electrically evoked contraction (acute 1C 50): adenosine 1.7 ± 0.3 x 10^{-6}M; 2-CA, 5.4 ± 0.7 x 10^{-8}M; CHA, 7.2 ± 0.8 x 10^{-8}M.

In normorphine-dependent preparations, 2-CA, given before naloxone challenge, almost completely prevented the occurrence of a withdrawal contracture (Fig. 1). After 2-CA, as expected, responses

to electrical stimulation were depressed, but those to acetylcholine were unchanged. Figure 2 shows that CHA, given 3 min after naloxone challenge, largely suppressed an existing withdrawal contracture.

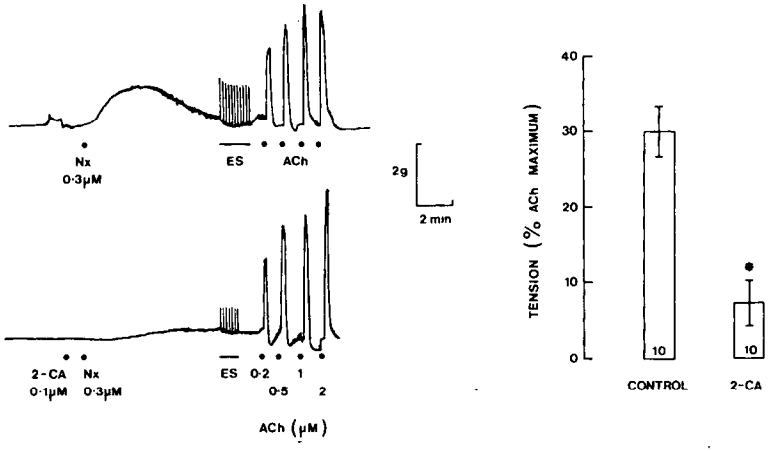

FIGURE 1. Prevention by 2-Chloroadenosine (2-CA) of Withdrawal Contracture Precipitated with Naloxone (Nx) in Guinea-pig Ileum in vitro.

Pieces of ileum were incubated in 10^{-6} M normorphine for 18-24 h at 4°C and for 30-40 min at 37°C. ES, electrical stimulation with 0.5 ms pulses at 0.1 Hz; ACh, acethylcholine; *$p<0.01$ for differences between mean responses of test and control preparations (n=10 pairs).

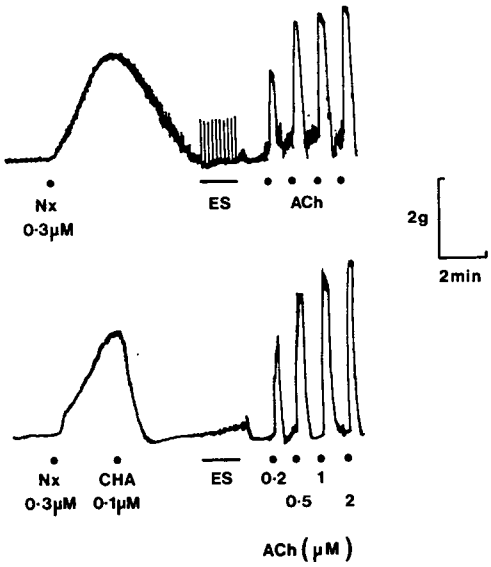

FIGURE 2. Suppression by N^6-Cyclohexyladenosine (CHA) of Withdrawal Contracture Precipitated with Naloxone in Guinea-pig Ileum in vitro. Details as in Fig. 1

Mouse in vivo

In total, 137 morphine-dependent mice were used in experiments with 2-CA, CHA and CD., Table 1 shows that 2-CA and CHA at low doses significantly inhibited withdrawal jumping, diarrhea and weight-loss; whereas CD was ineffective. In about half the dependent mice treated with CD, and in a few mice treated with 2-CA, CHA or saline, the jumping response to naloxone was replaced by a writhing response, with associated suppression of jumping. These animals were excluded from the counts of jumping in Table 1.

The lowest dose of 2-CA effective in inhibiting withdrawal responses in dependent mice (0.2 mg/kg i.p.) did not significantly worsen the rota-rod performance of opiate-naive mice (Figure 3). It may be noted that all doses of CHA completely suppressed withdrawal diarrhea. In general, the effects of the adenosine derivatives were dose-related, but a kink in the lower part of the dose-response lines consistently occurred.

TABLE 1. Inhibition with 2-Chloroadenosine (2-CA) and N^6-Cyclohexyladenosine (CHA) of Withdrawal Effects Precipitated with Naloxone in Morphine-Dependent Mice.

Drug	Dose (mg/kg i.p.)	n	No. of jumps/ 15 min ± SE	Incidence of diarrhea (%)	Weight-loss (mg±SE)
Control		25-27	214 ± 17	85.2	750 ± 67
2-CA	0.2	14	106 ± 29**	28.6*	441 ± 62*
	0.5	22	113 ± 9**	50.0*	641 ± 94
	1.25	9-10	70 ± 12**	10.0*	286 ± 72 **
	3.125	14	32 ± 7**	21.4*	191 ± 45**
CHA	0.5	6	77 ± 25**	0*	143 ± 76**
	1.25	8	84 ± 10**	0*	341 ± 79**
	3.125	6-7	35 ± 12**	0*	63 ± 32**
CD	5	8-14	285 ± 42	57.1	601 ± 94
	12.5	8.15	210 ± 15	80.0	789 ± 130

* P<0.01; ** P<0.001: for significance of difference from control value.

DISCUSSION

These experiments show that 2-CA and CHA potently inhibit opiate withdrawal effects both *in vitro* and *in vivo*. The effect *in vivo* was not due to sedation, since (i) 2-CA was effective at a non-sedative dose and (ii) sedative doses of CO did not inhibit withdrawal responses. If opiate dependence develops *in vivo*, as it probably does *in vitro*, in neurones bearing opioid receptors (Collier, 1980), this finding suggests that the neurones involved also bear receptors for adenosine, the activation of which would produce effects comparable to those of activating their opioid receptors.

That 2-CA and CHA potently and effectively inhibit opiate withdrawal effects raises the question: would these or other adenosine derivatives be useful in the clinical management of opioid withdrawal? This question generates a need for further experiments on these two compounds in dependent mice to determine (i) their side-effects and therapeutic ratios and (ii) their efficacy relative to clonidine. A second question also arises: would these adenosine derivatives themselves produce dependence *in vivo*, as 2-CA is known to do *in vitro* (Collier and Tucker, 1983). If so, would this be accompanied by drug-seeking behaviour? It seems unlikely, however, that the adenosine derivatives would produce drug-seeking behaviour, because the adenosine antagonist, caffeine, is euphorigenic.

That, in some mice, treatment with CD converted a precipitated withdrawal response of jumping to one of writhing is reminiscent of the observation that these two withdrawal effects appear to be mutually exclusive in morphine-dependent rats (Blasig et al. 1973).

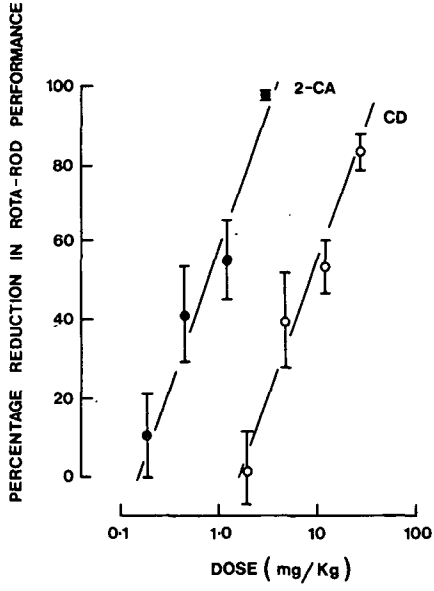

FIGURE 3. Dose/Response Lines of 2-CA and CD for Performance on the Rota-rod. Thirty minutes after drug treatment, mice were run on a spiral rota-rod. Performance was measured by the lateral distance that the mice traveled along the rota-rod before they fell off.

REFERENCES

Blasig, J., Herz, A., Reinhold, K., and Zieglgansberger, S. Development of physical dependence on morphine in respect to time and dosage and quantification of the precipitated withdrawal syndrome in rats. Psychopharmacologia (Berl), 33: 19-38, 1973.

Collier, H.O.J. Cellular site of opiate dependence. Nature (Lond), 283: 625-629, 1980.

Collier, H.O.J., Cuthbert, N.J., and Francis, D.L. Clonidine dependence in the guinea-pig isolated ileum. Br J Pharmacol, 73: 443-453, 1981a.

Collier, H.O.J., Cuthbert, N.J., and Francis, O.L. Model of opiate dependence in the guinea-pig isolated ileum. Br J Pharmacol, 73: 921-932, 1981b.

Collier, H.O.J., and Tucker, J.F. Novel form of drug-dependence - on adenosine in guinea-pig ileum. Nature (Lond), 302: 618-621, 1983.

Gustafsson, L., Hedqvist, P., Fredholm, B.B., and Lundgren, G. Inhibition of acetylcholine release in guinea pig ileum by adenosine. Acta physiol scand, 104: 469-478, 1978.

Hayashi, E., Mori, M., Yamada, S., and Kunitomo, M. Effects of purine compounds on cholinergic nerves. Specificity of adenosine and related compounds on acetylcholine release in electrically stimulated guinea-pig ileum. Europ J Pharmacol, 48: 297-307, 1978.

Moody, C.J., and Burnstock, G. Evidence for the presence of P_1-purinoceptors on cholinergic nerve terminals in the guinea-pig ileum. Europ J Pharmacol, 77: 1-9, 1982.

Paton, D.M. Structure-activity relations for presynaptic inhibition of noradrenergic and cholinergic transmission of adenosine: evidence for action on A,, receptors. J Auton Pharmacol, 1: 287-290, 1981.

Vizi, E.S., and Knoll, J. The inhibitory effect of adenosine and related nucleotides on the release of acetylcholine. Neuroscience, 1: 391-398, 1976.

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Abuse Liability and Behavioral Toxicity Assessment:

Progress Report From the Behavioral Biology Laboratories of the Johns Hopkins University School of Medicine

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A. Introduction

The initial report on the Johns Hopkins program presented at the 44th Annual Scientific Meeting of the Committee on Problems of Drug Dependence in Toronto, Canada, in June, 1982 summarized over a decade of progress in drug abuse liability assessment with laboratory baboons focused upon both drug self-administration procedures and sensory-motor psychophysical methodologies to evaluate the reinforcing properties and behavioral toxicology of chemical agents (Brady and Griffiths 1983). Against this background emphasizing the relationship between the reinforcing properties of drugs, on the one hand, and their behavioral effects, on the other, the present report will focus upon the progress made over the past year in such animal and human laboratory assessments with PCP analogues, both short- and long-acting benzodiazepines, and the somewhat "softer" drugs of abuse, caffeine, nicotine, and marijuana. Additional studies described in other sections of this volume (Stitzer et al., this volume; McCaul et al., this volume; Ensminger et al., this volume; Griffiths et al., this volume; Preston et al., this volume) augment this report of progress from the Johns Hopkins and Baltimore City Hospital drug abuse research programs in areas related to the sedative/anxiolytics and opiate agonist/antagonist compounds.

B. Phencyclidine Analogue Self-Administration in Laboratory Baboons

Consistent with the substantial body of knowledge demonstrating that animals will self-administer drugs that are abused by humans (Griffiths et al. 1979, 1980; Johanson and Balster 1978), PCP has been shown to be self-injected by monkeys (Balster et al. 1973;

Pickens et al. 1973), dogs (Jasinski et al. 1979), and rats (Carroll et al. 1981). In addition, studies on the structure-activity relationships of PCP analogues have demonstrated a good correlation between receptor binding potencies and pharmacological effects (Kalir et al. 1969, 1978; Vincent et al. 1979). The present study was undertaken in order to assess the reinforcing properties of PCP and four analogues in baboons under relatively larye fixed-ratio schedules (i.e., fixed ratio 160) and during 24 hr access to the drugs. In addition, the effects on food intake, as a possible measure of anorexia and/or behavioral toxicity, were characterized.

Eight male baboons (Papio anubis) weighing 18-28 kg served as subjects, using a harness-tether restraint system (Lukas et al. 1982) to protect surgically implanted venous silastic catheters (Lukas 1983). Drug injection was available upon completion of a 160 response fixed ratio requirement on a Lindsley lever with a 3-hr time out period following each injection, permitting a maximum of 8 injections per day. Self-injection performance was first established with cocaine at a dose of 0.32 mg/kg. After 3 consecutive days of cocaine availability during which six or more injections were taken each day, a dose of test drug or vehicle was substituted for the cocaine for a period of 15 days. Cocaine was then reinstated, and when the criterion of 3 consecutive days of six or more injections per day had been met (typically 3-5 days), another dose of a test drug was substituted.

The following drug doses expressed as mg/kg/injection of the salt were studied: cocaine hydrochloride (0.32), phencyclidine hydrochloride (0.01, 0.032, 0.1, 0.32), ketamine hydrochloride (0.01, 0.032, 0.1, 0.32, 1.0), N-methyl-1-phenylcyclohexylamine hydrochloride or NMPCA (0.0032, 0.01, 0.032, 0.1, 0.32). 1-(n-butyl)-1-phenylcyclohexylamine hydrochloride or NNBPCA (0.01, 0.032,0.1, 0.32, 1.0), and 1-(1-(2-thienyl) cyclohexyl) pyrrolidine hydrochloride or TCPY (0-01, 0.032, 0.1, 0.32). The vehicle for NNBPCA was 0.5% ethanol in 0.9% NaCl; the vehicle for all other drugs was 0.9% NaCl.

Figure 1 shows the mean number of injections during the last 5 days for PCP, ketamine, NMPCA, NNBPCA, and TCPY. Cocaine generally maintained self-injection levels of 7-8 injections per day while values for vehicle were in the range of 0-3 per day. Low doses of all drugs were associated with self-injection levels similar to vehicle control; increasing doses were generally associated with increasing numbers of injections per day. These relationships observed with number of injections per day also generally held true for response rates. Response rates for vehicle and low doses of all drugs were typically in the 0.003-0.006 res/sec range. Response rates increased to 0.25-0.45 res/sec as the dose was increased for all compounds except TCPY which were approximately 0.07 res/sec. The maximal rates of responding maintained by PCP and its analogues were similar to those maintained by cocaine (0.32 mg/kg). As measured by the number of injections per day, PCP was 1.3 to 1.4 times more potent than TCPY and NMPCA, 3 times more potent than NNBPCA, and 15 times more potent than ketamine.

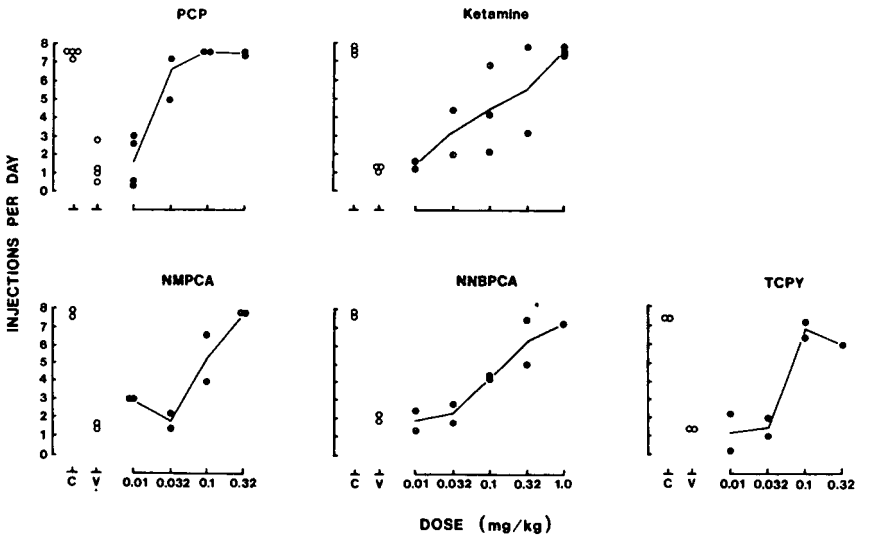

FIGURE 1. Mean number of injections per day maintained by PCP, ketamine, NNBPCA, NMPCA, and TCPY. Y-axis: injections per day; X-axis: dose (mg/kg/injection, log scale. C indicates mean of all 3-day periods with cocaine that immediately preceded every substitution of a drug dose or vehicle. V indicates mean of the last 5 days after substitution of the drug vehicle. Drug data points indicate mean of the last 5 days after substitution of a drug dose. Lines connect means at indicated doses of drug.

C. Sensory/Motor Effects of Chronic Diazepam Administration

Previous reports of a procedure for determining behavioral toxicity using a reaction time (RT) methodology with laboratory baboons (Brady et al. 1979; Hienz and Brady 1981; Hienz et al. 1981; Brady and Griffiths 1983) have focused upon sensory and motor effects following acute administration of a range of drugs. Dose-dependent elevations in both visual and auditory thresholds as well as increases in response latencies following single doses of diazepam have been described, and studies conducted over the past year have provided an extended analysis of chronic diazepam administration in relationship to such psychophysical effects. The procedures for determining such sensory/motor toxicity involved training baboons to press and hold a lever until presentation of a sound burst or light flash signalled availability of food delivery contingent upon lever release (e.g., reaction time paradigm). Auditory and visual thresholds were determined in separate sessions by randomly varying the intensity of the test stimuli from trial to trial in accordance with the method of constant stimuli, and examining detection frequencies at each intensity. Drugs were administered intramuscularly (i.m.) immediately before each experimental session, followed by 15 minutes of dark adaptation and 15 minutes of "warm-up" on the reaction time task before threshold determination was begun.

Figure 2 presents changes in visual thresholds (top) and median reaction times for visual stimuli (bottom) over 21 consecutive days of daily i.m. administration of 0.32 mg/kg diazepam, and for the following days of saline administration. Successive within-session estimates of visual thresholds and median reaction times are plotted for each session, and these within -session points are connected with solid lines for drug days, and with dotted lines for saline days. Thus each series of two to five connected data points represents one day's data for changes in visual threshold (top graph) and changes in visual reaction time (bottom graph). Day 1 shows the acute effects of 0.32 mg/kg diazepam on visual reaction time, a small but consistent increase in reaction time, with no apparent changes in visual thresholds. Tolerance to the drug's effects on reaction times developed gradually, and by Day 6 reaction times were back to normal values. Starting at Day 15 an anomalous decrease in visual reaction times occurred, possibly related to the appetite-inducing properties of the drug (we have previously shown that large changes in deprivation can shift baseline reaction times). Diazepam was withheld from the animal starting on Day 22, and reaction times then approximated normal values, although the within-session variability increased considerably during the withdrawal period. Visual thresholds continued to show little change.

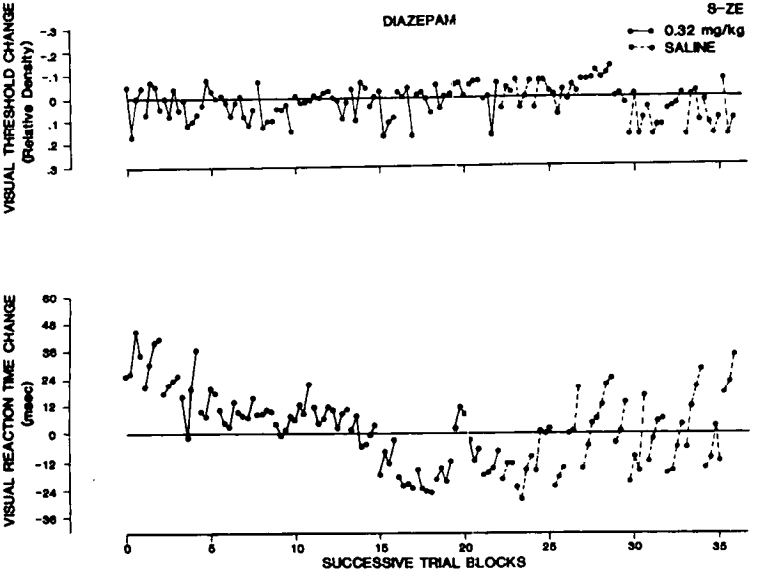

FIGURE 2. Changes in visual thresholds (top graph) and visual reaction times (bottom graph) over 21 consecutive days of daily i.m. administration of 0.32 mg/kg diazepam, and for subsequent days of saline administration. Threshold and reaction time changes from baseline are plotted for each successive block of trials within each session, and within-session points are connected by solid lines on drug days, and by dashed lines on saline days.

This general picture of reaction time effects, the development of tolerance to these effects, and highly variable changes in reaction times during withdrawal, became magnified at the daily dose of 1.0 mg/kg diazepam shown in Figure 3. Again, visual thresholds showed little or no change during either drug administration or withdrawal periods, while the initial effect of this dose of diazepam on reaction time was slightly greater, with tolerance to this reaction-time-increasing effect developing over 10 days as compared to 6 days for the 0.32 mg/kg dose. When the drug was stopped, reaction times showed a progressive and dramatic increase that peaked at Day 10 following cessation of the drug, with a gradual recovery following. Again, during this withdrawal period the variability of within-session estimates of reaction times was

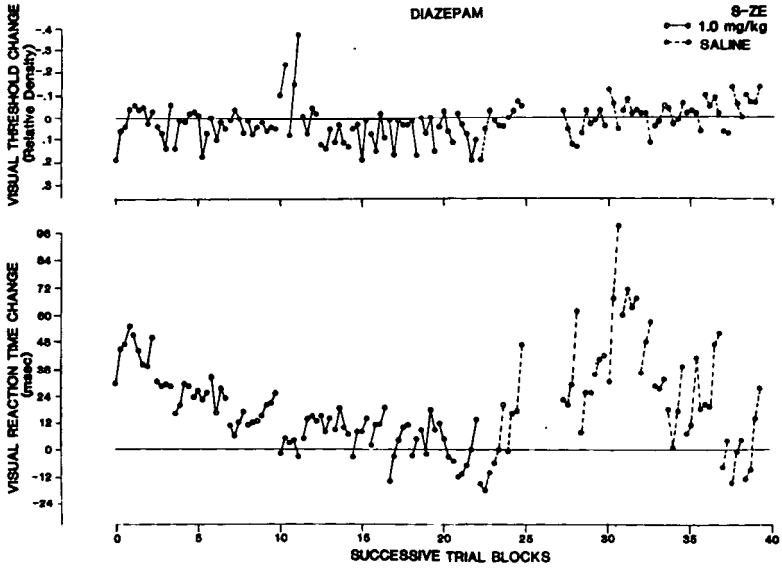

FIGURE 3. Changes in visual thresholds (top graph) and visual reaction times (bottom graph) over 21 consecutive days of daily i.m. administration of 1.0 mg/kg diazepam, and for subsequent days of saline administration. Further description as in Figure 2.

Increasing the dose again to 3.2 mg/kg diazepam produced even more dramatic effects on visual reaction times. The cumulative effects of the drug on reaction time peaked at Day 2, while the first clear effects on visual thresholds occurred on Days 4 and 5. Initial recovery from the reaction time effects was evident by Day 6, but not completed until Day 21. Stopping drug administration again produced an immediate effect upon reaction time, with complete recovery from this effect not occurring for the 19 days shown after drug administration was stopped.

D. Relationship between Reinforcing Properties and Sensory/Motor Toxicity of CNS Stimulants and Depressants

In a previous report (Brady et al. 1983), comparisons between the relative potency of a drug as a reinforcer maintaining self-administration, on the one hand, and its relative potency as regards disruptive effects upon sensory and motor functions, on the other, were described in terms of a "reinforcement/toxicity ratio." The reinforcing properties of three barbiturates (amobarbital, pentobarbital, secobarbital) and two dissociative anesthetics (ketamine and PCP), were determined using the procedure described in section B above. The same drugs were also evaluated to determine the criterion dose which produced a 50% change in auditory and/or visual thresholds, and/or a 10% change in motor reaction time using the procedures detailed in section C above. Over the past year, additional reinforcement/toxicity ratios have been determined for a short- (triazolam) and a long- (diazepam) acting benzodiazepine and for the stimulant d-methylamphetamine.

Figure 4 summarizes in graphic form the relationship between the criterion sensory and motor change doses and the criterion self-

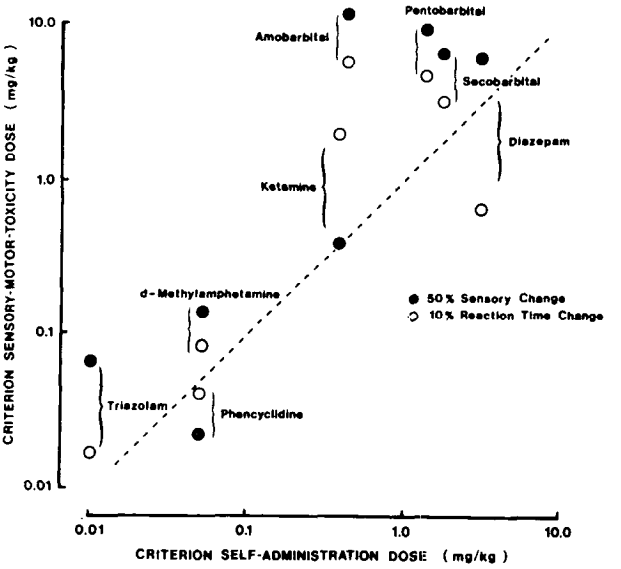

FIGURE 4. Relationship between criterion sensory and motor toxicity doses and criterion self-administration doses for three barbiturates, two benzodiazepines, two dissociative anesthetics, and a stimulant. The broken diagonal line represents equality between the reinforcing and toxic doses.

administration dose for the five previously reported compounds and the three drugs added over the past year. With the broken diagonal line representing equality between the reinforcement and toxic doses, all three barbiturates, the short-acting benzodiazepine triazolam, and d-methylamphetamine are characterized by ratio values which fall above the diagonal, indicating that the doses which produce disruptive sensory/motor changes are generally higher than the doses required to maintain self-administration of these compounds. There is also a consistent relationship between the sensory and motor effects of these compounds which is also shared by the long-acting diazepam in that the motor effects appear at lower doses than the sensory effects. In' the case of diazepam, however, the disruptive motor effects (i.e., slowed reaction times) appear at doses below those required to maintain self-administration as indicated by the reaction time value falling well below the diagonal line. In the case of both dissociative anesthetics, ketamine and PCP are readily differentiated by the consistent appearance of sensory changes at doses below those which produce motor effects, while PCP appears unique among the compounds thus far studied in that both the sensory and motor change values fall below the diagonal, indicating that the doses required to maintain self-administration are generally higher than the doses which produce significant behavioral toxicity.

E. Comparison of Benzodiazepine Behavioral and Subjective Effects in Humans

A study with 14 outpatient volunteers with histories of recreational benzodiazepine use compared the effects of diazepam (0, 10, 30, and 40 mg) and lorazepam (0, 1.5, 3, and 6 mg) on a series of performance (digit symbol substitution, saccadic fixation) and subjective self-report (drug liking scale) tasks. Drugs were administered double blind over 4 test days (one each for the above indicated 4 doses) with experimental sessions for both drug groups (i.e., diazepam and lorazepam) separated by at least one week.

The dose-related changes in performance and subjective self- report of drug liking were generally similar for the two drugs. The higher doses of both diazepam and lorazepam were associated with higher drug liking scores and with larger decrements on psychomotor performance and digit symbol substitution tasks. Lorazepam proved to be significantly more potent than diazepam, however, with 6 mg of lorazepam having consistently more marked effects than 40 mg of diazepam. Lorazepam also tended to produce effects of longer duration than diazepam. Figure 5 presents illustrative results on a psychomotor performance task. These results demonstrate that the magnitude and duration of benzodiazepine effects can be differentiated by such performance and subjective self-report measures, and that the direction and extent of these changes are not predicted by the fact that lorazepam is more rapidly eliminated than diazepam.

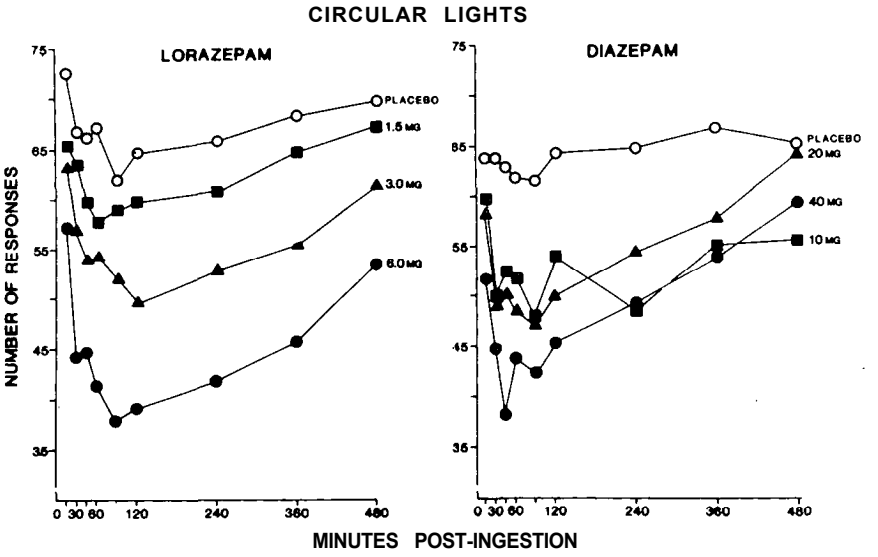

FIGURE 5. Effects of oral doses of lorazepam and diazepam on a circular lights psychomotor performance task.

A second study completed over the past year with an additional twelve human volunteers with histories of sedative drug abuse compared the effects of diazepam and oxazepam on psychomotor performance and subjective self-report measures. Drugs were administered orally every third day under double-blind conditions with the order of the drugs counterbalanced over the dose range for 10-160 mg for diazepam (i.e., 0, 10, 20, 40, 80 and 160 mg), and 30-480 mg for oxazepam (i.e., 0, 30, 60, 120, 240, 360 and 480 mg).

Area under the curve (AUC) analysis showed diazepam was 2.6 to 5.7 times more potent than oxazepam on a variety of psychomotor, behavioral, and subjective self-report measures. Comparison of relative potencies across measures showed diazepam to be relatively more potent in producing liking than in producing psychomotor and behavioral effects. Diazepam produced greater peak effects than oxazepam on a number of staff- and subject-rated measures, including liking. Analysis of time course showed that onset of effect was more rapid and time to maximal effect was shorter (1-2 hr vs. 4-12 hr) with diazepam than oxazepam, while time to offset of effect was similar for the two drugs. Diazepam was categorized as producing barbiturate-like subjective effects (38.3%) more frequently than was oxazepam (13.8%), while oxazepam was identified as placebo more often than diazepam. Repeated administration of 160 my of diazepam and 480 mg oxazepam showed that AUC liking was greater for diazepam than oxazepam, and that tolerance to psychomotor and behavioral effects occurred with oxazepam but not diazepam. This study suggests that diazepam may have a higher abuse liability than oxazepam.

F. Human Residential Programmed Environment Studies of Caffeine, Nicotine, and Marijuana Use

These studies are being conducted in a self-contained human programmed laboratory environment which has been previously described in detail (Brady et al. 1975), and which provides accommodations for small groups of three or more human volunteer research participants during periods of continuous residence from several days to several weeks. The overall floor plan of the laboratory and its arrangement within the external building shell are illustrated in Figure 6. The three identical private

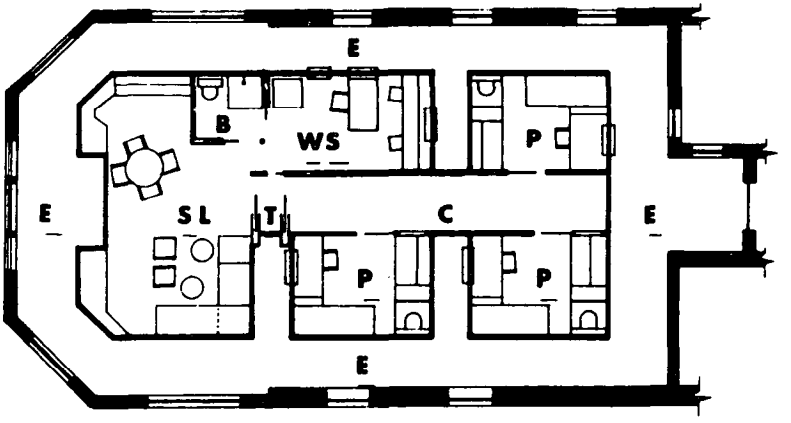

FIGURE 6. Diagrammatic representation of the overall floor plan of the laboratory and its arrangements within the external building shell.

rooms (P - each 2.6 x 3.4 x 2.4 m) are similar to small efficiency apartments containing kitchen and, bathroom facilities, bed, desk, chair, rug, and other furnishings. The social living area (SL - 4.3 x 6.7 x 2.7 m) is equipped with tables; chairs, sofa beds, storage cabinets, and a complete kitchen facility. The workshop (WS - 2.6 x 4.1 x 2.7 m) contains benches, stools, storage cabinets, tools, and a washer-dryer combination. A common bath (B, Figure 6) serves the social living area and the workshop. Access to the exterior walls of the laboratory is provided by a four- to six-foot corridor between the residential chambers and the external building shell that permits transfer of supplies and materials through two-way storage facilities accessible from both sides. Remotely controlled solenoid locks on doors and cabinets throughout the environment provide for experimental programming of access to various facilities and resources, though at least one unlocked door in each compartment permits departure from the laboratory at any time in case of emergency and preserves the right of subjects to terminate their participation in an experiment at any time. The electro-mechanical control devices throughout the environment are interfaced with a computer system located in an adjoining laboratory support facility that provides for experimental

monitoring, programming, recording, and data analysis. Each of the private rooms of the residential laboratory is equipped with a microcomputer which is also linked to the central computer system. A communication panel in each individual chamber includes both a cassette tape player and a telephone intercom for exchanges between subjects within the environment. Audio and video equipment in each of the residential chambers permits continuous monitoring during conduct of an experiment.

A series of three-person groups, both males and females, were initially studied over periods of 7 to 12 days of continuous residence in the programmed environment. During the experiments, the participants engaged a behavioral program of contingently scheduled activities which determined the sequence in which over twenty activities were selected (Emurian et al. 1978; Emurian et al. 1982). Throughout the experiments, cigarettes (each subject's preferred brand) and coffee (instant Chase & Sanborn) were freely available. Cigarette-smoking and coffee-drinking events were defined by their onset, as indicated by (1) the operation of a cigarette lighter or the first puff recorded by an automated puff-detecting device, and (2) by the requisition of a cup of coffee. An event time-series technique, the cross-interval histogram (Sayers 1970), was used to reveal possible relationships between the two series of events defined by cigarette smoking and coffee drinking. For any given inter-cigarette interval in which coffee drinking occurred, a coffee-drinking act was measured in time from the immediately preceding cigarette ("backward waiting time") and from the immediately succeeding cigarette ("forward waiting time"). From many such observations, frequency distributions of backward waiting times and forward waiting times were constructed. This event time-series analysis based upon instances in time of each substance's use revealed a relationship between cigarette smoking and coffee drinking: a coffee-drinking event tended to occur late in the inter-cigarette interval, and a cigarette-smoking event was most probable during the twenty minutes immediately following a coffee-drinking event.

In a second series of studies, 3 female and 5 male participants were exposed to a highly structured schedule in the programmed environment which required activity changes every hour on the hour. The program permitted selection in any sequence from a variety of different recreational (e.g., reading, arts and crafts, etc.) and work (e.g., computer tasks, manual labor, etc.) activities, thus permitting examination of the frequency and distribution of cigarette smoking, in particular, within equivalent 1-hr blocks of time in which markedly different activities were engaged. The rate and patterning of smoking were markedly different during activities in which subjects were paid for their performance (work activities) and during activities in which subjects engaged in recreational and leisure pursuits (non-work activities). The majority of cigarettes smoked during work occurred during the last 10 minutes of the hour; during non-work activities, a larger number of cigarettes were smoked and were more evenly distributed throughout the 1-hr period. Seven of the eight subjects displayed this pattern, with slight variations. The first cigarette was rarely ever smoked during the

first 50 minutes of a work period, whereas during non-work activities, the first cigarette tended to occur early in the 1 hr period. An examination of the rate of smoking during 1 hr periods preceding and following work periods revealed that the suppression of smoking during work periods was not followed by a compensatory increase in smoking rate during the succeeding activity.

A pilot study on smoked marijuana effects has recently been conducted in the programmed human residential laboratory focusing upon a number of continuous behavioral and physiological parameters. Two NIDA-supplied standard experimental cigarettes were self-administered on each of 4 consecutive days during 8-hr continuous residences in the programmed environment by a volunteer participant. In an ABBA design, the subject smoked a placebo marijuana cigarette in the morning and afternoon of each "A" day and an active mariiuana cigarette (approx. 8 mg delta-9-THC) in the morning and afternoon of each "B" day. During -approximately 25% of the time, the subject completed a performance battery and made subjective mood ratings. During the remaining time, the subject freely engaged in ordinary everyday activities (e.g., reading, writing). Heart rate, general activity, and behavioral performances were monitored continuously.

Figure 7 shows the cumulative number of activity transitions as a function of day and time of day. The ordinate represents responses

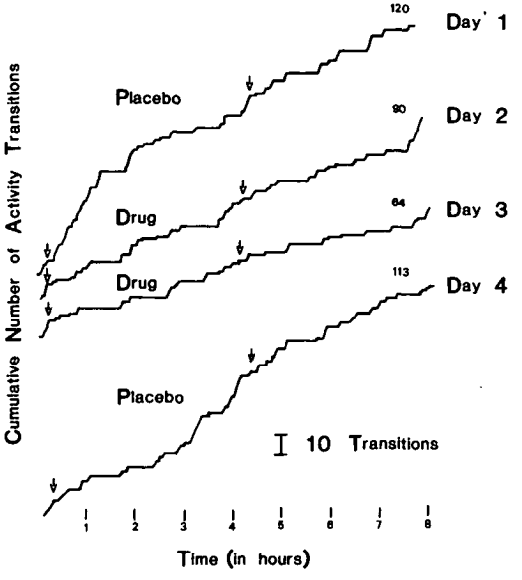

FIGURE 7. Cumulative number of activity transitions as a function of day and time of day. Arrows indicate cigarette smoking onset.

(activity transitions) and the abscissa represents time (8 hr). Each record covers a daily session, beginning at approximately

10:30 a.m. and extending to 6:30 p.m. The subject's behavior was continuously monitored via a television camera mounted in the subject's private living chamber with his knowledge. A mutually exclusive and exhaustive set of activity categories was defined (e.g, computer games, reading, experimental tasks, general maintenance etc.), and the time of onset and offset of each activity was entered in an Apple II computer by trained observers. The cumulative records shown in Figure 7 reflect a general suppression of activity during the two drug sessions as compared to the two placebo days. The total number of daily activity transitions was consistently greater during the two placebo conditions (M=119.5) than during the two drug conditions (M=77).

Figure 8 shows the heart rate and general activity level during each daily session. Heart rate was continuously measured with a

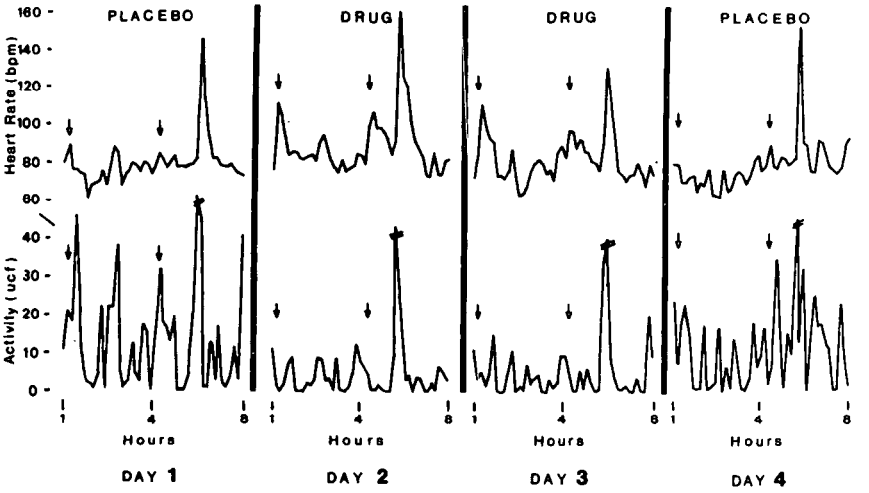

FIGURE 8. Heart rate in beats per minute (bpm) and general activity level in ultrasonic count frequencies (ucf) presented as lo-minute averages over sessions. Arrows indicate cigarette smoking onset. Brief physical exercise periods are reflected in the activity peak near the end of each daily session.

Lafayette heart rate monitor. The output of the monitor was detected and counted by a SKED system and is presented in Figure 8 (upper section) as lo-minute averages. General activity was measured with an ultrasonic motion detector which was sampled once per second by the SKED system and is also presented in Figure 8 (lower section) as lo-minute averages. Persistent heart rate accelerations were elicited by active marijuana cigarette smoking (see arrows at Days 2 and 3), though no such heart rate accelerations were observed following placebo cigarette smoking (see arrows at Days 1 and 4). Figure 8 also shows that general activity levels were higher and more variable during the two placebo conditions than during the two drug conditions. This

latter finding, together with the suppression of activity transitions, suggests that marijuana smoking had a general effect on the rate of behavior. Interestingly, cross-correlation analysis between the heart rate and general activity series suggests that the commonly observed relationship between activity and heart rate was diminished in magnitude during marijuana smoking.

Figure 9 shows the within-session self-ratings on the subjective "high" scale collected at hourly intervals. Clear drug effects obtained immediately following active marijuana smoking were also seen in the subject's ratings on the ARCI marijuana short form (MAR-15-2). Immediately prior to smoking active marijuana, the subject's average MAR score was 3.0 compared to an average score of 8.75 following active marijuana smoking. The average score preceding placebo marijuana smoking was 1.0 and the average score following placebo marijuana smoking was 1.75. These self-report findings are consistent with the results obtained in a companion study at the Addiction Research Center in which smoking this 8 mg marijuana cigarette produced subjective "liking" scores comparable to a 20 mg oral dose of delta-9-THC or a 15 mg subcutaneous dose of morphine.

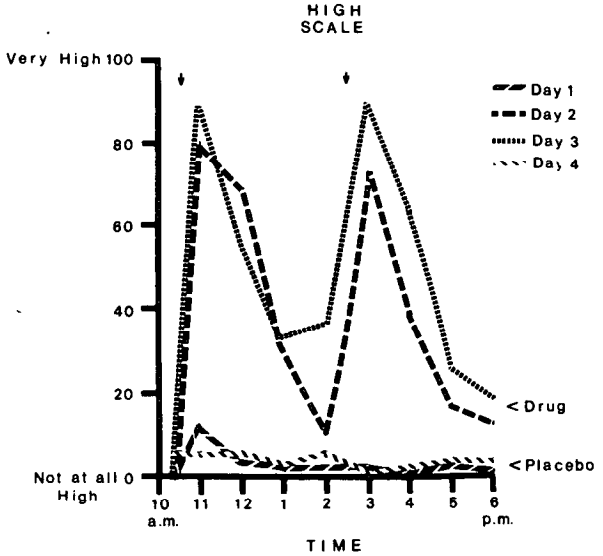

FIGURE 9. Subjective rating of "high" as measures on a 100mm visual line analog scale. A score of 0 indicates "not at all high" and a score of 100 indicates "very high." Arrows indicate cigarette smoking onset.

In the present pilot programmed environment study, each cigarette was smoked through an automatic puff-detecting device consisting of a cigarette holder connected to a pressure transducer. The output of the puff detector was hard wired to a PDP-8 computer where the onset and offset of each puff was timed and stored. A preliminary

analysis of the data suggests that patterns of puffing differ for self-administered active marijuana compared to placebo marijuana. Figure 10, for example, shows the puffing pattern from an active marijuana cigarette (upper record) compared to the puffing pattern from a placebo marijuana cigarette (bottom record), both smoked as the first cigarette of the day. Although the number of discrete puffs did not differ markedly, there was a clear difference between the two cigarettes in the patterning of puffs and in the total duration of smoking time.

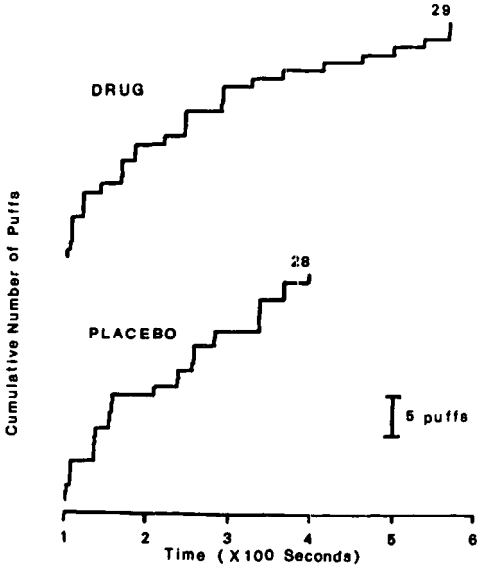

FIGURE 10. Cumulative number of puffs as a function of time from smoking onset for a single marijuana cigarette and a single placebo marijuana cigarette.

A battery of performance tasks was administered after each cigarette in both conditions. While performance on a computerized multiple task battery (e.g., pattern identification, probability monitoring, mathematical calculations, etc.) was not systematically affected -by marijuana smoking, a time estimation task did show differential drug effects. Intervals of 3, 5, and 10 seconds were consistently underestimated following active marijuana smoking (mean error = -.43, standard error = .10) while the same intervals of 3, 5, and 10 seconds were overestimated following placebo marijuana smoking (mean error = .15, standard error = .10).

The concurrent and continuous recording of general activity level, behavioral performance transitions, and autonomic function, as well as discrete measures of smoking topography, provide for a unique analysis of the interacting processes which characterize the abuse potential of this widely used substance.

REFERENCES

Balster, R.L., Johanson, C.E., Harris, R.T., and Schuster, C.R. Phencyclidine self-administration in the rhesus monkey. Pharmacol Biochem Behav, 1:167-172, 1973.

Brady, J.V., Bigelow, G.E., Emurian, H.H., and Williams, D.M. Design of a programmed environment for the experimental analysis of social behavior. In: Carson, D.H., ed. Man-Environmental Interactions: Evaluations and Applications. 7: Social Ecology. Milwaukee: Environmental Design Research Association, 1975. pp. 187-208.

Brady, J.V., Bradford, J.D., and Hienz, R.D. Behavioral assessment of risk-taking and psychophysical functions in the baboon. Neurobehav Toxicol, 1(Suppl. i)173-,84, 1979.

Brady, J.V., and Griffiths, R.R. Testing drugs for abuse liability and behavioral toxicity: Progress report from the laboratories at the Johns Hopkins University School of Medicine. In Harris, L.S., ed. Problems of Drug Dependence; 1982: Proceedings of the 44th Annual Scientific Meeting, The Committee on Problems of Drug Dependence. (NIDA Research Monograph No. 43, p. 99, DHHS Publication No. (ADM) 83-1264). Washington, D.C.: Government Printing Office, 1983.

Brady, J.V., Lukas, S.E., and Hienz, R.D. Relationship between reinforcing properties and sensory/motor toxicity of CNS depressants; implications for the assessment of abuse liability. In Harris, L.S., ed. Problems of Drug Dependence; 1982: Proceedings of the 44th Annual Scientific Meeting, The Committee on Problems of Drug Dependence. (NIDA Research Monograph No. 43, p. 196, DHHS Publication No. (ADM) 83-1264). Washington, D.C.: Government Printing Office, 1983.

Carroll, M.E., France, C.P., and Meisch, R.A. Intravenous self-administration of etonitazene, cocaine and phencyclidine in rats during food deprivation and satiation. J Pharmacol Exp Ther, 217:241-247, 1981.

Emurian, H.H., Emurian, C.S., and Brady, J.V. Effects of a pairing contingency on behavior in a three-person programmed environment. J Exptl Anal Behav, 29:319-329, 1978.

Emurian, H.H., Nellis, M.J., Brady, J.V., and Ray, R.L. Brief report: Event time-series relationship between cigarette smoking and coffee drinking. Addict Behav, 78:441-444; 1982.

Griffiths, R.R., Bigelow, G.E., and Henningfield, J.E. Similarities in animal and human drug-taking behavior. In: Mello, N.K., ed. Advances in Substance Abuse. Vol. 1. Greenwich (CT): JAI Press, 1980. pp. 1-90.

Griffiths, R.R., Brady, J.V., and Bradford, L.D. Predicting the abuse liability of drugs with animal drug self-administration procedures: Psychomotor stimulants and hallucinogens. In: Thompson, T., and Dews, P.B., eds. Advances in Behavioral Pharmacology. Vol. 2. New York: Academic Press, 1979.

Hienz, R.D., and Brady, J.V. Psychophysical profiles differentiate drugs of abuse. In Harris, L.S., ed. Problems of Drug Dependence: 1980: Proceedings of the 42nd Annual Scientific Meeting, The Committee on Problems of Drug Dependence. (NIOA Research Monograph No. 34, p. 226, DHHS Publication No. (ADM) 81-1058). Washington, D.C.: Government Printing Office, 1981.

Hienz, R.D., Lukas, S.E., and Brady, J.V. The effects of pentobarbital upon auditory and visual thresholds in the baboon. Pharmacol Biochem Behav, 15:799-805, 1981.

Jasinski, D.R., Cone, E.J., Gorodetzky,C.W., Risner, M.E., Shannon, H.E., Su, T.P., and Vaupel, D.B. Progress report from the NIDA Addiction Research Center. In Harris, L.S., ed. Problems of Drug Dependence: 1979: Proceedings of the 41st Annual Scientific Meeting, The committee on Problems of Drug Dependence. (NIDA Research Monograph No. 27, p. 61, DHHS Publication No. (ADM) 80-901). Washington, D.C.: Government Printing Office, 1980.

Johanson, C.E., and Balster, R. A summary of the results of a drug self-administration study using substitution procedures in rhesus monkeys. Bull Narc, 30:43-54, 1978.

Kalir, A., Edery, H., Pelah, Z., Balderman, D., and Porath, G. 1-phenyl cycloalkylamine derivatives. 2. Synthesis and pharmacological activity. J Med Chem, 12:473-477, 1969.

Kalir, A., Maayani, S., Rehavi, M., Elkavets, R., Pri-bar, I., Buchman, O., and Sokolovsky, M. Structural activity relationships of some phencyclidine derivatives: In-vivo studies in mice. Europ J Med Chem, 13:17-24, 1978.

Lukas, S.E. Subcutaneous splicing of intravenous and intragastric catheters. Pharmacol Biochem Behav, 18:267-268, 1983.

Lukas, S.E., Griffiths, R.R., Bradford, L.D., Brady, J.V., Daley, L., and Delorenzo, R. A tethering system for intravenous and intragastric drug administration in the baboon. Pharmacol Biochem Behav, 17(4):823-829, 1982.

Pickens, R., Thompson, T., and Muchow, D.C. Cannabis and phencyclidine self-administration by animals. In: Goldberg, L., and Hoffmeister, F., eds. Psychic Dependence, Bayer Symposium IV. New York: Springer-Verlag, 1973. pp. 78-86.

Sayers, B. Inferring significance from biological signals. In: Clynes, M., and Milsum, J., eds. Biomedical Engineering Systems. New York: McGraw Hill, 1970. pp. 84-163.

Vincent, J.P., Kortalovski, B., Geneste, P., Kaminka, J.M., and Lazdunski, M. Interaction of phencyclidine ("angel dust") with a specific receptor in rat brain membranes. Proc Natl Acad Sci, 76:4678-4682, 1979.

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Probes for Narcotic Receptor Mediated Phenomena 3. Oxide Bridged 5-Phenylmorphans

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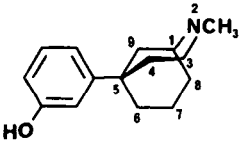
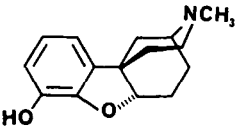
In one phase of our program to characterize the opiate receptor subpopulations on a molecular basis and obtain insight into the elusive question of the physiological role of these subpopulations in the overall modulation of the CNS (Rice et al. 1983, Klee et al. 1983), we are preparing a series of conformationally restricted 5-(m-hydroxyphenyl)morphans to map topological requirements for in vitro binding to receptor subpopulations and for in vivo activity. Racemic 5-(m-hydroxyphenyl)morphan (1), first synthesized by May (May and Murphy 1954) shows morphine-like in vivo activity (May and Murphy 1955) and is resolvable into enantiomers which exhibit different degrees of physical dependence capacity (May and Takeda 1970). Examination of 1 shows it to contain a piperidine ring with a freely rotating m-hydroxyphenyl substituent in the "4" position of the piperidine ring. (This is the 5 position of the 2-azabicyclo [3.3.1] nonane ring system.) The equatorial orientation of the phenyl ring relative to the piperidine ring is in contrast to the axial phenyl orientation of most rigid opiates.



1



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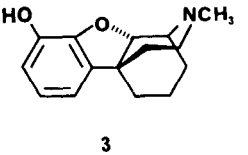
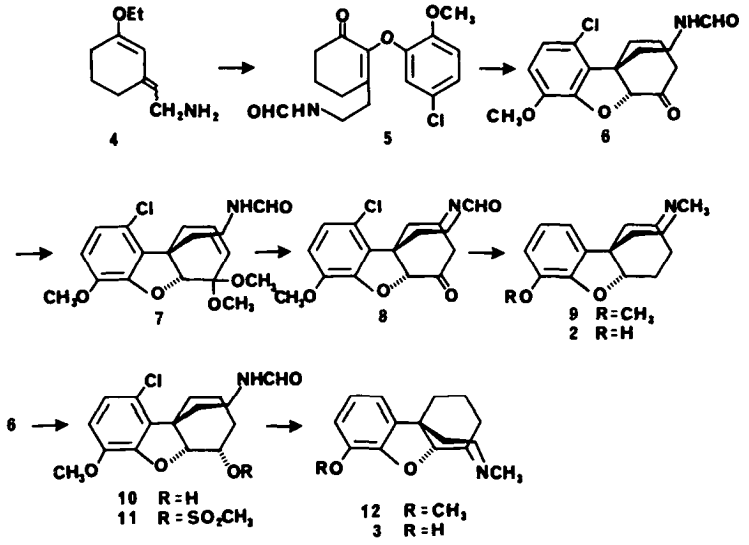


3

Since the phenyl ring of 1 is freely rotating, any angle relative to the piperidine ring can be adapted when binding to the receptor matrix. The importance of the phenyl ring torsion angle has previously been cited as one factor affecting the enantiomeric difference in potency between prodine isomers (Larson and Portoghese 1973). We are utilizing an oxide bridge to restrict the phenyl ring to a known conformation relative to the piperidine ring. An oxide bridge between the phenyl ring and atoms 4, 6 or 9 of 1 allows a possibility of six isomeric oxide bridged phenylmorphans since each site of attachment offers an alpha or beta orientation. Such a series of compounds retains the basic 5-(m-hydroxyphenyl)morphan skeleton while successfully changing the angle of the phenyl ring in 60° increments relative

to the previous member of the series. This paper describes the general synthesis, preliminary biological evaluation and receptor binding of the first two isomers of the series, 2 and 3. The detailed chemical synthesis of 2 is presented elsewhere (Burke et al.).

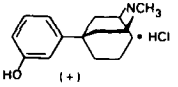
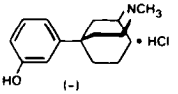
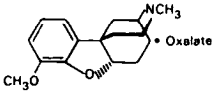
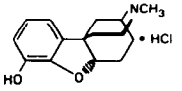
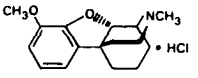
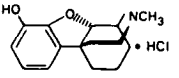
SYNTHESIS



Construction of both the methanobenzofuro[3, 2-d]azocine skeleton of 2 and the propanobenzofuro [2, 3-c] pyridine skeleton of 3 made use of the heteroatom directed photoarylation developed by Schultz and Fu (1976) and subsequently used in the synthesis of lycoramine (Schultz et al. 1977). Both 2 and 3 were prepared from 6, which in turn was obtained in six steps from starting amine 4 via aryloxyenone 5. The synthesis of 2 required formation of a carbon-nitrogen bond at the position ß to the carbonyl function in 6. This ring closure was achieved by first introducing the double bond present in 7 (four steps from 6 by the method of Weller and Rapoport, 1976) then allowing acid catalyzed 1, 4 Michael-type addition of the nitrogen to give 8 Treatment of 8 with $LiAlH_4$ reduced the ketone to an epimeric mixture of alpha, beta alcohols with accompanying partial hydrogenolysis of the aromatic chlorine in addition to reduction of the N-formyl function to N-methyl. Dechlorination was completed by subjecting the crude product to hydrogenation over 10% Pd/C. The resulting mixture of epimeric alcohols was deoxygenated by conversion to the corresponding mesylate esters followed by hydride displacement of the methanesulfonate groups with $LiEt_3BH$ to yield methyl ether 9. O-Demethylation with BBr_3 under conditions similar to those employed for the high yield conversion of codeine to morphine (Rice 1977) gave an excellent yield of the phenolic product 2.

For the synthesis of 3, reduction of 6 with $NaBH_4$ gave alcohol 10 which was converted to the methanesulfonate ester 11. Reduction of the N-CHO function with diborane yielded the corresponding N-CH compound which underwent intramolecular displacement of the methanesulfonate group. Hydrogenolysis of the chlorine atom gave methyl ether 12 which was O-demethylated with BBr_3 as in the case of 9 to provide the desired phenolic derivative 3. Single crystal X-ray analysis confirmed the structures of 2 and 3 and showed that the phenyl ring was held at angles of 86° and 8° respectively relative to least squares planes through atoms 1, 3, 4 and 9 of 1. Results of biological evaluation are shown below.

PHARMACOLOGICAL AND BIOCHEMICAL PROPERTIES OF 5-(m-HYDROXYPHENYL-MORPHANS AND OXIDE BRIDGED 5-(M-HYDROXYPHENYL)MORPHANS

Compound	Hot Plate[a] (mg/kg)	Tail Flick Antagonism *vs* Morphine[b](mg/kg)	Receptor Binding[c] (nM)
	0.63	inactive	5.2
	2.0	inactive	9.6
	inactive[d]	——	3,280
	inactive[d]	inactive	1,766
	inactive[d]	——	4,820
	inactive[e]	Ca30[f]	96

[a]Atwell and Jacobson, 1978. [b]Aceto et al. 1983. [c]Method: according to Itzhak et al. 1981. Only 10% of. mice were affected at 50 mg/kg. [e]Only 20% of mice were affected at 50 mg/kg. [f]53% of mice were affected at 30 mg/kg and 62% were affected at 60mg/kg at which doses convulsions were observed.

CONCLUSION

While both 2 and 3 are inactive in the hot plate assay for antinociceptive activity, 3 does exhibit appreciable binding to opiate receptor preparations from whole rat brain homogenate (IC_{50} = 96 nM for 3 as compared to IC_{50} = 5.2 nM for (+)-1 and IC_{50} = 1,766 nM for 2.) This may indicae that the 8° phenyl torsion angle of 3 is approaching that required for optimal binding. Work is in progress on the synthesis of the remaining four isomers in the series. It seems likely that completion of the series will provide valuable insight concerning the requirements of the phenyl torsion angle for binding to opiate receptors.

REFERENCES

Aceto, M.D., Harris, L. S., Dewey, W. L. and May, E. L. **Problems of Drug Dependence**, 1983. National Institute on Drug Abuse. Research Monograph 49. This volume.

Atwell, L., and Jacobson, A. E. The search for non-dependence producing analgesics in man: Ode to a mouse. Lab. Animal, 7:42-47, 1978.

Burke, T.R., Jr., Jacobson, A.E., Rice, K.C., and Silverton, J. V. Probes for narcotic receptor mediated phenomena 4. Synthesis of 2, 3, 4, 5, 6, 6a-hexahydro-3-methyl-8-hydroxy-1H-4, 11b-methanobenzofuro[3,2-d]azocine, an oxide bridged 5-(m-hydroxyphenylmorphan. J. Org. Chem. (submitted).

Itzhak, Y., Kalir, A., Weissman, B. A., and Cohen, S. Receptor binding and antinociceptive properties of phencyclidine opiate-like derivatives. Eur. J. Pharm. 72:305-311, 1981. A modified procedure was employed, using 20 mM, HEPES-KOH, pH 7.4 buffer, incubated at 25°C for 30 minutes.

Klee, W. A., Simmons, W. F., Sweat, F. W., Burke, T. R., Jr., Jacobson, A. E., and Rice, K. C. Identification of a Mr 58,000 glycoprotein subunit of the opiate receptor. FEBS Letters 150: 125-128, 1982. (Paper 1 in this series).

Larson, D. L., Portoghese, P. S. Stereochemical studies on medical agents. 12. The distinction of enantiotopic groups in the interaction of 1-methyl 4-phenyl-4-propionoxy piperidine. J. Med. Chem. 16:195-198, 1973.

May, E. L., and Murphy, J. G. Structures related to morphine. II. An isomer of N-methylmorphinan. J. Org, Chem. 19:618-624, 1954.

May, E. L., and Murphy, J. G. Structures related to morphine. IV. m-Substituted phenylcyclohexane derivatives. J. Org. Chem., 20:1197-1201, 1955.

May, E. L., and Takeda, M. Optical isomers of miscellaneous strong analgesics. J. Med. Chem. 13:805-807, 1970.

Rice, K. C. A rapid high-yield conversion of codeine to morphine. J. Med. Chem. 20: 164-165, 1977.

Rice, K. C., Jacobson, A. E., Burke, T. R., Jr., Bajwa, B. S., Streaty, R. A., and Klee, W. A. Irreversible ligands with high selectivity toward μ or δ opiate receptors. Science, 220:314-316, 1983. (Paper 2 in this series).

Schultz, A. G., and Fu, W. Y. Heteroatom directed photoarylation. Stereochemistry of aryloxyenone photocyclization. J. Org. Chem., 41:1483-1484, 1976.

Schultz, A. G., Yee, Y. K. and Berger, M. H. Total synthesis of d,l-lycoramine. J. Am. Chem. Soc., 99:8065-8067, 1977.

Weller, D. D., and Rapoport, H. A practical synthesis of codeine from dihydrothebainone. J. Med. Chem., 19:1171-1175, 1976.

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An *In Vivo* Pharmacological Analysis of Benzomorphan Binding Sites in Rats

Alan Cowan and Debra E. Gmerek

INTRODUCTION

Bombesin, a tetradecapeptide which was originally isolated from frog skin, causes excessive scratching when given i.c.v. to rats. Within 1 min of injection, low doses of the peptide (A = 0.013 µg, i.c.v.) elicit vigorous scratching (of mainly the ace, head and neck) which continues, almost constantly, for at least 45 min (Gmerek and Cowan, 1983a). Scratching is not a behavior that has commonly been monitored in preclinical opiate research. This situation may change, since pruritus is often an annoying side effect of epidural or intrathecal analgesia in humans. It is known that several opioids and opioid peptides antagonize the reciprocal hindlimb scratching response associated with intra-cerebral or intrathecal substance P in mice (Share and Rackham, 1981; Hylden and Wilcox, 1983). Also, when monkeys that have received cyclazocine, nalorphine or certain oripavines chronically are challenged with naloxone, or are abruptly withdrawn from these agents, scratching is very prominent (Cowan, 1973).

We report here that scratching caused by bombesin in rats is attenuated in a stereospecific and dose-related manner by several important benzomorphan analgesics but is essentially unaffected by a wide range of opioids. Our pharmacological analysis of this antagonism has provided *in vivo* evidence for benzomorphan-selective binding sites in rats. Such sites have previously been postulated fran binding studies with, for example, rat brain membranes (Chang et al. 1981) and homogenates of rat lumbo-sacral spinal cord (Gouarderes et al. 1982).

MATERIALS AND METHODS

Animals and Surgery

Male Sprague Dawley albino rats (Zivic-Miller, 180-200 g) were each implanted stereotaxically with a stainless steel cannula into the right lateral cerebral ventricle. They were then housed individually and allowed 4-7 days for recovery prior to testing. Each rat was used on only one occasion.

Compounds and Injections

The following agents were dissolved in saline: azidomorphine bitartrate. (Dr. J. Knoll, Semmelweis University of Medicine, Budapest), buprenorphine HCl (NIDA), codeine phosphate (MS & D), dextrorphan (NIDA), ethylketocyclazocine (EK) methanesulfonate (Sterling-Winthrop), heroin HCl (NIDA), ketocyclazocine methanesulfonate (Sterling-Winthrop), levorphanol tartrate (Roche), meperidine HCl (Sterling-Winthrop), methadone HCl (NIDA), morphine sulfate (Mallinckrodt), nalbuphine HCl (Endo), nalorphine HCl (MS & D), d-naloxone HCl and l-naloxone (Dr. A.E. Jacobson, NIH), naloxone HCl (Endo) and thebaine (NIDA).

Bremazocine (Dr. D. Romer, Sandoz, Basel), cyclazocine, d- and l-EK, l-pentazocine (all Sterling-Winthrop), phenazocine HBr, d- and l-SKF 10,047 (N-allylnormetazocine HCl) (all SK & F) and Win 44,441-3 (2α,6α,11S*)-(l)-1-cyclopentyl-5-(1,2,3,4,5,6-hexahydro-8-hydroxy-3,6,11-trimethyl-2,6-methano-3-benzazocin-11-yl)-3-pentanone methanesulfonate (Sterling-Winthrop) were dissolved in a minimal volume of glacial acetic acid, the pH adjusted to 5 with sodium bicarbonate and made up to volume with saline.

Aliquots of the following peptides were each dissolved in saline daily as needed: ß-endorphin (Dr. A.A. Manian, NIMH), synthetic bombesin (Sigma and Boehringer Mannheim), DADLE (D-Ala^2, D-Leu^5-enkephalin) (Sigma), dynorphin (1-13) (U.S. Biochemical), ICI 154,129 (N,N-bisallyl-Tyr-Gly-Gly-ψ-(CH_2S)-Phe-Leu-OH) (DR. M.J. Turnbull, ICI, England) and metkephamid (Dr. R.C.A. Frederickson, Eli Lilly).

ICI 154,129, metkephamid and the alkaloid opioids were injected s.c. in a volume of 1 ml/kg 15 min before a standard submaximal scratch-inducing dose of bombesin (0.10 µg, i.c.v.; Gmerek and Cowan, 1983a). ß-Endorphin and dynorphin (1-13) were given i.c.v. 15 min before bombesin; DADLE, was administered i.c.v. immediately before the bombesin. I.c.v. injections to hand-held, conscious rats were in volumes of 3-4 µl (over 20 sec) followed by 0.5-1 µl saline washes.

Doses of the enantiomers of naloxone were calculated in terms of the freebase. Doses of other agents refer to the particular salt.

Dosing schedules that involved injecting rats with EK, morphine, phenazocine or saline twice daily (at 8 a.m. and 5 p.m.) for 4 consecutive days are shown in table 1. Challenge with bombesin took place 19-20 hr after the last of the multiple injections.

TABLE 1 Dosing schedules for test compounds

Compound	Day 1	Day 2	Day 3	Day 4
Ethylketocyclazocine	5[a]	10	20	20
morphine	10	30	100	100
Phenazocine	1	3	10	10

[a](mg/kg, s.c.)

Quantitation of Behavior

The rats were placed singly in Plexiglas observation boxes (22 an long; 18 cm wide; 25 an high) at least 15 min before testing. Monitoring sessions began immediately after the last bombesin injection. Four rats were observed at a time with the help of a microcomputer, as described previously (Murray et al. 1981). Each rat was monitored for 5 sec out of every 20 sec for 30 min. Positive scores were given for the demonstration of grooming behavior during any 5 sec observation period. There was consequently a maximum of 90 positive grooming scores per rat in each 30 min session. The percent of the maximum number of grooming episodes (%MGE) was calculated for each rat. Groups containing a minimum of 5 rats were used for each data point. Absolute groaning scores were examined statistically by analysis of variance followed by Dunnett's test or the Mann-Whitney U test, as appropriate. A values were determined by linear regression analysis of absolute data from which saline control values had been subtracted. Confidence limits (95%) of A_{50} values are shown within parentheses.

RESULTS

Classification of Opioids

Behaviorally nondepressant doses of 6 benzomorphan analgesics significantly attenuated the excessive scratching elicited in rats by a standard dose of bombesin (0.10 µg, i.c.v.). The benzomorphans (and highest nondepressant doses) were bremazocine (10 mg/kg), cyclazocine (5 mg/kg), EK (0.75 q/kg), ketocyclazocine (1 mg/kg), ℓ-pentazocine (20 mg/kg) and phenazocine (1 mg/kg). The A_{50} values for EK and phenazocine were 0.36 (0.33-0.40) mg/kg and 0.29 (0.19-O.43) mg/kg, respectively.

Even at a dose as high as 10 mg/kg, d- and ℓ-enantiomers of the benzomorphan SKF 10,047 (the prototype agonist at sigma opiate receptors; Martin et al. 1976) did not markedly affect bombesin-induced scratching. The rats were behaviorally excited by the drug combinations used in this experiment. A high dose of Win 44,441-3 (20 mg/kg), a benzomorphan that is a pure opioid antagonist (Ward et al. 1983), had no great influence on the scratching.

The opioids and opioid peptides listed below were tested against bombesin at their highest behaviorally nondepressant dose. None are benzomorphans. None had a statistically significant attenuating effect on the scratching. The inactive agents were as follows: azidomorphine (0.05 mg/kg), buprenorphine (0.50), codeine (40), dextrorphan (30), heroin (0.50), levorphanol (1), meperidine (25), methadone (1), morphine (10), nalbuphine (10), nalorphine (50), naloxone (10), thebaine (25), ICI 154,129 (30), metkephamid (30) and ß-endorphin (10 µg). Classification of dynorphin (1-13) and DADLE could not be accomplished because these peptides caused excessive grooming by themselves.

Further Studies with EK and Phenazocine

The antagonism of bombesin-induced scratching in rats by EK was stereospecific, with ℓ-EK (0.50 mg/kg; %MGE = 14±4, $P<0.001$), but

not d-EK (0.50 mg/kg; %MGE = 71±6, P>0.05), being active. l-Naloxone (50-100 µg/kg) prevented the antagonizing effect of EK (0.50 mg/kg) in a dose-related manner. The A_{50} value for l-naloxone was only 76 (68-86) µg/kg; d-naloxone was essentially inactive.

EK (10 µg) and phenazocine (10 µg) lost their ability to attenuate the scratching if they were given i.c.v.

Pretreatment with Buprenorphine

Buprenorphine (0.50 mg/kg), given 0.5 hr before EK and phenazocine (both at 0.50 mg/kg), or 24 hr before EK, did not counter the ability of the benzomorphans to antagonize the scratching.

Tolerance and Cross-Tolerance Studies

Four twice-daily injections of EK (5-20 mq/kg) caused tolerance to the anti-bombesin action of EK. Similarly, twice-daily injections of phenazocine (1-10 mg/kg) for 4 days caused tolerance to the anti-bombesin action of phenazocine (figure 1). When rats were given 8 injections of morphine (10-100 mg/kg) across 4 days, bombesin still elicited excessive scratching. The same dosing schedule with morphine did not affect the ability of either EK or phenazocine to attenuate the scratching (figure 1).

DISCUSSION

In this study, we found that bombesin-induced scratching in rats is attenuated in a stereospecific and (dose-related manner by benzomorphan analgesics. Other commonly used opioids and opioid peptides were ineffective against bombesin when tested at *behaviorally nondepressant* doses.

We used EK and phenazocine as prototype benzomorphans for further study of the inhibitory opiate link modulating bombesin-induced scratching. The s.c. A_{50} value for EK (0.36 mg/kg) against scratching is comparable to the corresponding antinociceptive A_{50} value (0.40 mg/kg) in the tail compression test with OUT strain of rat. The activity of EK resides largely in the *levo* enantiomer in both procedures. The ability of EK to attenuate scratching was prevented by naloxone in a potent and stereospecific manner. These results, together with our demonstration of tolerance development to the inhibitory action of EK (and phenazocine), suggest that stereospecific opiate receptors are involved. Recall, however, that opioids other than benzomorphans are not active in this model. Mu opiate receptors (Martin et al. 1976) can probably be excluded from consideration. Thus, an eight-injection dosing schedule of morphine that, in our hands, causes tolerance to even the gastrointestinal slowing effect of morphine, had no marked influence on the ability of either EK or phenazocine to antagonize scratching. Furthermore, when mu receptors were occluded by buprenorphine, a partial agonist that dissociates very slowly from mu receptors, EK and phenazocine could still antagonize bombesin-induced scratching.

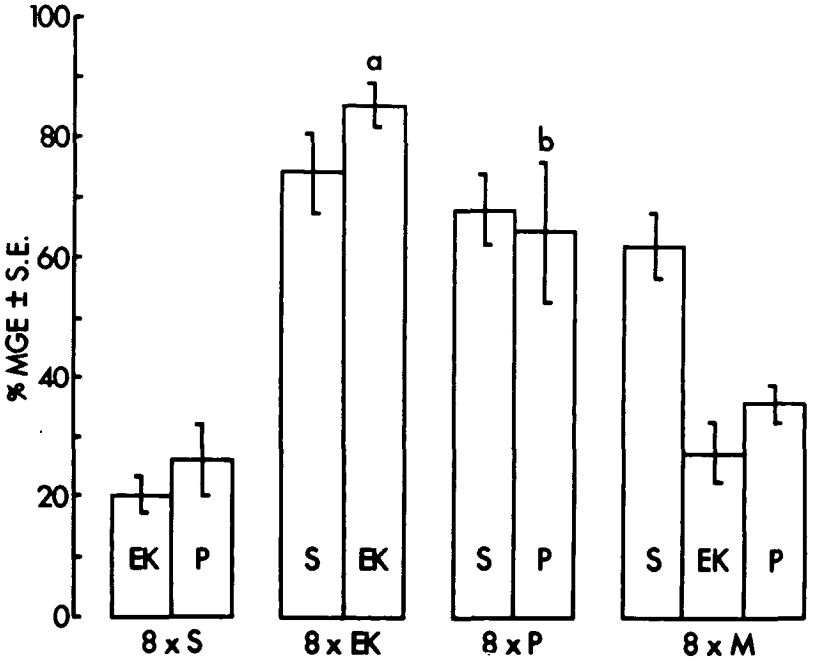

FIGURE 1 Effect of 4 twice-daily injections of saline (S), ethylketocyclazocine (EK), phenazocine (P) or morphine (M) on the ability of EK (0.50 mg/kg, s.c.) and P (0.50 mg/kg) to attenuate grooming induced by bombesin (0.10 µg, i.c.v.) in rats. %MGE is the percent of the maximum possible number of grooming episodes in 30 min.

[a] *P<0.001 compared to (8xS) + EK controls.*

[b] *P<0.001 compared to (8xS) + P controls (ANOVA and Mann-Whitney U test).*

EK and phenazocine show site specificity in their ability to antagonize scratching elicited by bombesin. When given i.c.v., neither benzomorphan influenced scratching. It therefore appears that, despite bombesin being given into the lateral cerebral ventricle, the sites mediating anti-bombesin effects are not readily accessible to EK or phenazocine after i.c.v. injection.

Five of the six benzomorphans found to be active in our study (bremazocine, cyclazocine, EK, ketocyclazocine and pentazocine) are conventionally regarded as kappa agonists, that is, they are ligands for the kappa subtype of opiate receptor (Martin et al. 1976). Recent findings from *in vitro* ligand competition studies support the existence of distinct binding sites for kappa agonists in the brains of several species (see Gmerek and Cowan, 1983b for references). Binding sites showing selectivity for benzo-

morphans (both kappa and sigma agonists) have been identified in the brains of rats, toads and humans (see Gmerek and Cowan, 1983b for references). Interestingly, Gouarderes et al. (1982) claim that benzomorphan and kappa sites in rat lumbo-sacral spinal cord are distinct, with the former resembling benzomorphan sites of rat brain.

The parallel activities of EK and phenazocine in our study suggest that (a) phenazocine interacts with kappa binding sites to influence rat behavior, and/or (b) the sites mediating inhibition of bombesin-induced scratching are selective for benzomorphan-derived agonists (Win 44,441-3, a benzomorphan that is a pure opioid antagonist, is not active). The latter view may be the more valid since nalorphine and nalbuphine are both inactive in the test yet possess kappa-like features. Further experiments with non-benzomorphan kappa agonists may help to clarify this issue. Also, cross-tolerance studies between phenazocine and EK will provide useful information on the mode of action of these agents in our test.

In summary, we have used classic pharmacological approaches for inferring opiate receptor mediation *in vivo*. Since bombesin-induced scratching in rats is antagonized by benzomorphan analgesics, but not by other standard opioids, our results provide *in vivo* evidence for the existence of previously postulated benzomorphan selective binding sites. The model affords a simple, yet novel, behavioral endpoint that can be used when defining the pharmacological profile of new benzomorphans and their antagonists.

REFERENCES

Chang, K.-J., Hazum, E., and Cuatrecasas, P. Novel opiate binding sites selective for benzomorphan drugs. Proc Natl Acad Sci, 78: 4141-4145, 1981.

Cowan, A. Evaluation of the physical dependence capacities of oripavine-thebaine partial agonists in patas monkeys. Adv Biochem Psychopharmacol, 8:427-438, 1973.

Gmerek, D.E., and Cowan, A. Studies on bombesin-induced grooming in rats. Peptides, 4: In press, 1983a.

Gmerek, D.E., and Cowan, A. *In vivo* evidence for benzomorphan-selective binding sites in rats. Submitted for publication, 1983b.

Gouarderes, C., Audigier, Y., and Cros, J. Benzomorphan binding sites in rat lumbo-sacral spinal cord. Eur J Pharmacol, 78:483-486, 1982.

Hylden, J.L.K., and Wilcox, G.L. Intrathecal opioids block a spinal action of substance P in mice: functional importance of both μ- and δ-receptors. Eur J Pharmacol, 86:95-98, 1983.

Martin, W.R., Eades, C.G., Thompson, J.A., Huppler, R.E., and Gilbert, P.E. The effects of morphine- and nalorphine-like drugs in the nondependent and morphine-dependent chronic spinal dog. J Pharmacol Exp Ther, 197:517-532, 1976.

Murray, R.B., Gmerek, D.E., Cowan, A., and Tallarida, R.J. Use of a programmable protocol timer and data logger in the monitoring of animal behavior. Pharmacol Biochem Behav, 15:135-140, 1981.

Share, N.N., and Rackham, A. Intracerebral substance P in mice: behavioral effects and narcotic agents. Brain Res, 211:379-386, 1981.

Ward, S.J., Pierson, A.K., and Michne, W.F. Multiple opioid receptor profile of WIN 44,441-3, a potent opioid antagonist. Int Narc Res Conf (Garmisch-Partenkirchen, F.R.G.), Abstr 173P, 1983.

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Differential Tolerance and Cross-Tolerance to Chronic Treatment with Mu and Kappa Opioid Agonists in the Rat

Gerald A. Young and Naim Khazan

INTRODUCTION

We recently assessed the acute effects of mu, kappa and sigma ooioid agonists on cortical EEG and EEG power spectra in the rat and reported on differential dose-related effects of morphine, ketocyclazocine and SKF-10,047 (N-allyl-normetazocine) on these parameters (Young et al. 1981; Young and Khazan 1983). Intravenously administered morphine produced high-voltage cortical EEG bursts associated with increases in EEG spectral power in the zero to 10 Hz range. Ketocyclazocine produced high-voltage EEG bursts associated with a predominant spectral peak in the 5-8 Hz band, SKF-10,047 produced desynchronized cortical EEG along with frequent theta wave activity whose EEG power spectra peaked at about 7.5 Hz and had the least power. Furthermore, the (-) enantiomers of methadone (mu agonist) and ketocyclazocine were active, producing EEG and EEG power spectral effects qualitatively similar to those produced by the respective racemic mixtures. The (+) enantiomers were found to be inactive. The (+) enantiomer of SKF-10,047, however, produced behavioral changes that were, in part, similar to those produced by the psychotomimetic agent dimethyltryptamine (Kovacic and Domino 1976).

Another characteristic of the opioid agonists is their ability to produce tolerance with variable degrees of cross-tolerance. In this regard, it has been reported that chronic administration of morphine, ketocyclazocine and SKF-10,047 to the chronic spinal dog produced tolerance to their agonistic effects (Martin et al. 1976). Using the same preparation, tolerance development to the agonistic effects of cyclazocine, an opioid with purported kappa and sigma agonistic and mu antagonistic oroperties, was also demonstrated. However, morphine-dependent dogs demonstrated no cross-tolerance to the agonistic effects of cyclazocine (Gilbert and Martin 1976). On the other hand, ketocyclazocine-dependent dogs seemed to have developed cross-tolerance to morphine. Furthermore, in an *in vivo* preparation utilizing tissue from guinea pig ileum, while tolerance to the effects of mu and kappa opioid agonists was produced,

cross-tolerance was not demonstrated (Schulz et al. 1981).

The objective of the present study was to further assess the development of tolerance and cross-tolerance to chronic administration of mu and kappa opioid agonists (morphine and ethylketocyclazocine, respectively) by studying their effects on EEG power spectra and behavior in the rat.

METHODS

Sixteen female Sprague-Dawley rats (250 - 300 g) were implanted with bipolar epidural frontoparietal EEG and temporalis EMG electrodes and with chronic indwelling jugular cannulae. Surgical procedures. have been previously described (Khazan 1975). The i.v. cannulae were prepared and implanted according to the method of Weeks (1962, 1972). During the experiments, rats were housed in individual cages (12" x 12" x 24"). To permit free movement of the rat, each cage was equipped with a swivel connector having concentric mercury pools which served as noise-free sliding contacts (Khazan et al. 1967). These freely-moving rats were allowed to acclimatize to the experimental cages for two to three days before experimentation. Lighting conditions consisted of a timer-regulated lights-off period from 10 pm to 6 am.

For each rat, direct EEG activity was filtered to pass frequencies between 1 and 35 Hz. The EEG and integrated EMG activities were continuously recorded on a Grass polygraph. The EEG was simultaneously recorded on FM magnetic tape using a Hewlett-Packard. Model-3940-A recorder. Power spectral analysis was performed offline using a Nicolet MED-80 minicomputer system; EEG power spectra were derived from 10-sec samples of EEG that were digitized at a sampling rate of 100/sec, and power spectral densities were estimated at 0.05 intervals from zero to 50 Hz (Young et al. 1978; Khazan and Young 1980). Average power spectra were obtained by averaging spectra derived from six 10-sec epochs; weighted geometric smoothing over three neighboring frequencies was used.

Behavioral states of sleep, REM sleep and wakefulness were identified by corresponding changes in EEG and EMG recordings (Khazan et al. 1967; Khazan 1975). Occurrences of opioid-induced high-voltage EEG bursts and of hyperarousal were also identified from EEG and EMG recordings.

One day prior to the initiation of chronic morphine treatment, each of eight rats was given an i.v. dose of morphine (10 mg/kg), and the effects of the injection on EEG, EEG power spectra and behavior were studied. These eight rats were then chronically treated with morphine by a series of automatic i.v. injections. Morphine sulphate was dissolved in isotonic saline at a concentration of 10 mg/ml. On the first day, rats received hourly injections of 1.25 mg/kg of morphine. The dose was then increased to 2.5, 5.0 and 10.0 mg/kg/hr on successive days. On the fifth day, the rats received 10.0 mg/kg of morphine every three hours and were subsequently maintained on this injection schedule. Effects of 10 mg/kg doses of morphine on

EEG, EEG power spectra and behavior during the seventh day were compared with those before initiation of chronic morphine treatment. Challenge doses of ethylketocyclazocine (EKC, 10 mg/kg) were substituted for morphine on the eighth 1 day. Effects of EKC on EEG, EEG power spectra and behavior in rats chronically treated with morphine were compared to the effects of EKC in non-tolerant rats and in EKC-tolerant rats.

In a second group of eight rats, one day prior to the initiation of chronic EKC treatment, each rat was given an i.v. dose of EKC (10 mg/kg). The same experimental parameters were assessed in these rats and in the same way as those in the first group, These eight rats were then chronically treated with EKC by a series of automatic i.v. injections. EKC methanesulfonate was dissolved in a small amount of 0.5 N NaOH and brought up to a concentration of 2.5 mg/ml with isotonic saline. On the first day, rats received a dose of 2.5 mg/kg/2 hrs. The dose was then increased to 5.0 and 10.0 mg/kg/2 hrs every other day. On the eighth day, challenge doses of morphine (10 mg/kg) were given in the place of regularly scheduled automatic EKC injections.

RESULTS

Representative effects of morphine administration (10 mg/kg) on EEG and behavior before and after chronic treatment are depicted in Figure 1 for an individual rat, In the non-tolerant state, morphine induced a predominance of behavioral stupor that was associated with high-voltage EEG bursts for 90 min, followed by a predominance of behavioral arousal associated with low-voltage desynchronized EEG for another 90 min. The first occurrence of slow-wave sleep emerged three hrs after morphine administration. In the morphine-tolerant state, however, morphine produced behavioral stupor for only ten min followed by an arousal state for 80 min. In this case, the first occurrence of slow-wave sleep after morphine injection emerged after 90 min. All eight rats demonstrated similar degrees of tolerance to the EEG and behavioral effects of morphine challenge. Mean latency for the eight rats to the occurrence of the first REM sleep episode after morphine administration was found to be 246 ± 5 (mean ± s.e.m.) min in the non-tolerant state and 90 ± 4 min in the tolerant state.

Representative examples of EEG and derived EEG power spectra during the morphine-induced stuporous state are shown in the too of Figure 2 during non-tolerant and tolerant states. Five minutes after morphine injection in the non-tolerant state, the EEG consisted of almost continuous high-voltage bursting, the spectral power of which predominated over the zero to 10 Hz range. Five minutes after morphine injection to the morphine-tolerant rat, however, the amount of EEG bursting and the peak-to-peak amplitude of individual bursts were substantially less than that which occurred in the non-tolerant rat. This is reflected by a difference in the magnitude of the respective power spectra; the power spectra associated with high-voltage EEG bursts in the tolerant state consist of only about 25% of total density area compared to that associated with the non-tolerant state.

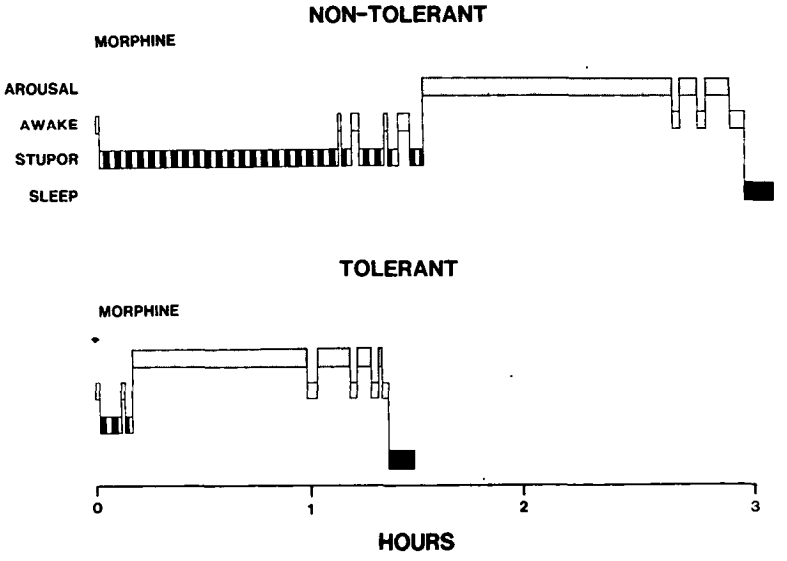

FIGURE 1

EEG and behavioral responses of an individual rat to a 10 mg/kg dose of morphine during the non-tolerant and tolerant states.

In the case of EKC in the non-tolerant state, an injection of EKC (10 mg/kg) typically produced a predominance of behavioral stupor and associated high-voltage EEG bursts for 70 min followed by a predominance of behavioral arousal and associated low-voltage desynchronized EEG for 50 min. The first occurrence of slow-wave sleep emerged 2 hrs after the EKC injection. In the tolerant state, EKC typically induced oscillations between behavioral stupor and wakefulness for 25 min, and the first occurrences of slow-wave sleep emerged in about 30 min. All eight rats demonstrated similar degrees of tolerance to EEG and behavioral effects of EKC. Mean latency to the first REM sleep occurrence after EKC administration was 220 ± 9 min in the non-tolerant state and 68 ± 3 min in the MC-tolerant state.

Representative examples of EEG and derived EEG power spectra during the EKC-induced stuporous state are shown in the bottom of Figure 2 in both the non-tolerant and tolerant states. During the first five min after EKC injection in the non-tolerant state, the EEG consisted of closely spaced high-voltage EEG bursts. The correlated EEG power spectra had a predominant peak in the 5-8 Hz band. During the first five min after EKC injection in the tolerant state, the amount of EEG bursting and the peak-to-peak amplitude of individual bursts were substantially less than that which occurred in the non-tolerant state. This is reflected by a difference in the size of the respective power spectra; the power spectra associated with high-voltage EEG bursts in the tolerant state decreased to about one-third of the total power density area compared to that associated with the non-tolerant state.

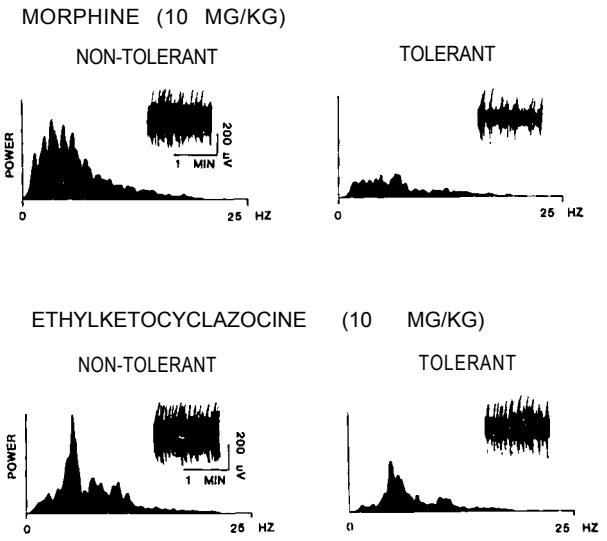

FIGURE 2

Cortical EEG samples and associated EEG power spectra are shown for individual rats during opioid-induced behavioral stupor. Effects of morphine are shown in the top of the figure during the non-tolerant and tolerant states. Similar data are shown after MC administration in the bottom of the figure.

During cross-tolerance assessment, EKC administration (10 mg/kg) to the morphine-tolerant rats induced a predominance of behavioral stupor and associated high-voltage cortical EEG bursts for about 70 min, followed by a oredominance of behavioral arousal and associated low-voltage desynchronized EEG for about 50 min. This overall effect of EKC administration in the morphine-tolerant rats was analogous to that seen when EKC was given to the non-tolerant rats; thus, no cross-tolerance to EKC was seen in the morohine-tolerant rats. Furthermore, mean latency to the first REM sleep occurrence after EKC was 220 ± 10 min in the morphine-tolerant rats, In the EKC-tolerant rats, on the other hand, morphine administration (10 mg/kg) produced behavioral stupor and associated high-voltage EEG bursts for about 10 min, followed by a predominance of behavioral arousal and low-voltage desynchronized EEG for about 75 min. This overall effect of morphine in the EKC-tolerant rats was similar to that seen when morphine was administered to the morphine-tolerant rats; thus, cross-tolerance was demonstrated. Mean latency to the first occurrence of REM sleep after morphine administration was 98 ± 5 min in the EKC-tolerant rats compared to 90 ± 4 min in the morphine-tolerant rats.

DISCUSSION

Studies dealing with opioid-receotor interactions have demonstrated that in the rat morphine does not readily displace radiolabeled EKC, but that both ketocyclazocine and EKC displace radiolabeled dihydromorphine (mu agonist) and EKC equally well (Wood et al. 1981; Wood 1982). However, the nature of the interaction of EKC with mu receptors is not clear at present. Based upon these in- vitro data, one might predict that chronic morphine treatment would have an effect on the pharmacodynamics of kappa receptors, and that, therefore, no cross-tolerance to EKC in morphine-tolerant rats should develop. On the other hand, one might predict that chronic EKC treatment would alter characteristics of mu receptors, and, therefore, cross-tolerance to morphine in EKC-tolerant rats might develop if EKC has mu receptor agonistic activity. Our EEG and EEG power spectral data support these predictions, While EKC-tolerant rats demonstrated full cross-tolerance to the effects of morphine on EEG and EEG power spectra, no cross-tolerance to effects of EKC was observed in morphine-tolerant rats. Lack of reciprocal cross-tolerance between different opioids has been previously noticed in other in vivo experiments (Martin et al. 1976; Gilbert and Martin 1976). These authors noted that morphine-tolerant dogs demonstrated no cross-tolerance to the agonistic effects of cyclazocine, while ketocyclatocine-dependent dogs may have developed cross-tolerance to the effects of morphine.

Although it is generally accepted that EKC demonstrates a significant property to bind to mu receptors, the nature of the biological response resulting from such binding needs further evaluation. Thus, further studies will be necessary to more fully understand interactions between mu and kappa opioid agonists at both the behavioral and molecular levels.

REFERENCES

Gilbert, P.E., and Martin, W.R.. The effects of morphine- and nalorphine-like drugs in the nondependent, morphine-dependent and cyclazocine-dependent chronic spinal dog. J Pharmacol Exp Ther, 198:66-82, 1976.

Khazan, N. The implication and significance of EEG and sleep-awake activity in the study of experimental drug dependence on morphine, In: Ehrenpreis, S. and Neidle, A., eds. Methods in Narcotic Research. New York: Marcel Dekker, 1975. pp. 173-215.

Khazan, and Young, G.A. Use of neurophysiology in the study of drugs and chemicals. In: Gryder, R.M. and Frankos, V.H., eds. The Effects of Foods and Drugs on the Develonment and Function of the Nervous System: Methods for Predicting Toxicity. Washington, D.C.: U.S. Govt. Print. Off., 1980 70-79.

Khazan, N.., Weeks, J.R., and Schroeder, L.A. Electroencephalographic, electronlyographic and behavioral correlates during a cycle of self-maintained morphine addiction in the rat. J Pharmacol Exp Ther, 155:521-531, 1967.

Kovacic, B., and Domino, E.F. Tolerance and limited cross-tolerance to the effects of N,N-dimethyltryptamine (DMT) and lysergic acid

diethylamide-25 (LSD) on food-rewarded bar pressing in the rat. J Pharmacol Exp Ther, 197:495-502, 1976.

Martin, W.R., Eades, C.G., Thompson, J.A., Huppler, R.E., and Gilbert, P.E. The effects of morphine- and nalorphine-like drugs in the nondependent and morphine-dependent chronic spinal dog. J Pharmacol Exp Ther, 197:517-532, 1.976.

Schulz, R Wuster, M., Rubini, P., and Herz, A. Functional opiate receptor; in the guinea-pig ileum: Their differentiation by means of selective tolerance development. J Pharmacol Exp Ther, 219:547-550, 1981.

Weeks, J.R. Experimental morphine addiction: Method for automatic intravenous injections in unrestrained rats. Science, 138:143-144, 1962.

Weeks, J.R. Long-term intravenous infusion. In: Myers, R.D., ed. Methods in Psychobiology. New York: Academic Press, 1972. pp. 155-168.

Wood, P.L. Multiple opiate receptors: Support for unique mu, delta and kappa sites. Neuropharmacology, 21:487-497, 1982.

Wood, P.L., Charleson, S.E., Lane, D., and Hudgin, R.L. Multiple opiate receptors: Differential μ κ and μ agonists. Neuropharmacology, 20: 1215-1220, 1981.

Young, G.A., and Khazan, N. Differential stereospecific effects of mu, kappa and sigma ooioid agonists on cortical EEG power spectra in the rat. In: Harris, L.S., ed. Problems of Drug Dependence 1982. National Institute on Drug Abuse Research Monograph 43. DHHS Pub. No. (ADM) 83-1264. Washington, D.C.: Supt. of Docs., U.S. Govt. Print. Off., 1983. pp. 190-195.

Young, G.A., Neistadt, L., and Khazan, N. Differential neuropharmacological effects of *mu, kappa* and *sigma* opioid agonists on cortical EEG power spectra in the rat. Res Comm Psychol Psychiat Behav, 6:365-377, 1981.

Young, G.A., Steinfels, G.F., Khazan, N., and Glaser, E.M. Cortical EEG power spectra associated with sleep-awake behavior in the rat. Pharmacol & Behav, 8:89-91, 1978.

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Relationship of Plasma Level and Pharmacological Activity of Methadone

Shean-Jang Liu and Richard I. H. Wang

INTRODUCTION

The metabolism and disposition of methadone in experimental animals (Pohland et al. 1971; Misra et al. 1973; Ling et al. 1981) and man (Inturrisi and Verebely 1972; Anggard et al. 1975; Verebely et al. 1975) have been reported. However, no report has appeared on the quantitative relationship between the levels of methadone in brain and plasma and the analgesic activity of methadone. Based on several drug interaction studies in rats, we have shown that brain concentration of methadone may be directly related to the analgesic effect of methadone (Liu and Wang 1975; Liu et al. 1975; 1978).

In addition to being used as a potent analgesic, methadone is used for treatment of opiate dependence. It has been suggested that methadone maintenance patients with steady-state plasma concentrations of methadone above 200 ng/ml have a better record of rehabilitation (Holmstrand et al. 1978). However, there are conflicting reports regarding the relationship between the concentrations of methadone in the plasma and several pharmacological effects of methadone (Inturrisi and Verebely 1972; Blake et al. 1973; Horns et al. 1975; Holmstrand et al. 1978). Whether monitoring of blood methadone levels is helpful in adjusting the dosage of methadone in patients maintained on methadone has not been established.

The present study was designed to investigate the quantitative relationship between the levels of methadone in the brain and plasma of rats and to provide insight into the possible utilization of monitoring methadone levels in the plasma as an index of the pharmacological effect of methadone. Analgesia was chosen as the pharmacological effect of methadone evaluated for this study. The results demonstrate that a good correlation exists between the analgesic activity of methadone and the levels of methadone in brain and plasma. This paper also gives evidence that decreased blood level of methadone in methadone maintenance patients, as a result of phenobarbital-induced acceleration of methadone biotransformation, precipitates opiate withdrawal symptoms.

METHODS

Analgesic studies. Male Sprague-Dawley rats (King Animal Labs, Oregon, WI) weighing 120-160 g were used. The rats were fasted overnight with free access to water before being used for experiments. Analgesia was measured by a modified hot-plate method of Eddy and Leimback (1953) as described previously (Liu and Wang 1975). The percentage of maximal possible effect (%MPE) of analgesia was calculated by the method described by Dewey and Harris (1971).

Analysis of methadone in brain and plasma. For tissue distribution study of methadone in rats, rats were given ^{14}C-dl-methadone hydrochloride (1.5-5.0 mg/kg, 15 µCi/kg) and killed by decapitation at different times. Concentrations of total ^{14}C in brain and plasma were determined by directly counting 0.5 ml of 20% (w/v) brain homogenate in distilled water or 0.3 ml of plasma in 15 ml of Aquasol scintillation solution (New England Nuclear Corporation, Boston, MA). The concentrations of ^{14}C-methadone and its major metabolites in brain and plasma were determined by high-performance liquid chromatography (HPLC) as described by Roerig et al. (1982). Samples of brain homogenate and plasma were lyophilized and extracted three times with methanol. The combined extracts were evaporated to dryness under a stream of nitrogen and the residue was redissolved in methanol. Portion of the methanol was injected into the HPLC system.

Methadone in the plasma of human subjects was extracted by n-butyl chloride as described by Inturrisi and Verebely (1972), except that methadol was used as an internal standard. The concentration of methadone was determined by gas-liquid chromatography (GLC). The GLC analysis was performed on a Hewlett Packard gas chromatograph model 5711A equipped with a hydrogen flame ionization detector and electronic integrator. The column is 1 m x 4 mm ID glass, packed with 3% OV-17 on 80-100 mesh chromsorb W. Temperature for the injector, detector and column oven were 250°, 300° and 220°, respectively. The carrier gas was nitrogen at a flow rate of 60 ml/min. Hydrogen and air flow rates were 60 and 240 ml/min, respectively.

Analysis of the regression line and calculation of ED50 and LD50 values were made according to the computer programs of Tallarida and Murray (1981) using an Apple II Computer. Significance was attributed at $p < 0.05$ using Student's t test.

RESULTS

Concentrations of methadone in the brain and plasma and the analgesic effect of methadone in rats. Figure 1 indicates that more than 90% of the ^{14}C in the brain was unchanged methadone while only about 7% of the ^{14}C was 2-ethyl-5-methyl-3,3-diphenylpyrroline (EMDP). The pyrrolidine metabolite, 2-ethyllidine-1,5-dimethyl-3, 3-diphenylpyrrolidine (EDDP) was not detectable in the brain. The percentage of ^{14}C in the brain as unchanged methadone was constant during a 3-hr period after administration, of ^{14}C-methadone. Therefore, the ^{14}C in the brain was considered to be unchanged methadone for practical purposes.

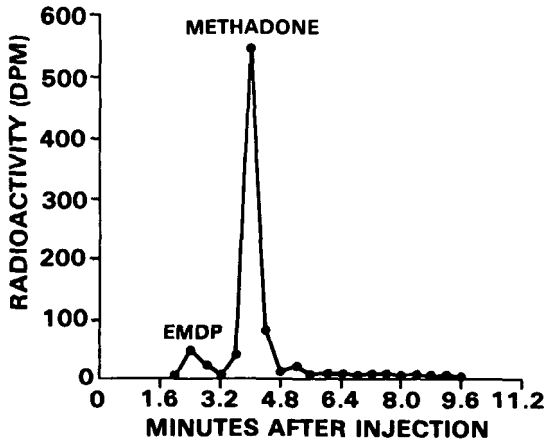

FIGURE 1. High-performance liquid chromatogram of rat brain extract after administration of ^{14}C-methadone. The abscissa shows the retention time of the sample.

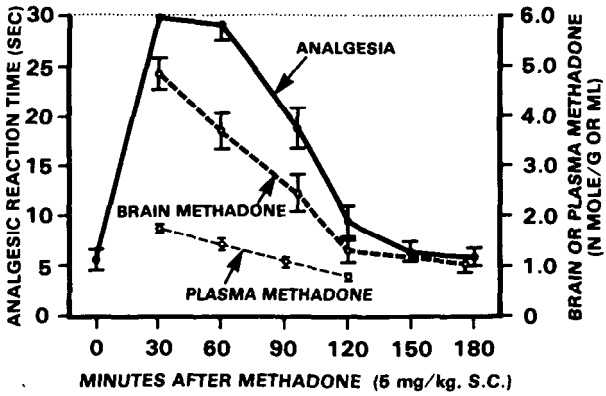

FIGURE 2. Time-response curves of analgesia and concentrations of methadone in brain and plasma. One group of 8 rats were given methadone (5 mg/kg, sc) for measurement of analgesia. Another four groups of 6 rats each were given ^{14}C-methadone (5 mg/kg, 15 µCi/kg, sc) and killed by decapitation 30, 60, 90 or 120 min after injection. Each point and vertical bar represents a mean ± SE for 6-8 rats.

Figure 2 illustrates the time-response curves of methadone over a 3-hr period after administration of methadone. It can be seen that both the analgesic reaction time and brain concentration of methadone fell sharply after reaching the peak time of 30 min. There were significant differences between the brain concentrations of methadone at 30-min vs 60-min and 60-min vs 90-min intervals. There were also significant differences between the analgesic reaction times at the same two time intervals.

The concentration of methadone in the plasma was much lower than that in the brain at all the corresponding times after administration of methadone *as* shown in figure 2. The percentages of ^{14}C in the plasma of rat as unchanged methadone were 78, 68, SO and 53% at 30, 60, 90 and 120 min, respectively, after administration of ^{14}C-methadone.

In order to test whether there are dose-response relationships between the dose of methadone and the concentrations of methadone in the brain and plasma, groups of rats were tested for analgesia 30 min after administration of several different doses of methadone and killed immediately after analgesia was measured. As illustrated in figure 3, the relationship between the observed

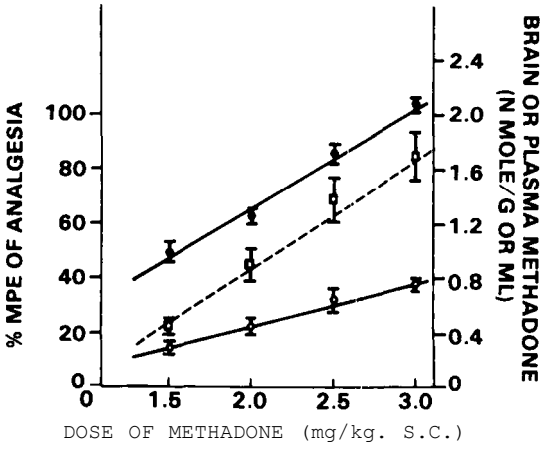

FIGURE 3. *Dose-response effects of methadone for brain and plasma methadone concentrations and analgesic activity of methadone. Groups of 5 rats each were injected with a dose of ^{14}C-methadone (1.5-3.0 mg/kg, 15 µCi/kg, sc) and analgesia was measured 30 min after injection. Immediately after the analgesic reaction time was taken the rats were killed by decapitation for &termination of brain and plasma methadone concentrations. Each point and vertical bar represents the mean ± SE for 5 rats.* ●——● *brain methadone concentration,* o——o *plasma methadone concentration,* □ - - - - □ *%MPE).*

analgesic response (expressed as %MPE) and the dose of methadone administered was linear. The analgesic ED50 was estimated to be 2.04 mg/kg (1.58-2.63, 95% confidence limit). Figure 3 also indicates that a linear relationship existed between the dose of methadone administered and the brain concentration of methadone. Regression analysis of these data indicates a highly significant correlation between the dose of methadone and the brain concentration of methadone (correlation coefficient r = 0.973, p <0.001). A linear relationship also existed between the dose and plasma concentration of methadone. Regression analysis of the data shows a significant regression (p <0.001) with a correlation coefficient 0f 0.748.

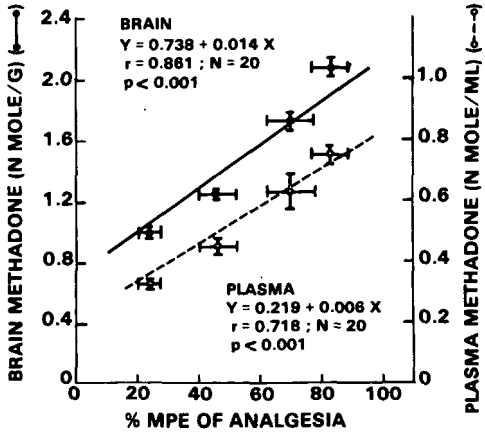

FIGURE 4. *Relationships between the analgesia and the concentrations of methadone in brain and plasma. Treatment of rats is as described in figure 3. Values are means ± SE for 5 rats.*

A highly significant correlation (r = 0.861, p <0.001) between the brain concentration of methadone and the analgesic %MPE was found as shown in figure 4. A 50% MPE methadone analgesia corresponded to a brain concentration of 1.44 nmole/g. Figure 4 also indicates a significant correlation between the plasma concentration of methadone and the analgesic %MPE (r = 0.718, p <0.001. A plasma concentration of 0.52 nmole/ml corresponded to SO% of the analgesic MPE.

Plasma concentrations of methadone in methadone maintenance patients. Blood samples of a few selected patient volunteers from our methadone maintenance program were analyzed to determine methadone plasma concentrations as a function of methadone dose. As shown in table 1 there are large individual differences in the plasma concentrations of methadone. However, the time course of methadone in the plasma was similar for each subject with peak methadone plasma concentrations occurring at about two hours. The concentration of methadone in the plasma of each subject at each time interval and the area under the plasma methadone concentration-time curve were all increased with increased doses of methadone.

Phenobarbital-induced decrease in methadone plasma levels and withdrawal syndrome in a methadone maintenance patient. While we were determining the plasma levels of methadone in patients maintained on different doses of methadone, we observed a case which provided evidence of metabolic drug interaction between, phenobarbital and methadone. A 28-year-old former heroin addict was maintained and stabilized on 95 mg methadone per day. Two months after the patient had been maintained on this dose of methadone his mean zero time plasma concentration was 283 ng/ml and the mean peak plasma methadone concentration was 591 ng/ml which occurred 2 hours after methadone administration as shown in figure 5. While the patient was being maintained on this stabilized dose he was comfortable and without any complaint of withdrawal symptoms. Three months after the blood methadone levels were monitored, the patient

TABLE 1

Plasma Concentrations of Methadone and Area Under the Plasma Methadone Concentration-time Curve (AUC) in Methadone Maintenance Subjects[a]

		METHADONE CONCENTRATION					AUC
		Hours After Methadone					
Subject	Dose (mg)	0[b]	1	2	4	8	0-8
T.L.	55	135	150	198	175	175	1375
	80	170	255	380	250	305	2083
T.W.	80	78	125	177	113	105	956
	100	75	345	356	306	220	2277
D.N.	45	26	145	135	87	75	775
	60	16	117	97	114	56	809
A.C.	50	90	195	260	218	147	1552
	70	115	188	318	275	205	1914

a Plasma concentrations of methadone are expressed as nanograms per milliliter. AUC is expressed as nanograms per milliliter x hour. Each figure is the mean obtained from 3 consecutive days.

b The zero time samples were taken 24 hours after the previous dose and just prior to the administration of next dose of methadone.

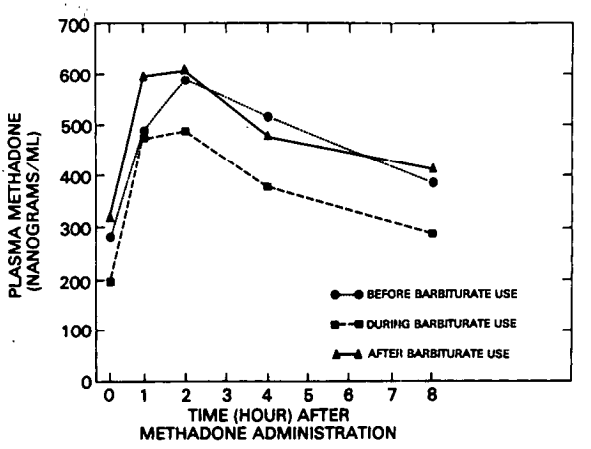

FIGURE 5. *Comparison of plasma levels of methadone in a 28-year-old patient before, during and after he abused barbiturates while maintained on methadone. Methadone was quantitated by gas-liquid chromatography method. Each point is the mean of 3 samples collected from 3 consecutive days.*

complained that his methadone dose was not holding him and withdrawal symptoms were appearing several hours before his next daily methadone dose. The withdrawal symptoms included abdominal cramps, nausea, sweating, restlessness, bone and muscle pain, anxiety and tremor. Both the mean zero-time and mean peak methadone plasma concentrations at this time were found to be lower than those observed three months previously as shown in figure 5. The area under the plasma methadone concentration-time curve at this time was 3.03 nmole/ml·hr as compared to 3.84 nmole/ml·hr three months ago, indicating the patient's plasma methadone level had declined. Routine bi-weekly urine monitoring revealed the patient had abused barbiturates constantly for the previous month and when confronted, the patient admitted constant use of phenobarbital for the previous month.

After counseling by his physician and social worker, the patient agreed to discontinue barbiturate abuse and this was confirmed by routine urine monitoring during the next six weeks. At this time the methadone plasma concentration at each time interval increased to the original level (figure 5) and the area under the plasma methadone concentration-time curve increased from 3.03 to 3.92 nmole/ml•hr. The patient reported feeling good with no more withdrawal symptoms before his next methadone dose.

DISCUSSION

The present study demonstrates that there is a good correlation between the brain concentrations of methadone and the subcutaneous administration of different doses of methadone in rats. In addition, our data demonstrate that there is a quantitative relationship between the concentration of methadone in the brain and its analgesic effect on the hot-plate test. This finding provides a basis for understanding changes in this relationship which may occur after administration of other drugs and after chronic administration of methadone. Both these conditions may change the brain concentration of methadone and result in a change of the analgesic effect of methadone. In this regard, we have previously reported that increased methadone-induced analgesia by desipramine or diazepam was associated with an increase in brain concentrations of methadone (Liu and Wang 1975; Liu et al. 1978). On the other hand, we showed that decreased methadone-induced analgesia after chronic pretreatment with phenobarbital or methadone was associated partly with a decrease in brain concentrations of methadone (Liu et al. 1978; 1979).

In our previous studies, we reported that measurements of changes in the concentration of total ^{14}C in plasma by TLC after administration of ^{14}C-methadone provided no practical information in determining changes in the pharmacokinetics of methadone induced by other drugs (Liu et al. 1978; Roerig et al. 1975). In the present study we separated and analyzed the plasma concentration of unchanged methadone by HPLC and showed that there is a good correlation between the plasma concentration of methadone and the different doses of methadone administered in rats. Similarly, the plasma levels of methadone in methadone maintenance patients, expressed as areas under the plasma concentration-time curves, were increased with increased doses of methadone, In addition, our data demonstrate

that levels of methadone in plasma correlated well with the analgesic activity of methadone in rats. The peak analgesia corresponded with the occurrence of peak levels of methadone in plasma and in the brain in our study. Therefore, measurements of changes in plasma methadone levels may reflect changes in methadone disposition and in pharmacological activity. The findings of the case in the methadone maintenance patient support this suggestion. Our findings clearly demonstrate that chronic phenobarbital intake results in an increase in methadone metabolism which was reflected in the decrease in plasma concentrations of methadone. As the level of methadone decreased, the duration of pharmacological action of methadone was shortened, resulting in a withdrawal syndrome. Our findings in this case thus confirmed our previous reports in rats that phenobarbital induction of methadone metabolism in man is possible, resulting in a decrease in the duration of methadone (Liu et al. 1978; 1979). Our findings are consistent with the reports of others that significant metabolic drug interactions with methadone occurred in methadone maintenance patients simultaneously receiving rifampin (Kreek et al. 1979) and phenytoin (Finelli 1976; Tong et al. 1981). Both rifampin and phenytoin have been shown to increase methadone biotransformation and cause narcotic withdrawal symptoms in patients previously well-maintained on the same dose of methadone (Kreek et al. 1979; Tong et al. 1981). These and our present findings thus provide some direct evidence that metabolic drug interactions with methadone which are sufficient to alter pharmacological action can be detected from changes in methadone plasma level, particularly from changes in the area under the plasma methadone concentration-time curve.

REFERENCES

Due to space limitations, a complete list of references may be obtained from the senior author.

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Irreversible and Reversible Narcotic Agonists: Discriminative and Analgesic Effects of Buprenorphine, Oxymorphazone, and Morphine

Charles P. France, Arthur E. Jacobson, and James H. Woods

Buprenorphine is a highly lipophilic thebaine derivative with a rapid onset and long duration of action. Classified as a partial agonist of the morphine type (Martin et al., 1976), buprenorphine has clearly been shown to have both agonist and antagonist behavioral actions--ranging from morphine-like analgesia in man (Houde, 1979; Jasinski et al., 1978) and animals (Bryant et al., 1983; Dum & Herz, 1981), to naloxone-like precipitation of withdrawal in opiate-dependent animals (Cowan et al., 1977b; Dum & Herr, 1981; Swain & Seevers, 1975). Some actions of buprenorphine appear to be irreversible in that they can be prevented but not reversed by opiate antagonists. While some dose-effect relations with buprenorphine are monophasic, in many cases, dose-effect curves are bi-phasic, e.g., rat tail flick (Cowan & Watson, 1980; Cowan et al., 1977b; Dum & Herz, 1981). In these latter cases, supramaximal doses of buprenorphine result in a partially or completely diminished behavioral response.

Drug discrimination procedures have been used extensively to investigate the stimulus properties of opiates (e.g., Colpaert, 1978), yet very little is known about the discriminative effects of long-acting agents; to date, the reversibility and long-term effects, as both an agonist and antagonist, have yet to be characterize-d for buprenorphine.

The purpose of these experiments was to compare buprenorphine, oxymorphazone, and the prototypic opioid agonist morphine, in the mouse hot plate analgesic assay and in pigeons trained to discriminate morphine from saline. Oxymorphazone was selected for comparison because unlike the mixed agonist-antagonist actions of buprenorphine, oxymorphazone is thought to be a pure, irreversible morphine-like agonist (Galetta et al., 1982; Hahn et al., 1982; Pasternak & Hahn, 1980).

METHODS

Potency (ED50) and time course data in mice were derived by procedures that have been described elsewhere (Atwell & Jacobson,

1978). Drugs were administered subcutaneously, and 8 mice were tested at each of five dose levels.

Five pigeons were trained to discriminate intramuscular injections of morphine from equal volume injections of saline for food reinforcement. A multiple-trial training procedure was used, and daily training sessions consisted of between 2 and 6 trials. Each trial was preceded by an injection (saline or morphine). After a 10-minute pretreatment period, two response keys were illuminated red and up to 10 reinforcements could be earned in 5 minutes, on a fixed-ratio 20 schedule of food presentation. For non-drug training trials, 20 consecutive responses on the right (saline-appropriate) key resulted in food delivery. On drug training days, a single injection of morphine (5.6 mg/kg) was administered randomly before one of the 2-6 trials, and only 20 consecutive responses on the left key resulted in food delivery. Incorrect responses reset the fixed-ratio requirement on the correct key.

Testing was conducted essentially the same as training, except that 20 consecutive responses on either key resulted in food delivery. A cumulative dosing procedure was used, and all drugs were tested up to doses that markedly suppressed responding. Dose-effect curves were determined for morphine, oxymorphazone, and buprenorphine, each when administered alone, and following pretreatments with various doses of naltrexone.

To test the reversibility of the acute effects of morphine, oxymorphazone, and buprenorphine, the smallest dose of each that reliably produced morphine-approrpriate responding within the 10-minute pretreatment period, was administered on the first trial, followed by increasing doses of naltrenone.

The duration of action for each drug was determined during two saline injection test trials (i.e., 20 responses on either key were reinforced) on days following a cumulative suppressing dose of each.

Finally, buprenorphine was evaluated for its ability to antagonize morphine. Buprenorphine was administered either 24 hours or 10 minutes before a cumulative dose-effect curve for morphine. Small (subthreshold) doses of buprenorphine were administered 10 minutes prior to morphine to test whether buprenorphine antagonized the discriminative or rate effects of morphine. However, because large doses (greater than 0.1 mg/kg) of buprenorphine occasion morphine-appropriate responding when given alone, and did so under these conditions, those doses could be evaluated only for their ability to antagonize the rate-suppressing effects of morphine.

RESULTS

Results of the hot plate test are given in Table 1. The ED50 for morphine and oxymorphazone did not differ significantly. Buprenorphine was approximately 30 times more potent than morphine in producing analgesia in mice. The mean onset for analgesia was

rapid for all three drugs. However, the time until peak analgesic effect was longer for buprenorphine than for morphine or oxymorphazone. Conversely, oxymorphazone had a longer duration of action than either morphine or buprenorphine.

TABLE 1
Mouse Hot Plate

	ED50 mg/kg (95% C.I.)	ED50 umol/kg (95% C.I.)	Time Course (minutes) Onset	Peak	Duration
Morphine sulfate	1.05 (0.8-1.4)	3.16 (2.40-4.18)	3.6	31.5	154
Oxymorphazone	0.76 (0.54-1.1)	(1.7-3.5)	3.8	34.4	273
Buprenorphine HCl	0.035 (0.028-0.045)	0.09 (0.07-0.12)	3.5	42.7	191

Figure 1 shows representative cumulative dose-effect curves for morphine, oxymorphazone, and buprenorphine from a single subject. All three drugs occasioned drug-appropriate responding in a dose-related manner, and at large doses suppressed food-reinforced responding completely. Greater than 90 percent of the trial responses were made on the drug-appropriate key at a cumulative dose of 0.32 mg/kg buprenorphine, 10.0 mg/kg morphine, or 1.0 mg/kg oxymorphazone. With increasing doses, subjects continued to respond on the morphine-appropriate key up to a suppressing dose of 32.0 mg/kg oxymorphazone. 56.0 buprenorphine, or 100.0 mg/kg morphine. Thus, oxymorphazone was 10 times, and buprenorphine 30 times, more potent than

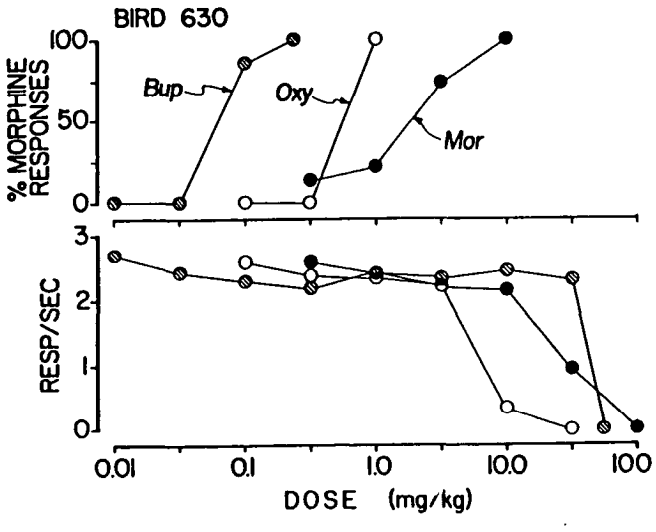

Figure 1. Dose-effect curves for cumulative doses of morphine (●) oxymorphazone (○), and bupernorphine (◍) in a pigeon trained to discriminate 5.6 mg/kg morphine from saline.

morphine as discriminative stimuli; in suppressing food-reinforced responding oxymorphazone and buprenorphine were approximately 2-3 times more potent than morphine. A much wider range of doses occasioned drug-appropriate responding without completely suppressing behavior for buprenorphine (0.32-32.0 mg/kg) than was the case for morphine (10.0-32.0 mg/kg) or oxymorphazone (1.0-10.0 mg/kg).

Naltrexone, administered 10 minutes prior to a test, antagonized the discriminative effects of morphine, oxymorphazone and buprenorphine. Small doses of naltrexone (0.01 and 0.1 mg/kg) shifted the morphine dose-effect curve approximately 1/2 log unit to the right. The oxymorphazone and buprenorphine dose-effect curves were shifted slightly to the right following 0.01 mg/kg naltrexone; however the dose necessary to occasion complete (greater than 90%) drug-appropriate responding was unchanged. Following a pretreatment injection of 0.1 mg/kg naltrexone, the oxymorphazone and buprenorphine curves were shifted in parallel to the right 1 full log unit. The largest dose of naltrexone (1.0 mg/kg) completely antagonized the discriminative effects of both morphine and oxymorphazone, in that subjects continued to 'respond on the saline-appropriate key up to a suppressing dose. In contrast to this apparent insurmountable antagonism, 1.0 mg/kg naltrexone caused a 100-fold shift to the right in the buprenorphine dose-effect curve, with all subjects responding on the morphine-appropriate key at a cumulative dose of 32.0 mg/kg buprenorphine. The surmountability of the antagonism with buprenorphine, but not with morphine or oxymorphazone, may be due to the wider range of doses for buprenorphine that occasion responding on the drug key without suppressing rates. The rate-decreasing effects of all three test drugs were not significantly altered by naltrexone pretreatments.

The smallest acute dose of each drug that occasioned responding on the drug key following a 10-minute preteatment period, and continued to produce drug-appropriate responding for the duration of the experimental session (1.5-2 hours), was 5.6 mg/kg morphine, 1.0 mg/kg oxymorphazone or 0.32 mg/kg buprenorphine. When naltrexone was administered on subsequent trials, it dose-dependently antagonized the discriminative effects of morphine and oxymorphazone. In the presence of 5.6 mg/kg morphine or 1.0 mg/kg oxymorphazone, a cumulative dose of 0.32 mg/kg naltrexone completely antagonized the drug cue in all subjects. The discriminative effects of buprenorphine however were not completely reversed by a dose of naltrexone 30 times greater than the effective dose in reversing morphine or oxymorphazone. Despite a cumulative dose of 10.0 mg/kg naltrexone , all but 1 of the subjects made greater than 90 percent of the trial reponses on the morphine-appropriate key. Figure 2 presents the data from a single subject showing naltrexone reversibility of an acute injection of morphine, oxymorphazone and buprenorphine.

Oxymorphazone displayed a slightly longer duration of action than morphine, as determined by drug-appropriate responding and rate suppression on days following a cumulative suppressing dose.

While no discriminative or rate effects of morphine were evident 24 hours after a suppressing dose of 100.0 mg/kg, the day after 32.0 mg/kg oxymorphazone approximately 70 percent of the responses were on the morphine-appropriate key. By 48 hours, however, the discriminative effects of oxymorphazone were no longer present and subjects once again responded on the saline key. Buprenorphine had a duration of action that was significantly longer than either morphine or oxymorphazone. Following a cumulative suppressing dose of buprenorphine, subjects continued to respond on the morphine-appropriate key for up to 5 days (mean=2.6 days).

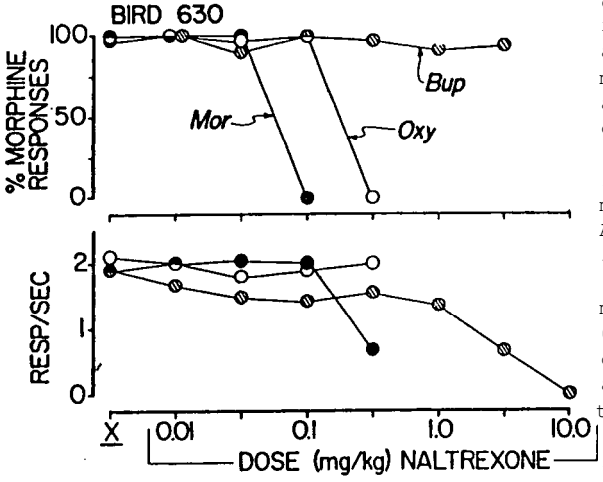

Figure 2. Dose-effect curves showing the reversibility of an acute injection of morphine, oxymorphazone or buprenorphine in a pigeon trained to discriminate 5.6 mg/kg morphine from saline. A single injection of 5.6 mg/kg morphine (●), 1.0 mg/kg oxymorphazone (○), or 0.32 mg/kg buprenorphine (◍) was administered prior to the first trial (X), followed by increasing doses of naltrexone on subsequent trials.

Buprenorphine failed to antagonize either the discriminative or rate effects of morphine. A wide range of doses (0.01-10.0 mg/kg) of buprenorphine were administered either 10 minutes or 24 hours before morphine, and in no case did the morphine dose-effect curve differ significantly from control determinations.

DISCUSSION

Duration of action in the mouse hot plate test was longest for oxymorphazone, shortest for morphine, and intermediate for buprenorphine. The duration of analgesia for oxymorphazone was 273 minutes, which is far less than the 24-48 hour duration of action reported by others (Galetta et al., 1982; Pasternak & Hahn, 1980). These investigators, however, used doses 130-260 times greater than the ED50 for oxymorphazone as determined in the present experiment. Thus, when equipotent doses were compared, the

duration of action for oxymorphazone was slightly less than twice that of morphine.

Oxymorphazone and buprenorphine displayed morphine-like actions in pigeons trained to discriminate morphine from saline. While there was no evidence of oxymorphazone acting as an irreversible agonist, the results with buprenorphine satisfy one criterion for irreversible agonists: the acute effects were prevented but not reversed by naltrexone.

The behavioral profile of oxymorphazone in this experiment does not establish it as a unique pharmacological agent. In comparison to morphine, oxymorphazone had a longer duration of action in the mouse and the pigeon, and was approximately 10 times more potent as a discriminative stimulus. The acute actions of oxymorphazone as a discriminative stimulus were both prevented and reversed by naltrexone, clearly demonstrating that it does not act irreversibly in the pigeon.

Once a discriminative effect was established with buprenorphine, it was not completely reversed by very large doses of naltrexone. Others (Cowan & Watson, 1980; Cowan et al., 1977a) have reported difficulty in reversing the acute effects of buprenorphine, and in subjects treated chronically with buprenorphine, opiate antagonists fail to precipitate withdrawal (Cowan et al., 1977b; Jasinski et al., 1978). Thus, while buprenorphine clearly acts via an opiate mechanism, as evidenced by parallel shifts in discriminative dose-effect curve after naltrexone pretreatments, it does so irreversibly.

Duration of action of the magnitude demonstrated for buprenorphine in the present experiment is a novel finding in behavioral pharmacology. After a cumulative suppressing dose of buprenorphine, pigeons continued to respond on the morphine-appropriate key for 24-120 hours; whereas, following suppressing doses of morphine or oxymorphazone, subjects were responding on the saline key within 48 hours, A long duration of action has been reported for buprenorphine in the rat, monkey, and man (Cowan et al., 1977b; Jasinski et al., 1978). Leander (1983) has reported that in pigeons trained to discriminate fentanyl from vehicle, 10.0 mg/kg buprenorphine results in 75% of subjects selecting the fentanyl-appropriate key 48 hours later. In opiate receptor binding (Hambrook & Rance, 1976; Rance & Dickens, 1978), and in isolated smooth muscle preparations (Kosterlitz et al., 1975), buprenorphine has been shown to have a much slower dissociation constant than morphine. The extremely slow pharmacokinetics of buprenorphine are thought to be responsible for the long delay, or even absence, or withdrawal signs upon termination of chronic treatment with buprenorphine (Cowan et al., 1977b; Jasinski et al., 1978).

No ceiling effect or bi-phasic dose-effect curve, suggestive of a partial agonist, was obtained with buprenorphine in these experiments. Buprenorphine also failed to antagonize either the

discriminative or rate-suppressing effects of morphine. While some investigators have shown buprenorphine to antagonize the effects. of morphine on schedule-controlled behavior (Leander, 1983), others have reported no antagonism (Dykstra, in press). In this experiment, both naltrexone and buprenorphine failed to antagonize the rate-suppressing effects of morphine. This negative result suggests that these animals are tolerant to the rate-decreasing effects of morphine, and thus under these conditions, morphine may suppress responding by a non-opiate mechanism. Narcotic-naive pigeons, responding on a schedule of food reinforcement similar to that used in the present experiment, are usually suppressed by 10.0 mg/kg morphine; pretreatment with 1.0 mg/kg naltrexone shifts the response rate dose-effect curve 1 full log unit to the right (unpublished observation). In these same animals, a wide range of doses of buprenorphine (0.1-10.0 mg/kg) antagonize the rate-suppressing effects of morphine. However, in both cases the shift to the right is no further than the control curves determined in the present study, Thus, tolerance to the rate-decreasing effects of morphine, in pigeons receiving a training drug on a regular basis, may account for the lack of antagonism by naltrexone and buprenorphine of this effect.

This comparison between morphine, oxymorphazone, and buprenorphine clearly shows that in the pigeon, buprenorphine has irreversible agonist actions; oxymorphazone, on the other hand, did not differ significantly in its profile of action from morphine, and therefore cannot be considered a prototypic irreversible agonist in the pigeon. The evidence in support of oxymorphazone acting as an irreversible agonist, has in large part, come from in vitro experiments. Thus, these data exemplify the problems involved in attempting to predict a behavioral response in vivo from in vitro data. Finally, these experiments demonstrate the application of drug discrimination procedures to the study of irreversible pharmacological agents, in such a way that the reversibility of a drug can be evaluated within a single experimental session.

ACKNOWLEDGEMENT

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REFERENCES AND ADDITIONAL DATA

A full set of references is available from the first author. In addition, a complete description of the observations referred to above is available from the first author.

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Morphine Abstinence Syndrome: Cholinergic Mechanisms in the Ventral Periaqueductal Gray of the Dog

Lawrence G. Sharpe and Wallace B. Pickworth

Central cholinergic systems appear to be involved in the opiate abstinence syndrome. Morphine decreases, whereas naloxone increases, acetylcholine (ACn) release in peripheral and central tissues (Domino and Wilson 1973; Jhamandas et al. 1971; Paton 1957; Waterfield and Kosterlitz 1975; Yaksh and Yamamura 1977). The cholinomimetic, physostigmine, potentiates abstinence signs in the morphine-dependent rat (Jhamandas et al. 1973) and mimics the morphine abstinence syndrome in the nondependent chronic spinal dog (Martin and Eades 1967; Wikler and Frank 1948). Atropine suppresses some manifestations of the morphine abstinence syndrome in the rat (Collier et al. 1972; Jhamandas et al. 1973; PinSky et al. 1973) and dog (Martin and Eades 1967). But results concerning the effectiveness of atropine to diminish opiate withdrawal signs are conflicting (Karczmar 1976; Way and Bhargava 1976). One problem is that atropine alone, through its peripheral action, produces some manifestations of the morphine abstinence syndrome, such as mydriasis, tachypnea and tachycardia. To evaluate central cnolinergic processes in the opiate abstinence syndrome we microinjected either carbachol, morphine, methylatropine or naloxane into the periaqueductal gray (PAG) and adjacent regions of 6 nondependent and 1 morphine-dependent dogs. Our purpose was to compare Carbachol-elicited responses with those elicited by morphine withdrawal and naloxone in the morphine-dependent dog, to map the distribution of carbachol-elicited responses in the PAG region and to test whether atropine could reduce any of the withdrawal-like signs through its action in the PAG and surrounding sites.

METHODS

Six beagle dogs (9-13 kg) were selected for their ability to adapt to a sling restraint. All procedures for surgery, microinjections, response measurements, data analysis and histology are described in detail elsewhere (Sharpe and Pickwort:. 1981).

The data far pupillary diameter, respiratory rate, pulse rate and temperature were analyzed by paired t tests in which the areas under the time-action curve (1 hr) for the different drug and control conditions were compared. All Other responses (shiver, activity, lick, whine, rhinorrhea, salivate, yawn and pant) were combined as one score for each animal by adding the

number of 15-min periods in which the response occurred. A score was assigned if the response was different from baseline and occurred in at least half of the 15-min time period. For example, salivation was given a maximum score of 4 if it was absent during baseline but was present during 50% or more of the time in the four 15-min time periods (1 hr). A maximum score of 12 could be assigned to shiver and activity because of the rated intensities from 0 to 3.

One animal (site 12, Fig. 1) was made dependent on 7 mg/kg/day morphine for 175 days. Except on test days, this animal received two subcutaneous injections of morphine *every* day; one at 0800 hr (2.3 mg/kg) and one at 1600 hr (4.7 mg/kg). The microinjection procedure (Sharpe and Pickworth 1981) was used as preliminary experiments to test whether methylatropine (1-3.µg) could antagonize any withdrawal signs precipitated by naloxone injected either intravenously or into site 12 (Fig. 1).

RESULTS

Fig. 1 snows that microinjections of carbacnol evoked several responses, the most susceptible being shiver or tremor, followed in order by mydriasis, tachypnea, tachycardia, licking, salivation, EEG signs of arousal, behavioral activation and temperature. Response susceptibility to carbachol was determined by ranking responses based on frequency, magnitude and. duration following the 3 doses of carbachol (0.05, 0.1 and 0.25 µg). The active carbachol sites (●) were clustered primarily in the ventral PAG (sites 4, 5, 8, 9, 10, 11, 12). Inactive Carbachol sites (○) included tne third ventricle (site l), dorsomedial PAG (site 2), dorsolateral PAG (sites 3, 7) and the ventral tegmental area (site 13). Microinjections of methylatropine (1 µg) significantly antagonized the responses evoked by 0.1 µg carbachol (Table 1). Microinjections of morpnine (5 µg) into the 4 ventral PAG sites (sites 4, 8, 9, 11, Fig. 1) produced a general depression in behavior, which, except for licking, appeared opposite in direction of that stimulated by carbachol.

In preliminary experiments on the morphine-dependent dog, we precipitated abstinence with several doses of either intravenous or microinjected naloxone at different times after the last morphine dose. At 19 to 21 hr after the last morphine dose, naloxone produced equivalent amounts of mydriasis when injected intravenously (33 µg/kg) and into site 12 (5 µg; Fig. 2). The 5 µg naloxone dose elicited no Other sign of witndrawal, whereas intravenous naloxone (33 µg/kg) elicited several typical abstinence signs that were equivalent to those caused by carbachol microinjections. Microinjections of metnylatropine (1 µg in 1 µl) antagonized the mydriasis precipitated by naloxone given by both routes of administration (Fig. 2). However, other abstinence signs produced by intravenous naloxone (33 µg/kg) were not antagonized by metnylatropine microinjections.

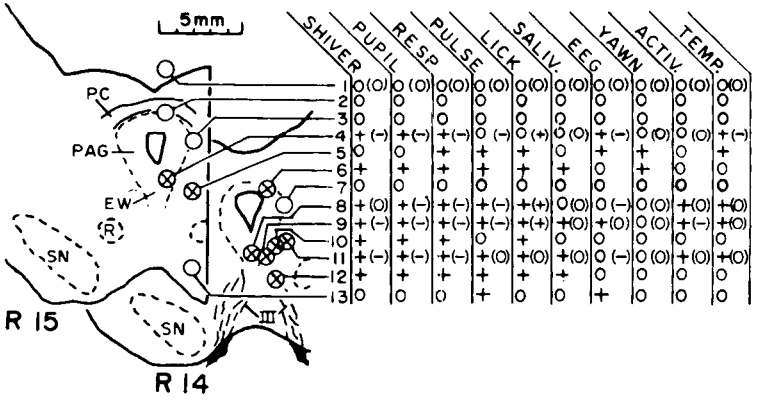

Fig. 1. Results from 6 dogs receiving a total of 31 microinjections of carbachol (0.05, 0.1 and 0.25 µg) into 13 sites at 2 levels of the rostral midbrain (R 14 and R 15, Lim et al. 1960). Responses are ordered from the most (shiver) to the least (temperature) sensitive in being stimulated, by carbachol (indicated by +). Except for lick, morphine (5 µg, in parentheses.) depressed (-) most of the responses that carbachol stimulated. Abbreviations: EW--Edinger-Westphal nucleus; PAG--periaqueductal gray; PC--posterior commissure; R--red nucleus; SN--substantia nigra; III--oculomotor nerve; 8--carbachol sensitive site; O--carbachol insensitive sites; RESP.--respiratory rate; SALIV.--salivation; EEG--+ denotes EEG signs of arousal and (-) denotes EEG signs of sedation; ACTIV.--Behavioral activation; TEMP.--colonic temperature.

Table 1. Responses produced by carbachol (0.1 µg in 0.5 µl) microinjected into the ventral PAG region of 4 dogs (sites 8, 9, 11, 12; Fig. 1) were antagonized by methylatropine (1 µg/µl in 1.0 µl) microinjected 30 min before carbachol.

RESPONSE	SALINE	CARBACHOL	METHYLATROPINE CARBACHOL
PUPILLARY DIAMETER (mm-min)[1]	2	129*	7**
RESPIRATORY RATE (breaths-min)1	37	2580*	173**
PULSE RATE (beats-min)[1]	61	849*	127**
OTHER RESPONSES ($\bar{x}$ score)[2]	3	14*	6**

[1]Areas under the time-action curve for 1 hr.

[2]Other responses (shiver, salivate, yawn, rhinorrhea, lick, pant, whine and activity) represent number of 15-min periods in which responses occurred. *$P < 0.05$ compared with saline. **$P < 0.05$ compared with carbachol.

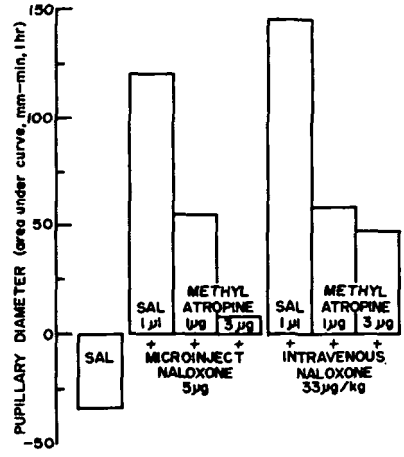

Fig. 2. Methylatropine (1 and 3 µg microinjected into site 12, Fig. 1, 30 min before haloxone) antagonized the mydriasis produced by naloxone either microinjected (MICRO-INJECT) into site 12, or injected intravenously in the morphine-dependent dog (N = 1).

DISCUSSION

Several years ago Paton (1969) and Crossland (1972) proposed that the morphine abstinence syndrome was caused in part by a sudden increase in the release of ACh. In this study, the striking parallelism between signs observed during microinjections of carbachol and those observed during morphine abstinence in the dog (Martin et al. 1974) promotes the proposal that increased ACh output in the PAG region may lead to a major portion of the morphine withdrawal syndrome. That microinjections of morphine produced opposite reactions supports this proposal because morphine has been shown to inhibit ACh release (Jhamandas et al. 1973; Paton 1957). The effects of 5 µg morphine microinjected in the ventral PAG resemble the effects produced by an i.v. dose of 1 mg/kg morphine--an amount 2,000 times the microinjection dose (Pickworth and Sharpe 1979).

In the morphine-dependent dog, naloxone, injected either intravenously or into site 12 (Fig. 1), produced mydriasis--one of the principal withdrawal signs in the dog (Martin et al. 1974). Since pretreatments with microinjections of methylatropine antagonized both instances of naloxone-precipitated mydriasis, it is reasonable to argue that mydriasis during withdrawal resulted from an increased release of ACh in this region. Given systemically, atropine would potentiate this mydriasis. The reason that changes in pupillary diameter were easily affected by microinjections in the ventral PAG is most likely because the Edinger-Westphal nucleus (EW) is located 0 to 2 mm from the sites yielding changes in pupillary diameter after carbachol and morphine (Fig. 1). A simple and testable neuronal model to explain morphine miosis and carbachol- or withdrawal-induced mydriasis is schematized in Fig. 3.

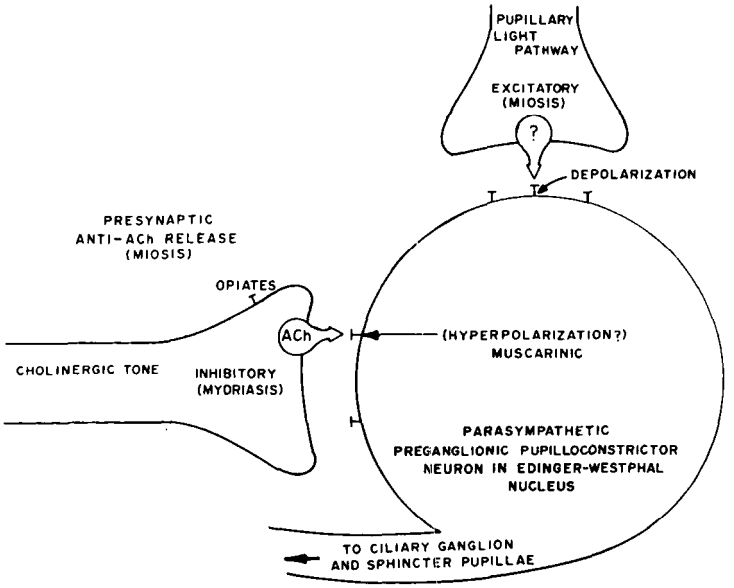

Fig. 3. Edinger-Westphal nucleus (EW) neuronal model. An increase in the muscarinic inhibitory tone to the EW (mydriasis) is caused by either carbachol microinjections or morphine abstinence (increased ACh release), perhaps through a mechanism of postsynaptic hyperpolarizing inhibition. A decrease in inhibitory tone (miosis due to increased activation of the excitatory pupillary light reflex path) is produced by morphine, perhaps through a mechanism of inhibiting presynaptically ACh release.

We found that, like morphine, clonidine (5 µg) produced miosis when microinjected in the ventral PAG of the dog (unpublished observations). The presynaptic action of morphine and clonidine to inhibit neurotransmitter release in multiple neuronal systems (see Vizi 1979) may partially explain the similar pharmacologic profiles of these two drugs in the dog (Pickworth and Sharpe 1979) and why clonidine suppresses opiate withdrawal in humans (Gold et al. 1978). We can argue that different but functionally redundant receptors exist at presynaptic sites if opioid and nonopioid drugs inhibit neurotransmitter release as a major function in their mechanism of action. Such redundant receptors would include the µ receptors (morphine), the α_2-adrenoceptors (clonidine) and more recently, the adenosine receptors (see Tucker this volume). Thus nonopioid drugs acting presynaptically as morphine does would be expected to be effective in the clinical management of opiate detoxification. Conversely, selective antagonists (i.e., atropine) that act postsynaptically on single neurotransmitter systems would be less effective in suppressing morphine withdrawal signs, especially if they produce mixed or opposite effects depending on their site of action in the central and peripheral nervous system. The preliminary evidence on atropine in this report lends support to this view.

When microinjected in the PAG of the morphine-dependent dog, naloxone failed to elicit withdrawal signs except for mydriasis and methylatropine failed to antagonize naloxone (i.v.) precipitated withdrawal signs except for mydriasis. However interesting and puzzling these results may be, reasonable explanations without further experimentation on more dogs would be highly speculative and premature. A "multipartite" basis for morphine dependence has been proposed (Collier et al; 1972) in which several neurochemical systems participate. The method of microinjecting opiates and transmitter agonists/antagonists into discrete brain regions is an attempt to understand these different "parts" of morphine dependence by elucidating how specific neurotransmitters in a given brain locus control one or more withdrawal signs.

REFERENCES

Collier, H.O.J., Francis, D.L., and Schneider, C. Modification of morphine withdrawal by drugs interacting with humoral mechanisms: some contradictions and their interpretation. Nature (Lond), 237: 220-223, 1972.

Crossland, J. Acetylcholine and morphine dependence. In: Kosterlitz, H.W., Collier, H.O.J., and Villarreal, J.E., eds. Agonist and Antagonists Actions of Narcotic Analgesic Drugs. London: Macmillan, 1972. pp. 232-234.

Domino, E.F., and Wilson, A.E. Effects of narcotic analgesic agonists and antagonists on rat brain acetylcholine. J Pharmacol Exp Ther, 184:18-32, 1973 .

Gold, M.S., Redmond, D.E., and Kleber, H.D. Clonidine blocks acute opiate-withdrawal symptoms. Lancet, 1:599-601, 1978.

Jhamandas, K., Phillis, J.W., and Pinsky, C. Effects of narcotic analgesics and antagonists on the in vivo release of acetylcholine from the cerebral cortex of the cat. Brit J Pharmacol, 43:53-66, 1971.

Jhamandas, K., Sutak, M., and Bell, S. Modification of precipitated' morphine withdrawal syndrome by drugs affecting cholinergic mechanisms. Eur J Pharmacol, 24:296-305, 1973.

Karczmar, A.G. Central actions of acetylcholine, cholinomemetics, and related drugs. In: Goldberg, A.M., and Hanin, I., eds. Biology of Cholinergic Function. New York: Raven Press, 1976. pp. 395-449.

Lim, R.K.S., Liu, C.-N., and Moffitt, R.L. A Stereotaxic Atlas of the Dog's Brain. Springfield, Ill.: Charles C. Thomas, 1960.

Martin, W.R., and Eades, C.G. Pharmacological studies of spinal cord adrenergic and cholinergic mechanisms and their relation to physical- dependence on morphine. Psychopharmacology, 11:195-223; 1967.

Martin, W.R., Eades, C.G., Thompson, W.O., Thompson, J.A., and Flanary, H.G. Morphine physical dependence in the dog. J Pharmacol Exp Ther, 189:759-771, 1974.

Paton, W.D.M. The action of morphine and related substances on contraction and on acetyicholine output of coaxially stimulated guinea-pig ileum. Brit J Pharmacol, 12:119-127, 1957.

Paton, W.D.M. A pharmacological approach to drug dependence and drug tolerance. In: Steinberg, H., ed. Scientific Basis of Drug Dependence. London: J. & A. Churchill, 1969. pp. 31-47.

Pickworth, W.B., and Sharpe, L.G. EEG-behavioral dissociation after morphine- and cyclazocine-like drugs in the dog: further evidence for two opiate receptors. Neuropharmacology, 18:617-622, 1979.

Pinsky, C., Frederickson, R.C.A., and Vazquez, A.J. Morphine withdrawal syndrome responses to cholinergic antagonists and to a partial cholinergic agonist. Nature (Lond), 242:59-60, 1973.

Sharpe, L.G., and Pickworth, W.B. Pharmacologic evidence for a tonic muscarinic inhibitory input to the Edinger-Westphal nucleus in the dog. Exp Neurol 71:176-190, 1981.

Waterfield, A.A., and Kosterlitz, H.W. Stereospecific increase by narcotic antagonists of evoked acetylcholine output in guinea-pig ileum. Life Sci, 16:1787-1792, 1975.

Way, E.L., and Bhargava, H.N. Assessment of the role of acetylcholine in morphine analgesia, tolerance and physical dependence. In: Ford, D.H., and Clouet, D.H., eds. Tissue Responses to Addictive Drugs. New York: Spectrum Publications, 1976. pp. 237-253.

Vizi, E.S. Presynaptic modulation of neurochemical transmission. Prog Neurobiol, 12:181-290, 1979.

wikler, A., and Frank, K. Hindlimb reflexes of chronic spinal dogs during cycles of addiction to morphine and methadon. J Pharmacol Exp Ther, 194:382-400, 1948.

Yaksh, T.L., and Yamamura, H.I. Depression by morphine of the resting and evoked release of [^{3}H]-acetylcholine from the cat caudate nucleus in vivo. Neuropharmacology, 16:227-233, 1977.

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The Discriminative Stimulus Properties of Cocaine and d-Amphetamine: A Comparison of Three Routes of Administration

Rene de la Garza, C. E. Johanson, and C. R. Schuster

The placement of new drugs into pharmacological classes is based on a comparison of the spectrum of action of a test drug with prototypic standards. One method designed to classify psychoactive drugs measures their subjective effects in humans. For instance, studies have demonstrated that morphine and related compounds produce a unique spectrum of subjective effects that can be reliably discriminated from subjective effects produced by other classes of psychoactive drugs (Goldberg et al., 1982). Other studies have shown that the methods for measuring the subjective effects of drugs are also useful for the classification of other abused substances (e.g., psychomotor stimulants, sedative-hypnotics). In recent years, drug discrimination procedures in animals have been used as an alternative method to compare the spectrum of action of drugs. A striking concordance has been found between drug classes obtained on the basis of similarities in subjective effects and those obtained on the basis of similarities as discriminative stimuli. These findings have led to the suggestion that the components of drug action responsible for discrimination among classes by animals are the same as those responsible for the subjective effects reported by humans (Schuster et al., 1981).

In drug discrimination procedures, reinforcement is made contingent upon the presence of a drug or its vehicle so that differential responding is maintained under stimulus control. When doses lower than the training dose are administered under test conditions, they produce a lower proportion of drug-appropriate responding, i.e., a generalization gradient is obtained. Furthermore, when drugs other than the training drug are substituted, they produce drug-appropriate responding to the extent that they resemble the training drug, i.e., there is drug class specificity. Drug discrimination studies can as well be used to compare the spectrum of action of the same drug or related drugs administered by different routes. Such comparisons may be especially useful for evaluating a drug such as cocaine which presumably has relatively little effect when given orally, or amphetamine which is used therapeutically by the oral route but often abused parenterally.

The purpose of the present experiment was to compare the discriminative stimulus properties of cocaine and amphetamine given orally and parenterally in rhesus monkeys using an avoidance/escape procedure

(Schaefer and Holtzman, 1977; Shannon and Holtzman, 1976, 1977). Rhesus monkeys were first trained to discriminate intramuscular cocaine from saline, and generalization gradients for cocaine administered by different routes (intramuscular, intravenous, intragastric) were determined during test sessions. In addition generalization gradients for c&amphetamine delivered intramuscularly or intragastrically were also determined.

METHODS

Subjects. Five rhesus monkeys were used in this study. The subjects were between 8 and 10 kg and were housed in stainless steel cages. They were given ad lib water and were fed approximately 180 g immediately after each experimental session. All subjects had previous experimental histories in self-administration and schedule-controlled studies with a variety of psychoactive drugs.

Apparatus. During experimental sessions, the subjects were seated in Plas-Lab primate restraining chairs and placed in a cubicle containing two response levers and stimulus lights. In order to decrease the likelihood of responding on both levers simultaneously, a Plexiglas plate was attached to the chair perpendicular to the body of the animal. The monkey's feet were placed into shoes fitted with brass plates that allowed electric shocks to be delivered to the feet.

Avoidance/escape training phase. The monkeys were initially trained to avoid/escape the delivery of electric shocks in a trial procedure with only the right lever present. Trials were initiated with the illumination of a houselight and the lights above the lever. Five seconds after the initiation of the trial, a shock period began. During this period shocks were delivered every 2 sec (250 msec in duration, 10 mA in intensity) until a response occurred (escape). Immediately after a response occurred, the houselight, lever lights and shock deliveries were terminated. If a response occurred before the 5-sec period had elapsed, the houselights and lever lights were terminated and no shocks were delivered (avoidance). In both cases, a 55-sec inter-trial interval (ITI) followed before a new trial was initiated. During the ITI the cubicle remained dark. The session lasted until 30 trials were completed or until 40 min had elapsed, whichever came first. When the avoidance response occurred on more than 90% of the trials for 3 consecutive days, the left lever was made operational and the right lever was covered. The same contingencies remained in effect until 90% of the trials were. avoidance trials for 3 consecutive days. Thereafter, the operational lever was changed daily. Concurrently, appropriate drug injections were administered 10 min prior to the session. The right lever was operative after the administration of 0.25 mg/kg cocaine (i.m.) for two of the monkeys and the left lever for the other three. The alternative lever was operative after saline. These conditions remained in effect until 5 consecutive days with more than 90% avoidance trials were obtained. Initially, the front of the chair was placed flush with the lever boxes. However, in order to decrease the probability of responding on both levers simultaneously, the distance between the chair and the lever boxes was increased so that the monkey had to extend its arm to depress the lever.

Drug discrimination phase. During this phase the subjects were presented with both levers simultaneously for the first time. The lever that terminated the trial was made conditional upon cocaine or saline administration. All other conditions were the same as previously described. If a correct response occurred, i.e., a response on the lever associated with the drug condition, the trial was terminated. If the monkey responded on the incorrect lever, a change-over delay (COD) period was started. During this period, responding on the correct lever did not terminate the trial until 2 seconds had elapsed since the last incorrect response (COD 2 sec). Further incorrect responses reset the COD timer. Correct responses during the COD period were signalled by a brief flicker of the houselight and lever lights. A trial was counted as correct when no incorrect responses were emitted.

An additional procedure was introduced during this phase to facilitate discrimination training. The intertrial interval was reduced to a few milliseconds at the start of the training session but all other contingencies remained the same. After several responses on the correct lever without incorrect responses, the intertrial was increased during the session in small steps (approximately 5 to 10 sec at a time) provided the subjects continued responding on the correct lever. This procedure was continued until the subject's performance remained above 90% over several sessions with the' interval reaching 55 sec for at least a portion of the session. Subsequently, the monkeys were reintroduced to the initial condition in which the intertrial interval was 55 sec throughout the entire session.

Initially, cocaine and saline injections alternated on a daily basis. When five consecutive sessions under the terminal contingencies occurred with more than 90% correct, the drug conditions were presented in a semirandom sequence. All other conditions remained the same.

Testing. When six consecutive sessions of more than 90% correct trials were obtained, the ability of other doses of cocaine administered by the intramuscular, intravenous or intragastric route to control cocaine-appropriate responding was determined during test sessions. Similar determinations were done with intramuscular and intragastric d-amphetamine. When these drugs were tested intragastrically, the solution was generally given 60 min prior to the session. For the other routes, drugs were administered 10 min pre-session. During testing conditions, responding on either lever terminated the trial. All other conditions remained the same. Each dose of a drug was given twice by each route. All doses of a compound were tested before a new route or drug was tested. Test sessions occurred no more frequently than twice a week and at least 2 training sessions (i.e., only one lever operative) intervened.

Data Analysis. The percentage of cocaine lever trials was used as a measure of drug substitution. The cumulative latency to the termination of the trial was used as measure of non-specific effects.

RESULTS

Figure 1 shows that cocaine administered intramuscularly to five monkeys (0.01 - 0.5 mg/kg) or intravenously to four monkeys (0.03 - 0.125 mg/kg) produced cocaine-appropriate responding in a dose-dependent manner. Regardless of route, the drug did not significantly increase the latency

to the first response compared to saline. Likewise, intragastric cocaine (1.0 - 16.0 mg/kg) produced cocaine-appropriate responding when administered 60 min pre-session. In one monkey tested with intragastric cocaine with a 10 min pretreatment (not shown), the same dose range led to saline-appropriate responding while the latency to the first-response remained unaffected.

d-Amphetamine delivered intramuscularly to three monkeys (0.03 - 0.25 mg/kg) or intragastrically to four monkeys (0.03 - 0.5 mg/kg) produced cocaine-appropriate responding in a dose dependent manner at doses that did not increase the latency to the first response.

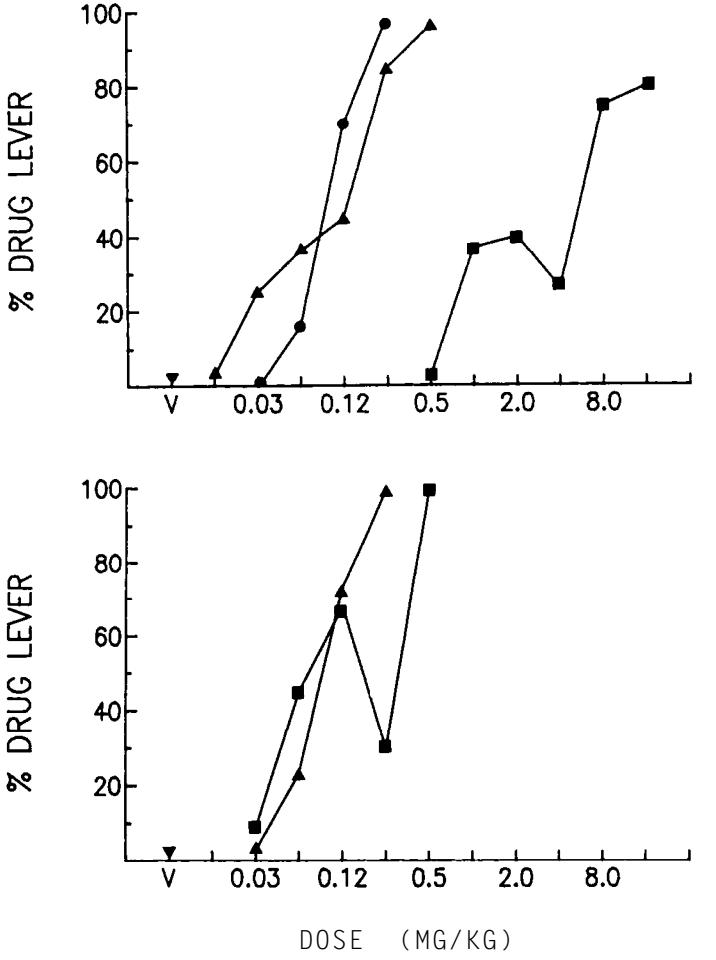

FIGURE 1. The percent of drug lever trials during test sessions with cocaine (top panel) and d-amphetamine (lower panel) averaged across all monkeys tested. Symbols indicate the route of administration: (▲) i.m.; (■) i.g.; and (●) i.v. All i.m. and i.v. administrations were given 10 min pre-session and the i.g. administrations were given 60 min pre-session.

Responding following both i.g. cocaine and d-amphetamine was more variable both within and across monkeys than when the other routes were tested. One monkey given 16 mg/kg of i.g. cocaine responded only 30% on the cocaine-appropriate lever but died unexpectedly of convulsions at least 8 hrs after the administration. A second monkey tested with 8 mg/kg cocaine responded only 47% on the cocaine-appropriate lever, but died 2 days later of kidney and liver complications.

Table 1 shows the ED50's of all the dose-response functions and the ratio of each over the ED50 of i. m. cocaine. Intravenous cocaine and both intramuscular and intragastric &amphetamine were not significantly different from i.m. cocaine in potency. In contrast, intragastric cocaine was approximately 40 times less potent than i.m. cocaine. Significantly, i.m. d-amphetamine was not different from i.m. cocaine whereas i.g. cocaine dramatically differed from i.g. &amphetamine.

TABLE 1: RELATIVE POTENCIES

DRUG	ROUTE	ED 50 (C.L.) IN MG/KG	RATIO
D-AMPHETAMINE	I.M.	0.09 (0.07-0.11)	0.9
D-AMPHETAMINE	I.G.	0.12 (0.02-4.9)	1.2
COCAINE	I.M.	0.10 (0.06-0.14)	1.0
COCAINE	I.V.	0.10 (0.08-0.13)	1.0
COCAINE	I.G.	4.37 (2.1-14.8)	43.7*

DISCUSSION

With cocaine there is a clear separation in potency between the intragastric route as compared to the i.m. and i.v. route of administration in the ability to produce drug-appropriate responding. Intragastric cocaine is at least 40 times less potent than i.m. cocaine. In addition, the data from one monkey suggest that intragastric cocaine is ineffective when given 10 min prior to a 30-min session. In rhesus monkeys, similar effects of cocaine have been reported in studies that compared the effects of different routes of administration on behavior maintained under a variable-interval schedule. Downs et a1.(1980) showed that oral cocaine was 16 times less potent than i.m. and i.v. cocaine.

In humans, van Dyke et al. (1978) showed that orally administered cocaine produced high cocaine plasma levels and subjective effects only 30 minutes after its delivery. These authors suggested that the early belief that cocaine was ineffective through the oral route (Ritchie and Cohen, 1975) may have been surmised from experiments that did not allow enough time for cocaine to pass from the highly acidic stomach to the small intestine where absorption of bases is more rapid. Similarly, the results in the present experiment can be explained by the low absorption rate of cocaine in the stomach, since approximately 1 hour had to pass before the discriminative stimulus effects were detectable.

d-Amphetamine was also tested by Downs et al. (1980) using different routes of administration and it was found that both i.m. and oral d-amphetamine produced similar dose-dependent changes. Likewise, in the present experiment i.m. and i.g. d-amphetamine were equipotent even though by the i.m. route drug was administered 10 min before the session and by the i.g. route, 60 min before the session.

REFERENCES AVAILABLE ON REQUEST

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Alcohol Self-Administration by Female Primates: Effects on Reproductive Function

N. K. Mello, M. P. Bree, J. H. Mendelson, and J. E. Ellingboe

Alcoholism in women is often associated with derangements of reproductive function. Amenorrhea, infertility (anovulatory cycles and short luteal phases) as well as spontaneous abortions are reported in the clinical literature (Harlap and Shiono, 1980; Hugues et al, 1980; Moskovic, 197.5; Ryback, 1977). The mechanism of amenorrhea in alcoholic women is unknown, and it is not clear if the toxic effects of alcohol occur primarily at the hypothalamic-pituitary level (Hugues et al, 1980); at the ovary (Valimaki and Ylikahri, 1981; Moskovic, 1975) or both in combination. Since alcoholic women are often malnourished and also have liver disease, it has been difficult to determine the relative contribution of alcoholism and these related disorders to the derangements of reproductive function observed clinically. Either malnutrition associated with profound weight loss (Frisch and MacArthur, 1974) or hepatic dysfunction can disrupt menstrual cycle regularity (Hugues et al, 1980). These clinical findings indicated that systematic evaluation of alcohol effects on female reproductive function under controlled conditions would require a model of alcoholism.

Since the reproductive physiology of female rhesus monkeys is most similar to human women, and the neuroendocrine regulation of primate reproductive function has been studied extensively (Knobil, 1974, 1980, 1981; Pohl and Knobil, 1982), we chose this species to develop a female model of alcoholism. The feasibility of using rhesus monkeys for alcohol self-administration studies (Woods et al, 1971; Winger and Woods, 1973; Woods and Winger, 1974; Karoly et al, 1978), as well as self-administration of other drugs (cf. Griffiths et al, 1980; Johanson and Schuster, 1981), has been well established.

This report describes the effects of chronic alcohol self-administration on reproductive fiction in female Macaque monkeys. We have previously shown that acute administration of alcohol (1.5, 2.5, 3.5 g/kg) that produced blood alcohol levels averaging 136, 260 and 344 mg/dl did not suppress 17-ß estradiol or lutein-

izing hormone (LH) levels at the premenstruum, menstruation, the periovulatory period or the mid-luteal phase (Mello et al, 1983a and b).

METHODS

Sexually mature female Macaque monkeys (4.6 to 7.5 kg) were housed individually in a cage room with adult males. A 12-hour light-dark cycle (7 a.m. to 7 p.m.) was in effect. Monkeys were maintained on ad lib food and water with daily supplements of fresh fruit, vegetables and multiple vitamins. Vaginal swabs were done daily to determine the onset and duration of menstrual bleeding. Blood samples were collected two or three times each week for radioimmunoassay of pituitary and gonadal hormones and to determine levels of alcohol in blood. Details of radioimmunoassay and blood alcohol analysis methods have been published (Mello et al, 1983a and b). Monkeys were periodically evaluated with laboratory tests to monitor the status of liver function, lipid and carbohydrate metabolism, electrolyte homeostasis and hematologic function.

Data are reported for five females, each of whom had occasionally received single doses of alcohol via nasogastric intubation to evaluate the acute effects of alcohol on pituitary and gonadal hormones (Mello et al, 1983a and b). All monkeys appeared to have normal ovulatory menstrual cycles as indicated by the mid cycle luteinizing hormone (LH) surge. Menstrual cycles were stable for at least 10 months before the initiation of the behavioral studies.

Animal maintenance and research was conducted in accordance with the guidelines provided by the Committee on Laboratory Animals Facility and Care, the National Research Council Institute of laboratory Animals Resources. The facility is licensed by the U. S. Department of Agriculture. The health of the monkeys was periodically monitored by a consultant veterinarian from the New England Regional Primate Center.

Monkeys were trained to work for food in an operant paradigm on gradually increasing response requirements on a variable ratio (VR) schedule in which the number of responses required for each reinforcement varied irregularly. An average of 16 responses (VR 16) produced a brief stimulus light (S+) and a 1 gram banana pellet. When response behavior was stable, a second order schedule was used where only a brief stimulus light (S+) was delivered after completion of each VI 16 response requirement. After four consecutive VR 16 components were completed, both the brief stimulus light and a food pellet were delivered. This is a second order fixed ratio (FR) of 4 schedule with VR 16 components (FR 4 [VR 16:S]).

Once food-maintained responding was stable, each monkey was surgically implanted with an intravenous catheter under ketamine anesthesia (25 mg/kg/i.m.) using aseptic procedures. Eight to 10 days after surgery, monkeys were given access to alcohol during menstruation or the late luteal phase of the menstrual cycle.

Monkeys learned to self-administer alcohol intravenously on the same operant schedule of reinforcement used for food acquisition. An average of 64 responses was required for each food pellet or alcohol injection (0.12 g/kg/inj) under a second order schedule of reinforcement (FR 4 [VR 16:S]).

Food and alcohol each were available during four 1-hour sessions each day. Food sessions began at 11 a.m., 3 p.m., 7 p.m. and 11 p.m.; alcohol sessions at 12 noon, 4 p.m., 8 p.m. and 12 midnight. The conditions of food and alcohol availability and time out (when responses had no programmed consequence) each were associated with a colored stimulus light (S+) projected on a translucent Plexiglas response key. Complete details of the apparatus and basic operant paradigm used in our previous studies of drug self-administration have been published (Mello and Mendelson, 1978).

RESULTS AND DISCUSSION

Alcohol produced an immediate and sustained disruption of menstrual cycle regularity in three monkeys. Each monkey began to self-administer high doses of alcohol as soon as it was available. During the first 30 days of alcohol access, these monkeys self-administered an average of 2.29 (± 0.26), 3.15 (± 0.27) and 3.24 (± 0.38) g/kg of alcohol per day. After the menstruation coincident with or within 10 days after initial access to alcohol, menstruation did not recur for 84 to over 180 days.

Each monkey that developed amenorrhea showed signs of physical dependence on alcohol. After the 11-hour interval between the midnight and noon alcohol session, these monkeys had gross tremor of the extremities and nystagmus which diminished appreciably after alcohol self-administration. Monkeys appeared intoxicated after alcohol sessions and blood alcohol levels measured immediately after a session ranged between 266 and 438 mg/dl.

Alcohol and food self-administration over successive 10-day intervals by the amenorrheic monkeys for 84, 93 and 160 consecutive days are shown in Figure 1. Despite daily alcohol intoxication, two monkeys showed no change in operant food self-administration from pre-alcohol baseline levels during the first 50 to 60 days of alcohol exposure. Monkey 10-80 worked for significantly more food than baseline ($p < .01$-$.001$) after 70 days of alcohol self-administrat ion. After 80 consecutive days of alcohol self-administration, Monkey T681 showed a significant decrease in food intake for 20 days ($p < .02$-$.001$). However, after 100 days of alcohol intoxication, food intake returned to levels that were not significantly different from baseline and then significantly exceeded baseline after 140 and 150 days of alcohol intoxication ($p < .02$-$.001$). Only Monkey B428 reduced operant food intake significantly ($p < .05$-$.001$) after the first 10 days of alcohol self-administration. After 60 days of alcohol self-administration, banana pellet intake returned to levels not significantly different from baseline.

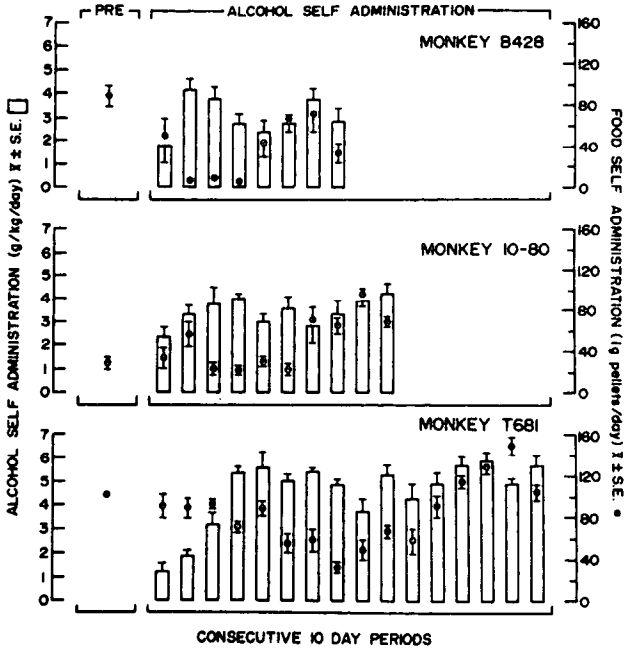

FIGURE 1. Reprinted with permission, from Mello et al., Science 221:677-679, 1983. Copyright 1983 by the AAAS.

Two of the three amenorrheic monkeys died after 84 and 93 consecutive days of alcohol self-administration. One monkey (10-80) apparently died of an overdose of alcohol; the postmortem blood alcohol sample was 502 mg/dl. A second monkey (B428) died of alcohol-related pulmonary disease. Necropsy revealed pathological changes in the reproductive system of both monkeys. The uterus was markedly atrophied and ovarian mass significantly reduced. Decreased ovarian mass appeared to reflect an absence of corpus lutea, which suggested that ovulation had not occurred (Mello et al, 1983c). Comparable pathology of the reproductive system has been reported in alcoholic women at autopsy (Jung and Russfield, 1972) and in rodents after chronic alcohol feeding (Van Thiel, Gavaler and Lester, 1978; Gavaler et al, 1980).

Luteinizing hormone (LH) levels were significantly depressed below pre-alcohol LH levels measured at menstruation ($p < .02$-$.001$) in each monkey that developed amenorrhea during chronic alcohol self-administration. Before the introduction of alcohol, LH values during menstruation ranged between 27 (± 1.06) and 36 (± 1.36) ng/ml. During chronic alcohol self-administration, LH values ranged between 18 (± 1.36) and 24 (± 1.41) ng/ml.

One amenorrheic monkey also had an unusual lesion of the adenohypophysis characterized by many small cells with hyperchromatic nuclei scattered among the the larger, polyhedral parenchymal cells. The origin of the cells in the pituitary lesion was undetermined.

Details of these pathological changes in brain, uterus and ovaries are described elsewhere (Mello et al, 1983c; Mendelson et al, 1983). It is possible that this unusual degenerative pituitary lesion occurred as a direct consequence of prolonged high dose intravenous alcohol self-administration. It is also possible that a derangement in pituitary gonadotropin secretory function induced the pathological changes observed in the ovaries and in the uterus.

Amenorrhea and the reproductive system pathology observed appear to be due to chronic alcohol intoxication and not to malnutrition or liver disease. Food self-administration remained relatively stable during alcohol self-administration (cf. Figure 1) and each monkey consistently ate daily supplements of fresh fruit, vegetables and biscuits. Laboratory tests were within normal range. After 98 days of alcohol self-administration, onemonkey had a slight elevation of alkaline phosphatase suggestive of mild hepatic impairment, but SGOT and SGPT levels were normal. No monkey had evidence of liver disease, i.e., hepatitis or cirrhosis.

The effects of alcohol on reproductive function were dose-related. Two monkeys self-administered relatively low doses of alcohol averaging 1.35 (± 0.26) and 1.66 (±0.38)g/kg/day for 119 and 173 days respectively. These monkeys continued to have stable menstrual cycles (28 days [± 0.89] and 31 days [± 1.35]) which were almost identical to the 10 menstrual cycles that preceded the alcohol self-administration studies (27 days [± 1.41] and 31 days [± 1.14]). LH levels did not differ significantly from pre-alcohol control levels.

This is the first report of alcohol-induced disruption of reproductive function in a female Macaque monkey model. Monkeys controlled their daily dose of alcohol in an operant paradigm so results cannot be attributed to non-physiological alcohol doses imposed by the investigators. The critical determining variable was the average dose of alcohol self-administered. High alcohol doses (2.95 - 4.41 g/kg/day) resulted in amenorrhea and pathological changes in the uterus and ovaries (Mello et al, 1983c) whereas lower alcohol doses (1.35 - 1.66 g/kg/day) were compatible with normal menstrual cycle function. This behavioral model of alcoholism in female monkeys provides an opportunity to systematically assess the critical alcohol dose and duration of exposure necessary to disrupt menstrual cycle regularity; to examine the neuroendocrine mechanisms underlying menstrual cycle disruption; and to evaluate the capacity for recovery of function following a period of chronic alcoholism.

REFERENCES

Frisch, R.E., and MacArthur, J.W. Menstrual cycles: Fatness as a determinant of minimum weight for height necessary for their maintenance or onset. Science, 185:949-951, 1974.

Gavaler, J.S., Van Thiel, D.J., and Lester, R. Ethanol: A gonadal toxin in the mature rat of both sexes. Alcoholism: Clin Exp Res, 4(3):271-276, 1980.

Griffiths, R.R., Bigelow, G.E., and Henningfield, J.E. Similarities in animal and human drug-taking behavior. In: Mello, N.K., ed. Advances in Substance Abuse, Behavioral and Biological Research. Vol. I. Greenwich: JAI Press, 1980, pp. 1-90.

Harlap, S., and Shiono, P.H. Alcohol, smoking and incidence of spontaneous abortion in the first and second trimester. The Lancet, 173-188, 1980.

Hugues, J.N., Cofte, T., Perret, G., Jayle, M.S., Sebaoun, J., and Modigliani, E. ,Hypothalamo-pituitary ovarian function in 31 women with chronic alcoholism. Clin Endocrinol, 12:543-551, 1980.

Johanson, C.E., and Schuster, C.R. Animal models of drug self-administration. In: Mello, N.K., ed. Advances in Substance Abuse, Behavioral and Biological Research. Vol. II. Greenwich: JAI Press, 1981, pp. 219-297.

Jung, Y., and Russfield, A.B. Prolactin cells in the hypophysis of cirrhotic patients. Arch Path, 94:265-269, 1972.

Karoly, A.J., Winger, G., Ikomi, F., and Woods, J.H. The reinforcing property of ethanol in the rhesus monkey. Psychopharmacology, 58:19-25, 1978.

Knobil, E. On the control of gonadotropin secretion in the rhesus monkey. Recent Prog Horm Res, 30:1-46, 1974.

Knobil, E. The neuroendocrine control of the menstrual cycle. Recent Prog Horm Res, 36:53-88, 1980.

Knobil, E. The role of the central nervous system in the control of the menstrual cycle of the rhesus monkey. Experimental Brain Research (Suppl. #3), 200-207, 1981.

Mello, N.K., and Mendelson, J.H. Self-administration of an enkephalin analog by rhesus monkey. Pharmac Biochem Behav, 9(5): 579-586, 1978.

Mello, N.K., Bree, M.P., Ellingboe, J., Mendelson, J.H., and Harvey, K. Lack of acute alcohol effects on estradiol and luteinizing. hormone in female Macaque monkey. Pharmacol Biochem Behav, 1983b, (in press).

Mello, N.K., Bree, M.P., Mendelson, J.H., and Ellingboe, J. Alcohol self-administration disrupts reproductive function in female Macaque monkeys. Science, 221:677-679, 1983.

Mello, N.K., Ellingboe, J., Bree, M.P., Harvey, K., and Mendelson, J.H. Alcohol effects on estradiol in female Macaque monkey. In: Harris, L.S., ed. Problems of Drug Dependence 1982. NIDA Research Monograph Series 43, pp. 210-216. Washington, D.C.: U.S. Government Printing Office, 1983a.

Mendelson, J.H., Mello, N.K., Bavli, S., Ellingboe, J., Bree, M.P., Harvey, K., King, N.W., and Sehgal, P.K. Alcohol effects on female reproductive hormones. In: Cicero, T.J., ed. Endocrinological Aspects of Ethanol Tolerance. NIAAA Monograph Series, 1983, (in press).

Moskovic, S. Effect of chronic alcohol intoxication on ovarian dysfunction. In: Srpski Arhiv za Celokupno Lekarstvo, V. 103, No. 9, 1975, pp. 751-758.

Pohl, C.R., and Knobil, E. The role of the central nervous system in the control of ovarian function in higher primates. Ann Rev Physiol, 44:583-593, 1982.

Ryback, R.S. Chronic alcohol consumption and menstruation. J Am Med Ass, 238:2143, 1977.

Valimaki, M., and Ylikahri, R. Alcohol and sex hormones. Scand J Clin Lab Invest, 41:99-105, 1981

Van Thiel, D.H., Gavaler, J.S, Lester, R., and Sherins, R.J. Alcohol induced ovarian failure in the rat. J Clin Invest, 61:624-632, 1978.

Winger, G.D., and Woods, J.H. The reinforcing property of ethanol in the rhesus monkey: I. Initiation, maintenance and termination of intravenous ethanol-reinforced responding. In: Seixas, F.A., and Eggleston, S., eds. Alcoholism and the Central Nervous System. Ann N Y Acad Sci. 215:162-175, 1973.

Woods, J.H., and Winger, G.D. Alcoholism and animals. Prev Med, 3:49-60, 1974.

Woods, J.H., Ikomi, F.I., and Winger, G. The reinforcing properties of ethanol. In: Roach. M.K., McIsaac. W.N., and Creaven. P.J.. eds. Biological Aspects of Alcoholism. Austin: University of Texas Press, 1971, pp. 371-388.

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Benzodiazepines: Drug Discrimination and Physiological Dependence

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The benzodiazepines are among the most widely used of all prescribed drugs. Concern about abuse of these drugs has prompted the development of preclinical methods for assessing various pharmacological effects of diazepam-like drugs which are relevant to their abuse and dependence liability. This abstract describes results from a series of ongoing experiments to assess discriminative stimulus effects and physiological dependence-producing properties of benzodiazepines.

Drug discrimination: In drug discrimination procedures, animals are trained to respond differentially depending on the nature of drug pretreatment. The procedure can provide information analogous to a human testing situation in which subjects catagorize drugs with respect to their subjective effects.

In ongoing drug discrimination experiments, four baboons were trained to discriminate lorazepam (1.0 mg/kg) and two baboons were trained to discriminate pentobarbital (5.6 mg/kg) in a two-lever drug versus no-drug discrimination procedure. Food delivery depended on 20 consecutive responses on one lever in sessions preceded by an intramuscular injection of the training drug (60-min pretreatment time), and on 20 consecutive responses on the other lever following no drug. All baboons completed 100% of the response runs on the appropriate level in training sessions. Test sessions were conducted in which a drug dose different from the training dose was administered (either orally or intramuscularly), and 20 consecutive responses on either lever produced food. Pentobarbital-trained baboons consistently generalized to lorazepam, diazepam, alprazolam, and pentobarbital. Although lorazepam-trained animals consistently generalized to lorazepam, diazepam, alprazolam, bromazepam, and triazolam, only one of four baboons generalized to pentobarbital (tested through a wide range of doses, and at 30- and 60-min pretreatment intervals). These results demonstrate an interesting asymmetry in cross- generalization with benzodiazepines and barbiturates. In related experiments, oral doses of Ro 15-1788 (a benzodiazepine antagonist), naltrexone (an opioid

antagonist), and caffeine were administered alone or immediately before an injection of lorarepam or pentobarbital. Ro 15-1788 (0.1-1.0 mg/kg) had no effect on the pentobarbital stimulus and produced a surmountable antagonism of the lorazepam stimulus. Naltrexone (1.0-10.0 mg/kg) had no effect on the pentobarbital or lorazepam stimulus. Caffeine (0.1-10.0 mg/kg) produced variable effects within and across animals. When administered alone, neither Ro 15-1788, naltrexone, nor caffeine generalized to the pentobarbital or lorazepam stimulus. The differential effects of Ro 15-1788 on the stimulus effects of lorazepam and pentobarbital along with the asymmetry in cross-drug generalization between lorazepam- and pentobarbital-trained baboons suggest that the stimulus effects of these two compounds depend upon different mechanisms of action.

Physiological dependence: Physiological dependence is an important aspect of the behavioral toxicity of benzodiazepines and may be related to the abuse liability of these drugs. In ongoing experiments to explore the development of physiological dependence on benzodiazepines in baboons, reliable observational procedures were developed for assessing benzodiazepine withdrawal by scoring a series of 15 withdrawal signs and body postures. In one study baboons implanted with intragastric catheters were given diazepam (20 mg/kg/day) continuously for 45 days. Termination of diazepam administration (spontaneous withdrawal) resulted in a mild to intermediate withdrawal syndrome over the next 15 days characterized by suppressed food intake, tremor and abnormal body posturing. Parametric studies have been undertaken to characterize precipitated withdrawal by administering intramuscular injections of the benzodiazepine antagonist, Ro 15-1788, 5.0 mg/kg, after periods of chronic benzodiazepine administration. Intensity and consistency of withdrawal signs are an increasing function of diazepam dose (0.125-20.0 mg/kg/day) and duration of diazepam administration (1 hr, 1, 3, 7, 35 days). Results to date indicate that reliabie precipitated withdrawal occurs with diazepam doses as low as 0.25 mg/kg/day administered for 7 days. Precipitated witndrawal after higher diazepam doses administered for longer durations is characterized by emesis, retching, tremor, and convulsion. Compared to spontaneous withdrawal, precipitated withdrawal is more intense, has a more rapid onset, and is of briefer duration.

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An Investigation of Brain Reinforcement Systems Involved in the Concurrent Self-Administration of Food, Water, and Morphine

Steven I. Dworkin, John D. Lane, and James E. Smith

Since the demonstration in 1954 by Olds and Milner that animals will self-administer minute electric currents into various brain regions, the existence of central reinforcement pathways has been under vigorous investigation. Studies of the neurobiological substrates of reinforcement have used two general approaches. The first procedure has investigated responding maintained by central pathways involved in the presentation of an intracranial electrical stimulus (intracranial self-stimulation).The second general procedure is drug self-administration where responding is maintained by the presentation of intravenous or intracranial drug infusions. Variations of these procedures have been used to investigate the neurochemical, neuroanatomical, and neurophysiological mechanisms of reinforcement. Results from these studies have implicated both dopamine-or norepinephrine-releasing neurons, although some controversy has existed as to which catecholamine is "most" important in central reinforcement mechanisms.

Generalization from studies of these two behavioral procedures to a theory of the neurobiological mechanisms of reinforcement is risky, especially considering the complexity of reinforcement demonstrated at the behavioral level. The behavioral effects of many stimuli, including intracranial electrical stimulation and drug presentations, have been shown to be influenced by several variables. These factors include the schedule of event presentation, the rate of ongoing behavior, and the behavioral and drug history of the organism.

Consequential events cannot be considered to have invariant properties. For example, experiments with squirrel monkeys have shown that responding can be maintained by a fixed-interval schedule of electric-shock presentation in one component of a multiple schedule and punished by the same intensity shock in a second component (Barrett and Glowa, 1977). Using the same species, cocaine has been shown to be both a negative and a positive reinforcer at the same time (Spealman, 1979). Furthermore, responding by rats can be maintained by the termination of self-produced rates of brain stimulation (ICSS) (Steiner et al., 1969). The behavioral effects of several variables. including psychoactive drugs have also been shown to depend on the event and/or schedule used to maintain its presentation (for review, see Barrett and Katz, 1981). To avoid

these problems in investigations of the neurobiological substrates of reinforcement, it is necessary to monitor more than one behavior.

The present study was designed to investigate the behavioral and neurobiological effects of neurotoxin lesions on responding concurrently maintained by food, water, and morphine reinforcement. Such procedures allow for assessment of the specific involvement of small populations of neurons in reinforcement processes associated with different reinforcers. The results from this study indicate that kainic acid lesions of the nucleus accumbens may selectively attenuate the reinforcing efficacy of morphine.

METHOD

Subjects

Seven adult male Fisher-344 rats between 90-150 days old at the beginning of the experiment were studied in an operant-conditioning chamber with three reinforcers.

Food and Water

The rats were initially trained to lever press on two separate retractable levers under a concurrent schedule of food and water presentations. After stable responding was observed, the schedule was then changed to a concurrent chained (conc chain) schedule (Autor, 1969). Under this schedule requirement, the first response on the food or water lever resulted in the retraction of the other lever (FR1 initial link). A fixed number of responses, that was gradually increased from 1 to 9, was then required on the extended lever (terminal link). Food presentations consisted of the delivery of one 45 mg pellet, and water reinforcers were 20 sec access to a 0.1 ml dipper of tap water containing tetracycline. Following completion of the schedule requirement, both levers retracted for 30 sec (time out or TO). A limited hold 100 sec contingency (from the first response) was also used. Elapse of the limited hold without completion of the terminal ratio resulted in the scheduling of a TO without reinforcer presentation. Following the TO, the levers were extended and the schedule contingencies were reset. The stimulus lights above each lever were only illuminated during lever extension. After stable performance was observed under the conc chain FR1 FR9 schedule, the animals were surgically prepared as described below.

Surgical Procedure

Each rat was implanted with a chronic jugular catheter using previously described methods (Weeks, 1962, 1972). Intracranial injection cannulae were also implanted into the nucleus accumbens using a previously described procedure (Myers, 1966). Guide cannulae (26 gauge stainless steel tubing) were implanted stereotaxically using the appropriate coordinates for the

nucleus accumbens (Konig and Klippel, 1963; 9.5 mm A to lambda, 1.2 mm L, 5.1 mmV). The guide cannulae were permanently cemented to the skull with dental cement and a stainless steel 32 gauge stylet inserted.

Morphine

The rats were placed in one-lever operant conditioning chambers and made physically dependent on morphine over a 12-day period by delivering hourly infusions of morphine. The dose ranged from 1.25 - 10 mg/kg/infusion and was increased every third day. A lever was then introduced and the rats were trained to respond under an FR10 schedule. Subjects were then placed back into the original conditioning chamber and a morphine reinforcer option added to the initial food and water schedule. Subsequent responding was maintained under a conc chain FR 1 FR9 schedule of food, water and morphine presentation.

Morphine Dose-Effect Study

Five subjects were used for the dose-effect determinations. Morphine sulfate was dissolved in a bacteriostatic 0.9% sodium chloride solution containing 0.83 USP units/ml of sodium heparin. Dosages were determined as the salt and were calculated using the average weight of 0.333 Kg. A range of doses from 2.5-40 mg/kg/infusion or dosages of 0/83-13.33 mg/infusions was investigated. Morphine presentations consisted of a 0.2 ml infusion delivered over a 5.5 sec period and were followed by a 30 sec tone presentation. Dose-effect curve determinations were made by replacing the daily morphine dose (10 mg/kg/infusion) with another dose of morphine or saline for 24 hours. Drug substitutions were always made at the start of the dark cycle (5 a.m.). At least two determinations of each dose or vehicle were made.

Kainic Acid Lesion

Chemical lesions were produced by the microinjection of kainic acid through the implanted cannulae using a previously described procedure (Myers, 1966) (see Surgical Procedures above). On the day of microinjection, the animal was anesthetized with Valium, the stylet removed and an injection cannula (32 gauge stainless steel tubing that extends 0.5 mm beyond the end of the guide cannula when inserted) that is attached to a 1 µl microsyringe by PE tubing, was inserted into the guide cannula and secured. A 0.2 µl injection of 2 µg kainic acid in artificial CSF was then made using a precision microinfusion pump that delivers 0.033 µl of solution per minute. The injection cannula was removed after 10 minutes and the stylet replaced. Artificial CSF alone was microinjected into control animals.

Histological Procedures

The histological method used to verify cannulae placement has been previously reported (Pisa et al. 1980). The rats were sacrificed by decapitation and 16 or 32 micron frozen sections

cut and stained with the Kluver and Barrera (1953) method for identification of both cell bodies and myelinated fiber bundles. Location and extent of lesions were assessed by light microscopy.

RESULTS

Control Performance

Characteristic rates and temporal patterns of responding were maintained by the conc chain FR1 FR9 schedule of food and water presentation. Responding consisted of a short pause after the TO followed by a high rate of responding. Similar local rates of responding were maintained by the two reinforcers. A large number of consecutively completed ratios (runs) on the food lever were followed by runs on the water lever. These runs on the food lever were longer than the runs on the water lever, resulting in twice the mean number of food presentations for the six rats per day (285 ± 39) (values are mean ± S.D.) compared to the mean number of water'presentations, 143 ± 57 per day. Over 90% of the responding occurred during the animals' dark cycle (5:00 a.m. to 5:00 p.m). The addition of the third reinforcer, morphine, to the schedule did not alter the local rate or temporal response patterns. The three reinforcers maintained characteristic fixed-ratio patterns of responding. Responding maintained by morphine presentations was evenly distributed over both light and dark cycles and the mean number of morphine presentations was 14 ± 3 per day for the six subjects. The addition of morphine to the food and water schedule produced a small increase in the number of food presentations (333 ± 68) and a decrease in the number of water presentations (115 ± 23) delivered per day.

Morphine dose-effect curve

The effects of substituting several doses of morphine (2.5-40 mg/kg/infusion) or saline for the fixed daily dose of morphine on the number of ratios completed on the three levers are shown in Figure 1A. Only the lowest dose produced a slight decrease in the number of ratios completed on the food and water levers. Higher doses did not affect responding on either the food or water lever.

Increasing the doses of morphine above the fixed daily dose of 10.0 mg/kg/infusion resulted in dose-related decreases in the number of morphine infusions delivered. Decreasing the dose below the daily dose increased the number of ratios completed on the morphine lever. The most prominent effect of saline substitution was the large increase in the number of ratios completed on the morphine lever and the almost complete elimination of responding maintained by water presentation (see Figure 1A).

Effects of lesions

The effects of the kainic acid lesion of the nucleus accumbens on the number of ratios completed (percent of control) on each

of the three levers are presented in Figure 1B. The lesion resulted in a small increase in responding on the water lever and no change in responding on the food lever. However, responding on the morphine lever was significantly decreased (see points above 10.0 mg/kg/inj). Furthermore, in contrast to the effects observed before the lesion, morphine dose (2.5 - 40 mg/kg/inj) after the lesion did not significantly alter responding on any lever. The lowest dose of morphine investigated resulted in occasionally increased responding on the morphine lever after the lesion. Moreover, the behavioral effects of saline substitution after the lesion were very different from the effects observed before the lesion. Saline substitution did not alter responding on either the food, water or morphine manipulanda.

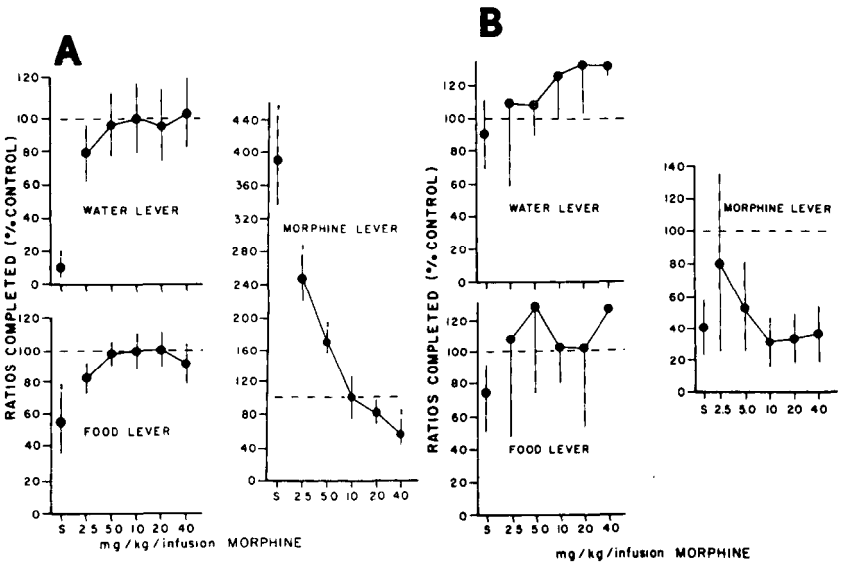

Figure 1. Morphine dose-effect curves for concurrent responding maintained by food, water and intravenous morphine before (A) and after (B) the kainic acid lesion. Data are expressed as percent of control or baseline intake determined by exposure to 10 mg/kg per infusion of morphine. "S" represents exposure to saline. Points are means for double determinations of 24-hours exposure to each dose (except 10.0 mg/kg - mean of 10 determinations) and the error values ± standard deviations for N=5 animals. Significant *differences determined by Student's t-tests comparing each point with the baseline condition were: +=p<0.01, =p<0.001.*

DISCUSSION

Investigations of the neurobiological substrates of reinforcement should include procedures for studying behavior maintained by several different consequent events. Studies in which responding is reinforced using a variety of different events (e.g., food, water, and drug presentation) permit the determination of specific neuronal mechanisms of reinforcement. For example, two recent studies have demonstrated the independence of neuronal systems mediating the reinforcement of opiates and stimulants (Ettenberg et al, 1982; Petit et al, 1982). Demonstration of the reinforcer-specific effects of neurobiological manipulations on behavior maintained by different consequent events questions the involvement of only a single neurotransmitter system in brain reinforcement processes. In the present study, responding was concurrently maintained by response-dependent food, water, and morphine presentation. Changing the morphine dose was shown to alter morphine responding, but had little or no effect on responding maintained by food or water. Furthermore, kainic acid lesions of the nucleus accumbens were shown to selectively reduce the reinforcing efficacy of morphine.

A second advantage of the procedure used in the present study was that non-selective effects due to motor impairment could be independently evaluated. The fact that the neurotoxin lesions only changed responding on the morphine lever indicated that the lesion did not produce a non-specific motor deficit. Several previous reports that suggest the involvement of dopamine reinforcement systems in mediating food reinforcement have not controlled for non-specific motor effects (Wise, 1980).

The dose-effect curves demonstrated that effects of morphine observed were not the result of a shift in the dose-effect curve. Behavior after the lesion was not sensitive to different doses of morphine. Kainic acid lesions destroy interneurons and outputs from the injected region. These data suggest that these interneurons and output from the nucleus accumbens are excitatory to opiate processes, but not necessary for food and water reinforcement.

Several recent studies have demonstrated the complexity of neurobiological substrates of reinforcement and have implicated the involvement of several different neurotransmitter systems in these processes. Furthermore, both common as well as independent neurotransmitter systems appear to be involved in the ability of different consequent events to maintain behavior. Studies that attempt to determine both the general and specific neuronal systems involved in responding maintained by different reinforcing events provide a more complete assessment of neurobiological substrates of reinforcement.

REFERENCES

Due to space limitations, a complete list of references may be obtained from the senior author.

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Buprenorphine, Heroin, and Methadone: Comparison of Relative' Reinforcing Properties

N. K. Mello, M. P. Bree, and J. H. Mendelson

Buprenorphine is a partial agonist of the morphine type. It is both a long-acting opiate antagonist, like naltrexone, and a potent opiate agonist with respect to analgesia, physiological and subjective reactions in man (Houde, 1979; Jasinski et al, 1978; Lewis et al, 1983) . However, buprenorphine does not induce physical dependence in several species and appears to produce only minimal physical dependence in man (Jasinski et al, 1978; Mello et al, 1982; Rance, 1979).

Buprenorphine's positive morphine-like agonist effects combined with its antagonist potency, low toxicity, and minimal capacity for producing physical dependence, suggested that it should be valuable for the treatment of opiate addiction (Jasinski et al, 1978). Clinical studies have shown that buprenorphine maintenance (8 mg/day s.c.) significantly suppressed self-administration of heroin (21 to 40.5 mg/day) by male heroin addicts over 10 days of heroin availability in comparison to buprenorphine placebo (Mello and Mendelson, 1980; Mello et al, 1982). Buprenorphine (0.282 to 0.789 mg/kg/day i.v.) also significantly suppressed opiate self-administration in the rhesus monkey drug self-administration model (Mello et al, 1983). Recent clinical studies have shown that sublingual administration of buprenorphine (1-2 mg) should be suitable for daily maintenance for the treatment of narcotic addiction (Jasinski et al, 1983).

The opiate agonist effects of buprenorphine resemble those of methadone and morphine in terms of reported subjective responses (Jasinski et al, 1978). The degree of euphoria and other positive subjective effects of 8 mg/day of buprenorphine were equivalent to those produced by 120 mg/day of morphine (30 mg q.i.d.) or 40 to 60 mg of methadone (Jasinski et al, 1978). There is usually a high concordance between subjective reports of opiate effects and animal drug self-administration data (Griffiths and Balster, 1979), and buprenorphine has also been shown to be a positive reinforcer in rhesus monkeys (Woods, 1977; Mello et al, 1981; Yanagita et al, 1982 and baboon (Lukas et al, 1983).

The abuse potential of buprenorphine relative to the abuse potential of the opiate agonist methadone is unknown, and the relative efficacy of buprenorphine and methadone in attenuating opiate self-administration has not been compared in inpatient or outpatient clinical studies. The accumulated clinical experience with methadone abuse (Kreek, 1978) argues for the importance of systematic evaluation of the potential abuse liability of new pharmacotherapies. One approach to evaluating relative reinforcing efficacy of various drugs, and inferring potential abuse liability, is the progressive ratio procedure. This procedure provides a quantitative index of the number of responses that a monkey will emit for a single drug injection, a measure sometimes described as "response cost." Different doses of a single drug or different drugs can be ranked according to the maximum number of responses emitted to acquire a single drug injection (Yanagita, 1976; Yanagita et al, 1965). This report describes preliminary data obtained on the relative reinforcing properties of buprenorphine, methadone, and heroin using progressive ratio procedures.

METHODS

Six male Macaque monkeys (1 Macaca mulatta and 5 Macaca nemestrina) weighing 6.0 to 8.4 kg were studied. Five monkeys had a history of opiate agonist and mixed agonist-antagonist self-administration and one monkey was drug-naive at the beginning of these studies. Monkeys were surgically implanted with chronic indwelling catheters to permit intravenous drug self-administration. All surgical procedures were performed under aseptic conditions. Monkeys were anesthetized with either pentobarbital (30 mg/kg/i.v.) or ketamine (25 mg/kg/i.m.) and a double lumen silicon rubber catheter (I.D. 0.028"; O.D. 0.088") was placed in a vein. Following surgery, animals were given 1 ml of combiotic every other day for a total of five injections.

Animal maintenance and research was conducted in accordance with the guidelines provided by the Committee on laboratory Animals Facility and Care, the National Research Council Institute of laboratory Animals Resources. The facility is licensed by the U.S. Department of Agriculture. The health of the monkeys was periodically monitored by a consultant veterinarian from the New England Regional Primate Center.

Monkeys worked at an operant task for food (1 gm banana pellet) and for buprenorphine (.01, .03, .05, .10 mg/kg/inj), heroin (.01, .05, .10 mg/kg/inj), methadone (.03, .10, .25 mg/kg/inj) and saline. Food and baseline drug self-administration were maintained under a second-order schedule of reinforcement [FR 4 (VR 16:S)]. An average of 16 responses on a variable ratio schedule (VR 16) produced a brief colored stimulus light (S+). However, a drug injection or a food pellet was delivered only after a fixed ratio of 4 (FR 4) of the VR 16 response requirements had been completed; i.e., each food pellet or drug injection required an average of 64 responses. Details of the apparatus have been published (Mello and Mendelson, 1978). Monkeys were maintained at ad lib weight and given multiple

vitamins, fresh fruit and vegetables daily to supplement a banana pellet diet.

Food sessions began at 11 a.m., 3 p.m., 7 p.m. and 11 p.m. each day, and drug sessions began one hour later at 12 noon, 4 p.m., 8 p.m. and midnight. Each food or drug session lasted either one hour or until 20 drug injections or 65 food pellets were delivered. The chamber was dark between 1 a.m. and 9 a.m.

Each dose of buprenorphine, heroin, and methadone was available for a minimum of 40 sessions over 10 days or until baseline drug self-administration was stable. Subsequently, the second order schedule response requirement was systematically increased by increasing the number of responses required in the Fixed Ratio (FR) component of the second order schedule in increments of two. The resulting progressive ratio response requirements per drug injection are summarized below.

Schedule Requirement	Average Response Requirement Per Drug Injection	Number of Sessions
	64	8
FR 4 (VR 16:S)	96	8
FR 8 (VR 16:S)	128	8
FR 10 (VR 16:S)	160	8
FR 12 (VR 16:S)	192	8
FR.. .N (VR 16:S)	. ..N	8

Each increase in the FR schedule component was run for eight sessions over two days. Progressive increases in the response requirement were continued until drug self-administration decreased significantly below baseline levels. The usual 'break-point" criterion is two consecutive days of no drug self-administration, The in-mediately preceding response requirement for a single drug injection indicates the maximum number of responses that the monkey will emit for that dose of the drug.

Once the "breakpoint" at a single drug dose was reached, the monkey was returned to the baseline schedule requirement (FR 4 VR 16:S) at the same drug dose until drug self-administration resumed. This procedure was useful to reduce the extinction-like effects of the progressive increases in response requirements for drug. Monkeys were then given access to the next dose of drug and run at the basic second order schedule response requirement until drug self-administration was stable over 40 consecutive sessions or 10 days. Response requirements were then increased progressively as before and continued until the monkey reached the "breakpoint" at that dose. All monkeys were not run at every dose of each drug.

RESULTS

These studies are still in progress and all 10 drug doses and saline have not yet been evaluated in all monkeys. The group average breakpoints for buprenorphine (.01-.10 mg/kg/inj), heroin (.01-.10 mg/kg/inj) and methadone (.03-.25 mg/kg/inj) are shown in Figure 1.

The reinforcing efficacy of buprenorphine appears to be greatest at low doses. Buprenorphine at doses of .01 and .03 mg/kg/inj maintained responding on progressively increasing values of second order schedules for longer than higher doses of buprenorphine (.05 and .10 mg/kg/inj) . Yanagita and co-workers (1982) also reported that rhesus monkeys worked longer for a low (.015 mg/kg/inj) than for a high (.06 mg/kg/inj) dose of buprenorphine on a progressive ratio schedule. This pattern is consistent with the notion that the antagonist component of this mixed agonist-antagonist drug attenuates its agonist effect at higher doses. Several agonist effects of buprenorphine such as euphoria (Jasinski et al, 1978), "stupor" (Kareti et al, 1980), antinociception and respiratory depression in rats (Cowan et al, 1977a and b) each are maximal at relatively low doses and are significantly reduced or fail to increase at higher doses.

These data are also concordant with observations in baboons that self-administered buprenorphine on an FR 160 schedule (Lukas et al, 1983). Buprenorphine self-administration tended to plateau over a dose range of 0.1 to 1.0 mg/kg/inj (Lukas et al, 1983). Changes in buprenorphine injections per day as a function of dose were less consistent in rhesus monkey when responding was maintained on a second order FR 3 (VR 16) schedule. Only 2 of the 5 monkeys showed a consistent decline in buprenorphine injections as the dose per injection was increased over a range of .005 to .10 mg/kg/inj (Mello et al, 1981).

Monkeys consistently emitted more responses for heroin than for buprenorphine across the range of doses studied. These data suggest that the abuse potential of buprenorphine relative to heroin is low. Moreover, in contrast to buprenorphine, the progressive ratio breakpoint for heroin increased as a function of increased heroin dose. These data are consistent with previous progressive ratio studies of heroin (.001 to 0.5 mg/kg/inj) and codeine (.01 to 16 mg/kg/inj) self-administration in rhesus monkey (Hoffmeister, 1979), but a number of procedural differences limit further comparisons between these studies.

The lowest dose of methadone (.03 mg/kg/inj) maintained less responding in a progressive ratio paradigm than either buprenorphine or heroin. At an intermediate methadone dose (.10 mg/kg/inj) progressive ratio breakpoints were almost twice as high as those for buprenorphine. However, the abuse potential of methadone relative to heroin and buprenorphine cannot be estimated with confidence until additional progressive ratio data for methadone self-administration are available.

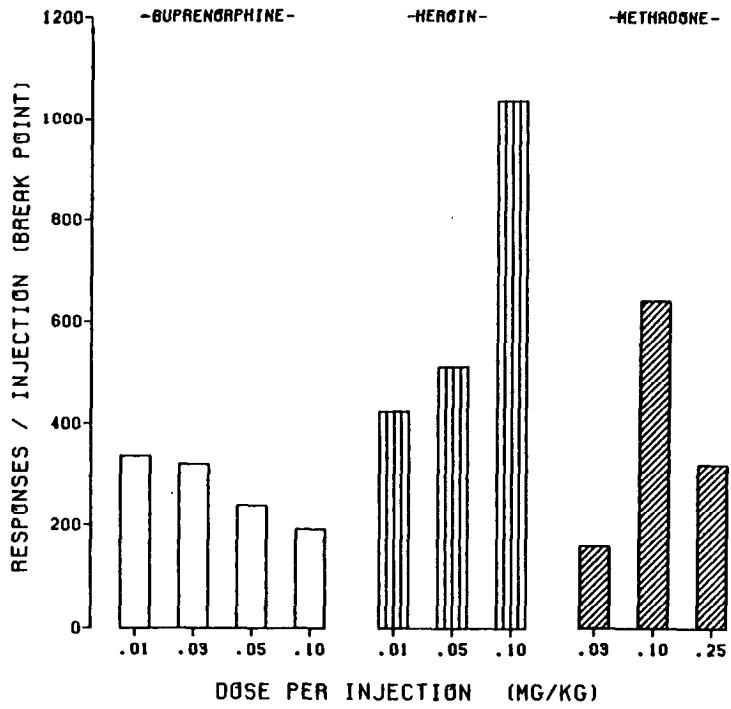

Figure 1: Group average maximum number of responses per injection emitted for ascending doses of buprenorphine (.01-.10 mg/kg/inj), heroin (.01-.10 mg/kg/inj) and methadone (.03-.25 mg/kg/inj) on a progressive ratio schedule.

REFERENCES

Cowan, A., Doxey, J.C., and Harry, E.J.R. The animal pharmacology of buprenorphine, an oripavine analgesic agent. Br J Pharmacol, 60:547-554, 1977a.

Cowan, A., Lewis, J.W., and MacFarlane, I.R. Agonist and antagonist properties of buprenorphine, a new antinociceptive agent. Br J Pharmacol, 60(4):537-545, 1977b.

Griffiths, R.R., and Balster, R.L. Opioids: Similarity between evaluations of subjective effects and animal self-administration results. Clin Pharmacol Ther, 25:611-617, 1979.

Hoffmeister, F. Progressive-ratio performance in the rhesus monkey maintained by opiate infusions. Psychopharmacology 62:181-186, 1979.

Houde, R.W. Analgesic effectiveness of the narcotic agonist-antagonists. Br J Clin Pharmacol, Supplement 3 to Volume 7, 297-308, 1979.

Jasinski, D.R., Pevnick, J.S., and Griffith, J.D. Human pharmacology and abuse potential of the analgesic buprenorphine. Arch Gen Psychiat, 35:601-616, 1978.

Jasinski, D.R., Henningfield, J.C., Hickey, J.E., and Johnson, R.E. Progress Report of the NIDA Addiction Research Center, Baltimore, Maryland, 1982. In: Harris, L.S., ed. Problems of Drug Dependence 1982, NIDA Research Monograph Series 43, pp. 92-98. Washington, D.C.: U.S. Government Printing Office, 1983.

Kareti, S., Moreton, J.E., and Khazan, N. Effects of buprenorphine, a new narcotic agonist-antagonist analgesic on the EEG, power spectrum and behavior of the rat. Neuropharmacology, 19:195-201, 1980.

Kreek, M.J. Medical complications in methadone patients. In: Kissin, B., Lowinson, 3. and Millman, R., eds. Recent Developments in Chemotherapy of Narcotic Addiction, Ann. N. Y. Acad. Sci., 311:110-134, 1978.

Lewis, J., Rance, M.J.; and Sanger, D.J. The pharmacology and abuse potential of buprenorphine, a new antagonist analgesic. In: Mello, N.K., ed. Advances in Substance Abuse, Behavioral and Biological Research, Vol. 3, Greenwich: JAI Press, Inc., 83, in press.

Lukas, S.E., Griffiths R.R., and Brady, J.V. Buprenorphine self-administration by the baboon: Comparison with other opioids. In: Harris, L.S., ed. Problems of Drug Dependence 1982, NIDA Research Monograph 43, pp. 178-183. Washington, D.C.: U.S. Government Printing Office, 1983.

Mello, N.K., and Mendelson, J.H. Buprenorphine suppresses heroin use by heroin addicts. Science, 207:657-659, 1980.

Mello, N.K., Bree, M.P., and Mendelson, J.H. Buprenorphine self-administration by rhesus monkey. Pharmac Biochem Behav, 15:215-225, 1981.

Mello, N.K., Bree, M.P., and Mendelson, J.H. Comparison of buprenorphine and methadone effects on opiate self-administration in primates. J Pharmacol Exp Ther, 225(2): 378-386, 1983.

Mello, N.K., Mendelson, J.H., and Kuehnle, J.C. Buprenorphine effects on human heroin self-administration: An operant analysis. J Pharmacol Exp Ther, 223(1):30-39, 1982.

Rance, M.J. Animal and molecular pharmacolgy of mixed agonist-antagonist analgesic drugs. Brit J Clin Pharmac, 7:281S-286S, 1979.

Woods, J.H. Narcotic reinforced responding: A rapid screening procedure. In: Proceedings Committee on Problems of Drug Dependence, 1977, pp. 420-437

Yanagita, T. Some methodological problems in assessing dependence-producing properties of drugs in animals. Pharmacological Reviews, 27(4) :503-509, 1976.

Yanagita, T., Deneau, G.A., and Seevers, M.H.: Evaluation of pharmacologic agents in monkey by long term intravenous self or programmed administration. Excerpta Med Int Congr Ser, 87:453, 1965.

Yanagita, T., Katoh, S., Wakasa, Y., and Oinuma, N. Dependence potential of buprenorphine studied in rhesus monkeys. In: Harris, L.S., ed. Problems of Drug Dependence 1981, NIDA Research Monograph Series 41, pp. 208-214. Washington, D.C.: U.S. Government Printing Office, 1982.

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Behavioral Interactions of Opioid Agonists and Antagonists With Serotonergic Systems

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Morphine interacts with brain serotonergic (5-HT) systems; these systems have been implicated in morphine analgesia and dependence see Cervo et al., 1981). The 5-HT agonist quipazine induces analgesia in rats that is attenuated by naloxone and 5-HT antagonists (Minnema et al., 1980; Samanin et al., 1976). Behavioral disruption by the hallucinogens LSD, DMT and mescaline, mediated primarily through brain 5-HT effects (Rech and Commissaris, 1982), is potentiated by naloxone and naltrexone (Commissaris et al., 1980; Ruffing and Domino, 1981) and is variably antagonized or potentiated by morphine and methadone (Ruffing and Domino, 1981). Cyclazocine causes a disruption of operant behavior similar to that of the hallucinogens which is reversed in part by naloxone and the 5-HT antagonist metergoline, and to a greater extent by the combination of naloxone and metergoline (Henck et al., 1983). These studies indicate that indole and phenethylamine hallucinogens interact to some extent with brain opioid mechanisms as well as brain 5-HT components, whereas opioid drugs influence behavior in part by actions on 5-HT systems.

We have extended these drug studies in an attempt to characterize interactions with 5-HT mechanisms and to identify the various types of opioid receptors involved.

METHODS

Male Sprague-Dawley rats were food deprived to 75-80% of free-feeding weights and trained to a fixed ratio-40 (FR-40) schedule of food reinforcement. The number of reinforcers (Bioserve 45 mg food pellets) and "pause intervals" (a 10 sec period without a response, Commissaris et al., 1980; Rech and Commissaris, 1982) were recorded for daily 40-min sessions. Changes in the patterns of responding were determined after combinations of lysergic acid diethylamide (LSD) or 2,5-dimethoxy-4-methylamphetamine (DOM) with naloxone (NAL), cyclazocine (CYCL) with quipazine (QUIP) or metergoline (MTG), ethylketocyclazocine (EKC) with NAL or MTG, and N-allyl-normetazocine (SKF 10,047; SKF) with NAL or MTG. Dose-response curves were analyzed by a one-way ANOVA using the least significant differences (lsd) test for comparing individual doses to baseline; multiple dose-response curves were compared by a two-

way ANOVA using the lsd test for individual comparisons. The significance level was set at $p < 0.05$.

RESULTS

LSD (12.5-100 μg/kg) or DOM (0.125-1.0 mg/kg) caused a dose-related increase in pause intervals (Fig. 1) that was reciprocally related to a decrease in reinforcers delivered. Pretreatment with 4 mg/kg NAL potentiated the disruptive effects of LSD and DOM, but neither dose-response curve was shifted in a parallel fashion.

CYCL disrupted FR-40 responding as shown in Fig. 2. Like the hallucino gens, CYCL caused a dose-related decrease in reinforcers that was reciprocally related to increases in pausing. This disruption was attenuated over the entire dose range, at least for pauses, by pretreating with a low dose of QUIP (0.5 mg/kg). Additional pretreatment with MTG (1.0 mg/kg) nullified the QUIP antagonism only at the highest dose of CYCL tested. A previous report indicated that the CYCL effects were antagonized over the middle-dose range by both NAL (4 mg/kg) and MTG (1 mg/kg) pretreatment, and combination of these pretreatments showed additive protection (Henck et al., 1983).

FR-40 disruption was also observed with QUIP (Fig. 3) and a dose-related reciprocal increase in pausing was again associated with the decrease in reinforcers. Therefore, the hallucinogens, certain opioids, and 5-HT agonists appear to affect FR-40 responding in this manner. Pretreatment with NAL (4 mg/kg) slightly potentiated the disruptive effects of low doses of QUIP but had no significant effect at higher doses. Likewise, pretreatment with a small dose of CYCL (0.5 mg/kg) slightly potentiated low dose QUIP without influencing the effect of higher doses. However, when the NAL and CYCL pretreatments were combined, the marked disruption observed after 2.0 mg/kg QUIP. alone was greatly attenuated. Thus, the disruptive effects of QUIP may involve both 5-HT and opioid mechanisms.

The prototype kappa agonist EKC (Fig. 4) decreased reinforcers in a dose-related manner with a reciprocal increase in pauses, but, unlike the hallucinogens, exhibited a steep dose-response curve. Pretreatment with NAL slightly potentiated the disruptive effects of low doses of EKC but prominently antagonized the effects of higher doses. Pretreatment with MTG also enhanced the effects of low doses of EKC and antagonized EKC disruption only at the 1.0 mg/kg dose. A low dose (0.5 mg/kg) of CYCL was also administered as a pretreatment (not illustrated) and interacted with EKC in a pattern very similar to that noted with MTG pretreatment.

Since SKF has hallucinogenic properties and may be a selective agonist at sigma receptors, its effects on the FR-40 operant pattern were examined (Fig. 5). Once more the hallucinogenic profile of decreased reinforcers with a reciprocal increase in pause intervals was obtained. In this case pretreatment with 4 mg/kg NAL did not significantly influence the disruptive pattern of the drug. Pretreatment with MTG slightly antago nized the effects of SKF at several intermediate doses. Therefore, SKF does not appear to exert these disruptive effects via NAL-sensitive opioid receptors, but may act to some extent through 5-HT mechanisms.

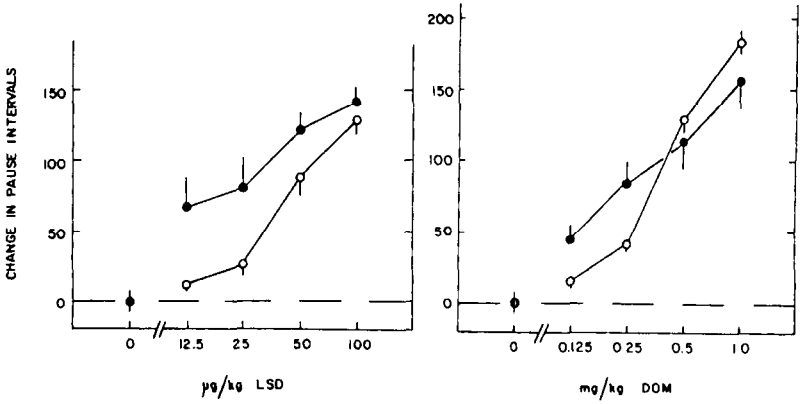

Fig. 1. Dose-response of LSD or DOM alone (open circles) or combined with 4 mg/kg naloxone (NAL) (closed circles) on FR-40 behavioral response pattern.

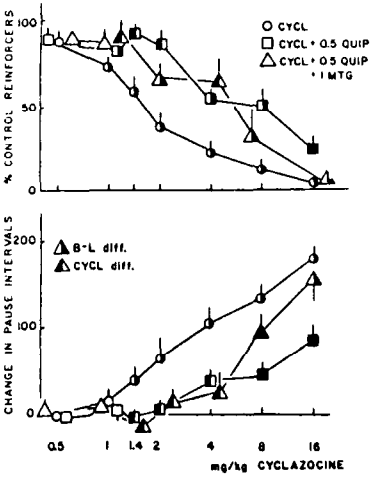

Fig. 2. Dose-response of cyclazocine (CYCL) alone, combined with 0.5 mg/kg quipazine (QUIP), or combined with 0.5 mg/kg QUIF" and 1.0 mg/kg metergoline (MTG).

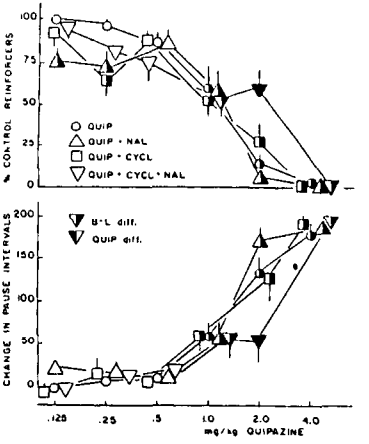

Fig. 3. Dose-response of quipazine (QUIP) alone, combined with 4 mg/kg naloxone (NAL), with 0.5 mg/kg cyclazocine (CYCL), or with both NAL and CYCL.

Pretreating with a low dose (0.5 mg/kg) of CYCL (results not illustrated) slightly attenuated the increase in pausing at the highest dose (16 mg/kg) of SKF, but otherwise had no appreciable effect.

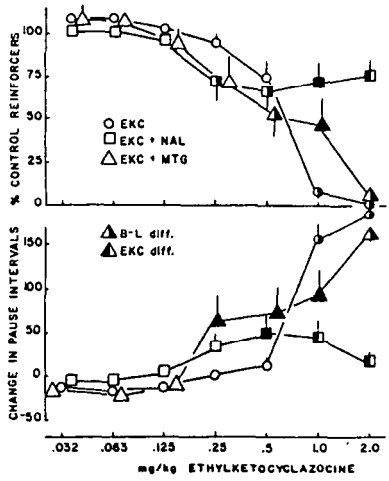

Fig. 4. Dose-response of ethylketocyclazocine (EKC) alone, combined with 4 mg/kg naloxone (NAL), or combined with 1 mg/kg metergoline (MTG).

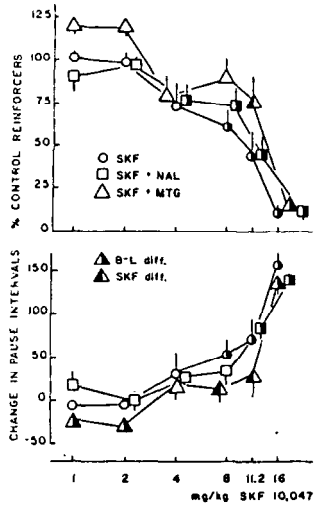

Fig. 5. Dose-response of SKF 10,047 (SKF) alone, combined with 4 mg/kg naloxone (NAL), or combined with 1 mg/kg metergoline (MTG).

DISCUSSION

The shifts of the dose-response curves of LSD, DOM (Fig. 1 and mescaline (Commissaris et al., 1980) to the left by pretreatment with 4 mg/kg NAL were not parallel, suggesting that the opioid interaction is modulatory and not exerted at the same receptors as those affected by the hallucinogens. Similar results were found for LSD and DMT by Ruffing and Domino (1981). Additionally, they observed antagonism of the operant behavioral disruption of hallucinogens by pretreating with low doses of morphine or methadone. The dose of NAL required to produce these interactions (2-8 mg/kg) is in the range optimal for kappa receptor antagonism rather than that which is optimal for mu antagonism (0.2-1.0 mg/kg).

Disruption of the FR-40 operant pattern by these hallucinogenic drugs is characterized by dose-related decreases in responses (reinforcers earned) correlated with increases in pause intervals (10-sec intervals without a

response) over the entire dose-response curve (Rech and Commissaris, 1982). This pattern of impairment is not observed with many other psychoactive drugs (chlorpromazine, d-amphetamine, pentobarbital), but is seen with other 5-HT agonists, such-as QUIP and lisuride. The operant behavioral effects of hallucinogens and non-hallucinogenic 5-HT agonists are attenuated by pretreating with MTG and other 5-HT antagonists, but the rate-decreasing effect of the other classes of psychoactive agents is not affected by these pretreatments. However, the opioid mixed agonist-antagonist CYCL was found to disrupt the FR-40 operant pattern in the same way as the hallucinogens did, i.e., a decrease in reinforcers correlated with a reciprocal increase in pause intervals (Henck et al., 1983). Furthermore, this impairment was partly antagonized by pretreating with NAL, MTG, or a combination of the two.

The behavioral effects of CYCL were attenuated by a low dose of QUIP (Fig. 2). This antagonism may relate in part to the opioid-like effects of QUIP (Minnema et al., 1980; Samanin et al., 1976). However, 5-HT influences may also pertain, since combined pretreatment with QUIP and MTG reversed the protection by QUIP at the higher doses of CYCL. QUIP itself, at higher dose levels, caused a decrement in FR-40 responding (Fig. 3), which was little affected by pretreatment with NAL or a low dose of CYCL. Nevertheless, the combined pretreatment with NAL and CYCL reversed both the decrease in reinforcers and the increase in pausing caused by the 2 mg/kg dose of QUIP. It seems likely that both opioid and 5-HT mechanisms are involved in this complex interaction.

If the CYCL effects on FR-40 behavior act in part through kappa opioid receptors, the more selective kappa agonist EKC might show similar effects. However, interactions of EKC with NAL or MTG were quite complex (Fig. 4). The slight potentiation of lower doses of EKC by NAL may relate to subtle opioid influences at other than kappa receptors. The prominent protection by NAL against higher doses of EKC probably does involve kappa receptors. The fact that MTG pretreatment enhanced the disruptive effects of low doses of EKC but attenuated those from a higher dose suggests that EKC interacts in a complex manner with 5-HT mechanisms that influence this behavior.

Lastly, the actions of SKF (Fig. 5) must be mediated via mechanisms different from those involved in the effects of indole and phenethylamine hallucinogens and QUIP and CYCL. NAL had no effect on the SKF dose-response pattern in keeping with a mechanism involving sigma receptors, which are considered NAL-insensitive (Simon and Hiller, 19781 MTG pretreatment exerted only a slight protection against the behavioral decrement caused by SKF, indicating that the latter drug does not act primarily via 5-HT receptors.

The results of this study show that the opioids CYCL and EKC produce their disruption of operant behavior by interacting, at least in part, with 5-HT and NAL-sensitive opioid systems. Thus, they appear to exert their effects in a manner related to the actions of indole and phenethylamine hallucinogens. On the contrary, the effects of SKF appear to be mediated mainly through mechanisms different from those of the indole and phenethylamine hallucinogens.

REFERENCES

Cervo, L., Rochat, C., Romandini, S., and Samanin, R. Evidence of a preferential role of brain serotonin in the mechanisms leading to naloxone-precipitated compulsive jumping in morphine-dependent rata. Psychopharmacol, 74:27l-274, 198 1.

Commissaris, R.L., Moore, K.E., and Rech, R.H. Naloxone potentiates the disruptive effects of mescaline on operant responding in rats. Pharmacol Biochem Behav, 13:601-603, 1980.

Henck, J.W., Mokler, D.J., Commissaris, R.L., and, Rech, R.H. Cyclazocine disruption of operant behavior is antagonized by naloxone and metergoline. Pharmacol Biochem Behav, 18:41-45, 1983.

Minnema, D.J., Dewitt, L., and Rosecrans, J.A. Antinociceptive comparison of quipazine and morphine. Commun Psychopharmacol, 4:115-120, 1980.

Rech, R.H. and Commissaris, R.L. Neurotransmitter basis‘ of the behavioral effects of hallucinogens. Neurosci Biobehav Rev, 6:521-527, 1982.

Ruffing, D. and Domino, E.F. Effects of selected opioid agonists and antagonists on DMT- and LSD-25-induced disruption of food-rewarded bar pressing behavior in the rat. Psychopharmacol, 75:226-230, 1981.

Samanin, R., Bernasconi, S., and Quattrone, A. Antinociceptive action of quipazine: Relation to central serotonergic receptor stimulation. Psychopharmacol, 46:219-222, 1976.

Simon, E.J. and Hiller, J.M. The opiate receptors. Annu. Rev. Pharmacol. Toxicol., 18:189-204, 1978.

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Bioassay of Subjective Effects Associated With Benzodiazepine Withdrawal in Animals: A Novel Direction in Dependence Research

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In humans, the earliest arising and most readily detectable symptom of withdrawal from drugs of dependence are subjective in nature. For example, cessation of high-dose use of a number of drugs, including benzodiazepine anxiolytics, initially produces complaints of anxiety *or* a general sense of uneasiness. For most substances with dependence liability, as withdrawal progresses, subjective symptoms are superseded by more vivid physical manifestations of abstinence, and it is only these latter phenomena that have been well characterized in the drug-dependence literature. Signif-icantly, however, continuation of the self-administration cycle in man is not contingent on the emergence of overt behavioral signs of withdrawal; therefore, subjective events occurring early during drug withdrawal may be an important factor in continued drug dependence.

By definition, subjective events are not directly verifiable by experimenter observation, and becauseo f the dangers of anthro-pomorphism, animal investigations of subjective events are particularly difficult. On the other hand, subjective events can be tested experimentally if behavioral responses can be made specifically contingent upon detection of the subjective occurrence by the test subject. For example, subjects can be trained to use the interoceptive discriminative stimuli (IDS) arising from drug injection as the basis for choosing which of several potential responses is correct. When only two responses are available, the response emitted, be it human-verbal or animal-choice behavior, resolves to "yes, the stimulus is present," or "no, it is not." The qualitative nature of such a binary decision can then be quantified through the method of population analysis; that is, the percent of subjects reporting the presence of the subjective event is a function of the stimulus intensity. In the past decade, many investigations have shown that drug effects thus measured are classified in parallel (e.g. LSD-like, narcotic-like) by humans (Altman et al. 1977) and animals (Glennon and Rosecrans 1981).

This paper illustrates application of drug discrimination technology to quantify in animals a subjective factor that frequently occurs in man during withdrawal from drugs of dependence. The rationale was to train animals to discriminate a drug possessing IDS properties that

can be related to a subjective symptom often noted in man during withdrawal: anxiety. Pentylenetetrazol (PTZ) administered in sub-convulsant doses was chosen as the training drug because the IDS produced by PTZ are correlated with the reported anxiogenic effect of this compound in man (Rodin and Calhoun 1970), and discrimination of these IDS has been proposed as an animal model of anxiety (Lal and Shearman 1980) because 1) clinically efficacious anxiolytics block PTZ IDS, 2) drugs known to be anxiogenic in man generalize to PTZ IDS, and 3) although PTZ is a convulsant, non-anxiolytic anti-convulsants do not block PTZ IDS (Lal and Emmett-Oglesby 1983).

In this paper we summarize results from pilot experiments designed to detect PTZ-like stimuli occurring following termination of short-term administration of diazepam and pentobarbital. The results show that PTZ-like stimuli are produced by diazepam withdrawal, whether precipitated by a benzodiazepine antagonist or produced by termination of diazepam administration, and that these stimuli are alleviated by diazepam and pentobarbital. Short-term administration of pentobarbital failed to produce similar signs.

METHODS

Male Long-Evans hooded rats were trained and tested in conventional behavioral chambers (Coulbourn Instruments) as described by Lal and Emmett-Oglesby (1983). Scheduling of reinforcement contingencies and-recording of data was done through TRS-80 microcanputers and printers (Radio Shack) connected to the chambers through LVB interfaces (Med Associates, Inc. using a program described by Emmett-Oglesby et al. (1982). Briefly, subjects were trained to press one of two levers to obtain a food reward. During training, the correct lever to press was determined by injection conditions: following administration of PTZ, 20 q/kg, only responses on one of theleverswererewarded; following non-PTZ treatments, only responses on the other lever were rewarded. In either case, 10 responses had to be emitted on the correct lever in order to obtain reinforcement. Once subjects had learned the PTZ-saline discrimination to the criterion of selecting the correct lever on ten consecutive training days, experimental testing was begun. On test sessions, independent of the injection condition, the lever upon which ten responses were first emitted was considered selected and was reinforced.

Following training, subjects were tested for the ability of various treatments to generalize to or block PTZ IDS. Subsequently, training and testing were stopped, and diazepam, 20 q/kg, was injected every 8 hours. After 5 days of this regimen, the ability of Ro 15-1788 to elicit PTZ-IDS was tested. Fifteen minutes following diazepam administration, Ro 15-1788 was administered, and rats were tested for lever selection 15 minutes later. Following these tests, chronic diazepam administration was continued, and on days 7-10, the ability of various treatments to generalize to or block PTZ IDS was tested at 8 hours after a preceding diazepam injection. After 11 days of diazepam administration, chronic injections were terminated, and lever selection was tested at various times over the next days. In a second experiment, pentobarbital was injected in doses of 160 mg/kg/day for 7 days

followed by 240 mg/kg/day for an additional 7 days. Subsequently, chronic injections were terminated, and generalization to the PTZ stimulus was tested at various times over the next 36 hours.

RESULTS

The PTZ discrimination was well established after training (Table 1). Prior to diazepam dependence, all rats selected the PTZ-correct lever following PTZ injection and the saline-correct lever following saline injection (Table 1). In addition, on acute administration, neither diazepam, clonidine (Table 1) nor Ro 15-1788 (Figure 1) generalized to the PTZ stimulus. Diazepam did, however, antagonize the PTZ cue (Table 1).

TABLE 1. Generalization of various treatments to the pentylene-tetrazol stimulus prior to chronic diazepam.

Test Drug	Dose (mg/kg)	Number of Rats Tested	% Selecting PTZ Lever(1)
Saline	--	26	0
Pentylehetetrazol	20.0	26	100
Diazepam	10.0	10	0
Clonidine	0.04	5	0
	0.16	4	0
Pentylehetetrazol + Diazepam	20.0 + 5.0	9	11
Pentylenetetrazol + Clonidine	20.0 + 0.04	5	100
Pentylenetetrazol + Clonidine	20.0 + 0.16	5	100

(1) % subjects emitting the first 10 responses on the PTZ-correct lever.

PTZ-like IDS occurred in diazepam-treated subjects after both precipitated and spontaneous withdrawal. In contrast to the failure of Ro 15-1788 to generalize to PTZ prior to dependence, in rats treated with diazepam for 5 days, Ro 15-1788 produced dose-dependent selection of the FTZ lever with an ED50 of 7.1 mg/kg (Fig. 1).

Termination of two weeks of chronic pentobarbital administration did not produce significant generalization to PTZ IDS (Table 2). However, spontaneous withdrawal from 11 days of 60 mg/kg/day of diazepam did produce generalization to the PTZ stimulus (Table 2). This generalization was greatest at 8 hours (chi square = 12.5, $p<.001$) of withdrawal (earliest time tested), and was still detectable 16 days after termination of chronic diazepam. At the 8-hour spontaneous withdrawal point, pentobarbital blocked the appearance of PTZ-like IDS, whereas clonidine did not (Table 3). When these subjects were moved from the chamber, injected with Ro 15-1788 and returned to the chambers, pentobarbital still blocked PTZ-lever selection, but clonidine did not (Table 3).

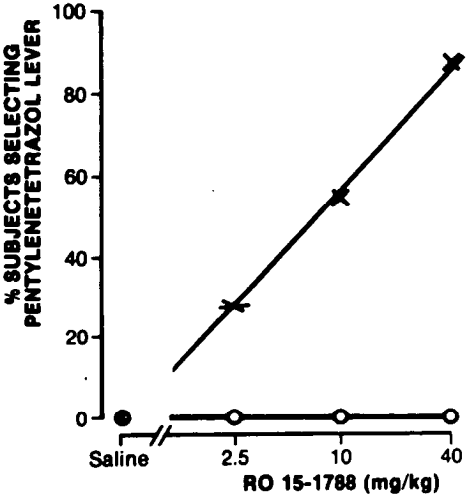

Figure 1. Dose-dependent generalization of Ro 15-1788 to the pentylenetetrazol stimulus. Abscissa: dose of Ro 15-1788. Ordinate: percentage of rats canpleting the first ten responses on the PTZ lever. 0 = non-diazepam dependent . X = diazepam dependent.

TABLE 2. Generalization to the pentylenetetrazol stimulus following termination of chronic pentobarbital(1) or diazepam(2).

Pentobarbital		Diazepam	
Hours After Last Dose	% selecting PTZ Lever(3)	Days After Last Dose	% Selecting PTZ Lever
2	22	0.33	60
6	11	1	55
12	33	2	35
24	0	3	40
36	11	8	30
			33
		16	25

(1) Pentobarbital was given i. p., 40 mg/kg/injection, 4 times per day for one week follwed by 6 times per day during the second week.
(2) Diazepam was given i. p., 20 mg/kg/injection, 3 times per day for eleven days.
(3) See Table 1 for testing criterion. Spontanous occurrence of PTZ-like stimuli during withdrawal was tested by determining lever selection following saline injection.

TABLE 3. Generalization of various treatments to the pentylene-tetrazol stimulus after 8-hour withdrawal fran chronic diazepam.(1)

Treatment(2)	Dose	Number of Subjects	% Selecting PTZ Lever(3)
Saline	-	16	56
Pentobarbital	10	6	0
Clonidine	0.04	7	43
Pentobarbital + RO 15-1788	10 10	6	0
Clonidine + Ro 15-1788	0.04 10	9	54

(1) Tests were conducted on days 7 through 10 of chronic diazepam administration, 20 q/kg, every 8 hours.
(2) Treatments occurred 8 hours post diazepam.
(3) See Table 2.

DISCUSSION

Physical dependence on diazepam has been reported in man (Marks 1978) and laboratory animals (Martin et al. 1982). What is novel in the present results is that a subjective aspect of withdrawal can be detected and quantified in laboratory animals. The discrimination of non-convulsant doses of pentylenetetrazol, an animal assay for drug effects related to anxiety in man (Lal and Emmett-Oglesby 1983), detects withdrawal from diazepam, whether precipitated or spontaneous. Previous studies using overt physical signs of withdrawal typically have found that benzodiazepine dependence in rats can only be obtained after high-dose and/or prolonged administration (Boisse et al. 1982; Martin et al. 1982). The relatively short period taken in this study to demonstrate withdrawal suggests that subjective aspects of withdrawal can be detected and quantified after far less dependence than is required to produce overt signs of abstinence.

Physical dependence on pentcbarbital has also been reported in laboratory animals Martin et al. 1982), although demonstration of physical manifestations of withdrawal requires prolonged and high-dose administration. Thus, the failure to detect pentobarbital withdrawal in the present experiment may be a reflection of inadequate dose or duration of pentobarbital treatment. These results can be contrasted with those for diazepam, suggesting that, benzodiazepines may have a greater propensity for producing subjective signs of withdrawal, even after relatively brief periods of administration.

Acute administration of Ro 15-1788, a benzodiazepine receptor antagonist (Mohler et al. 1981), generalized to PTZ only during chronic diazepam administration. When combined with the results

from spontaneous withdrawal, these data suggest that Ro 15-1788 precipitated diazepam withdrawal. This conclusion is supported by previous studies showing that Ro 15-1788 precipitates physical signs of withdrawal in animals exposed to prolonged high-dose benzcdiazepine treatment (Boisse et al. 1982).

Spontaneous withdrawal signs, as wellas those precipitated by Ro 15-1788, were blocked by pentobarbital, but not by clonidine. Pentobarbital is a non-benzodiazepine drug with anxiolytic properties, and there is substantial evidence that its effects are not mediated by benzodiazepine receptors (Davis and Ticku 1981). The present findings support the hypothesis that a non-benzodiazepine receptor mechanism is responsible for the pentobarbital alleviation of benzodiazepine withdrawal. Clonidine is effective in relieving signs of opiate abstinence in man (Gold et al. 1978) and animals (Fielding et al. 1978), but the ability of clonidine to allieviate benzodiazepine withdrawal signs is unknown. The present data suggest that clonidine would not be expected to relieve anxiety symptoms produced by benzodiazepine withdrawal.

Thedetection of PTZ IDS has been proposed as an animal modelof anxiety, and the appearance of PTZ-like IDS during withdrawal from diazepam is in agreement with reports in man that anxiety is a protracted and frequently seen symptom of benzodiazepine withdrawal (Marks 1978). The present results may thus aid in resolving a controversy surrounding the role of drug dependence in the appearance of subjective complaints when a drug is terminated. Clinically, there is debate as to whether such anxiety is a manifestation of drug withdrawal or is only a return of the patient's preexisting condition. Petursson and Lader (1981) have argued that anxiety often goes unrecognized as a true withdrawal sign because anxiety was usually the condition for which the benzodiazepine was initially prescribed. The present data support their conclusion that subjects do indeed experience witbdrawal-induced anxiety.

In summary, our data suggest that a subjective manifestation of drug withdrawal, related toanxiety in man, can be assayed in animals. This sign can be detected and quantified at levels of dependence much less than those necessary to produce more overt signs (e.g., convulsions) of withdrawal. Thus, the pentylenetetrazol disc rimination paradigm can be used to test subjective signs of drug withdrawal, and it can be employed to investigate neurochemical and behavioral factors mediating these signs.

REFERENCES

Altman; J., Albert, J., Milstein, S., and Greenberg; J. Drugs as discriminableevents in humans. Adv Behav Biol, 22: 187-206, 1977.

Boisse, N., Gay, M., Guarino, J., Kruger, H., and Ryan, G. Antagonist precipitated withdrawal following chronic chlor-diazepoxide dosing in the rat. Abs Soc Neurosci, 8: 396, 1982.

Davis, W.C., and Ticku, M. K. Picrotoxinin and diazepam bind to two distinct proteins: Further evidence that pentobarbital may act at

the picrotoxinin site. J Neurosci, 1: 1036-1042, 1981.

Emmett-Oglesby, M. W., Spencer, D. G., Jr., and Arnoult, D. E. A TRS-80-based system for the control of behavioral experiments. Pharmacol Biochem Behav, 17: 583-587, 1982.

Fielding, S., Wilker, J., Hynes, M., Szewezak, M., Novick, W., Jr., and Lal, H. A comparison of clonidine with morphine for antinociceptive and antiwithdrawal activity. J Pharmacol Exp Ther, 207: 899-905, 1978.

Glennon, R., and Rosecram, J. Speculations on themechanismof action of hallucinogenic indolealkylamines. Bio Behav Rev, 5: 197-207, 1981.

Gold, M. S., Redmond, D. E., Jr., and Kleber, H. D. Clonidine blocks acute opiate withdrawal symptoms. Lancet, 2: 599-602, 1978.

Lal, H., and Emmett-Oglesby, M. W. Behavioral analogues of anxiety: animal models. Neuropharmacol, (In Press).

Lal, H., and Shearman, G. Interoceptive discriminative stimuli in the development of CNS drugs and a case of an animal model of anxiety. Ann Rev Med Chem, 15: 51-58, 1980.

Marks, J. The Benzodiazepines: Use, Overuse, Misuse, Abuse. Lancaster, England, MTP Press, 1978.

Martin, W. R., McNicholas, L. F., and Cherian, S. Diazepam and pentobarbital dependence in the rat. Life Sci, 31: 721-730, 1982.

Mohler, H., Burkard, W., Keller, H., Richards, J., and Haefely, W. Benzodiazepine antagonist RO 15-1788: binding characteristics and interactionwithdrug-induced changes in dopamine turnover and cerebellar cGMP levels. J Neurochem, 37: 714-722, 1981.

Petursson, H., and Lader, M. H. Withdrawal fran long-term benzcdiazepine treatment. Br Med J, 283: 643-645, 1981.

Rodin, E. A. and Calhoun, H. D. Metrazol in a "normal" volunteer population. J Nerv Ment Dis, 150: 438-443, 1970.

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Self-Administration of Clonidine and Oxazepam by Methadone Detoxification Patients

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The benzodiazepines and clonidine have frequently been used to alleviate withdrawal symptoms during methadone detoxification. Clonidine has been shown to be effective in decreasing the symptoms of abrupt withdrawal from chronic methadone (Washton and Resnick 1981; Charney et al. 1981); however, its usefulness in gradual methadone detoxification, a procedure more commonly used in outpatient methadone clinics, has yet to be demonstrated. Because of the apparent efficacy of clonidine as a treatment agent in opioid withdrawal, it has been suggested that clonidine may possess some potential abuse liability -- either because of its opioid-substitution properties or because of other sedative or euphorigenic properties which it might have. Clonidine has been shown to support intravenous self-administration in both the rat (Davis and Smith 1977; Shearman et al. 1977) and the rhesus monkey (Woolverton et al. 1982) . The availability of clonidine in the illicit street market and the existence of some degree of drug abuse has been reported (Gold et al. 1980).

The use of benzodiazepines and related compounds as adjuncts in the treatment of the opioid withdrawal syndrome has been commonly recommended (Goldstein 1972; Kleber and Gold 1978) and is commonly practiced. Although insomnia, anxiety, and muscle cramps associated with methadone detoxification would appear to indicate the use of these drugs, their efficacy as adjuvants in methadone detoxification has not been demonstrated in controlled studies. It is well established that the benzodiazepines possess some degree of abuse potential and will be self-administered by opioid addicts (Woody et al. 1975; Stitzer et al. 1981). The reinforcing properties of these agents may account for their popularity in the treatment of methadone withdrawal symptoms.

The purpose of the present study was to examine the extent and pattern of self-administration of clonidine and oxazepam during methadone detoxification and to examine the extent to which these drugs alleviate the symptoms of opioid withdrawal. Two groups of methadone detoxification patients were used. In one group we compared clonidine and placebo, and in the second group we compared

oxazepam and placebo. The procedures used were the same for both groups and consisted of two components: (1) to assess efficacy, patients were given exposure to active drug and placebo, and the acute effects of the two agents and their effects on withdrawal symptoms were monitored; and (2) to assess drug preference, following the forced exposure, subjects were given the opportunity to self-administer the drug of their choice and to choose the dose of that drug. These preference data gave an indication of patient acceptability of the drug and possibly some measure of abuse liability. The two components were repeated six times over the course of the detoxification to assess the relationship between drug efficacy and preference and withdrawal symptomatology.

METHODS

Subjects and general procedures: The participants were 12 male methadone maintenance patients who requested inpatient detoxification from methadone, and who gave their informed consent to participate in this six-week research protocol. The average age of the subjects was 32 years (range 23 - 41 years). The subjects reported an average of 13 years (range 3 - 25 years) prior narcotic addiction. The average daily methadone maintenance dose was 39.6 mg (range 30 - 50 mg).

Subjects participated while residing on an 8-bed behavioral pharmacology research ward. On the basis of admission data subjects were assigned to either the clonidine (N=6) or oxazepam (N=6) group. The study was run in double-blind fashion; neither the subjects nor the staff were informed of the methadone detoxification schedule or group assignment.

Methadone detoxification schedule: Methadone was administered in a constant volume (60 ml) of cherry syrup solution once daily at 6:00 p.m. Subjects were maintained on their maintenance doses for the first 5 to 6 days that the protocol was in effect, followed by five days at one-half of the maintenance dose. The methadone dose was then decreased to zero over the next 10 days. The subjects continued to receive a constant volume of methadone placebo (cherry syrup) throughout the rest of the study (15 days).

Adjunct medications: Placebo (lactose) and commercially available preparations of oxazepam and clonidine hydrochloride were administered in colored opaque capsules and given an arbitrary letter code (e.g., A and B). Codes and colors varied between subjects but were held constant over each subject's protocol.

The adjunct medication schedule began on the first day of methadone dose reduction (50% maintenance dose) and was arranged into six blocks of five days. Due to occasional early subject dropout, some subjects completed fewer than six blocks. On day 1 of each block (baseline) no adjunct medications were given. On days 2 and 3 of each block (no-choice trials), the subjects were administered three capsules of placebo or drug (oxazepam 10 mg/capsule or clonidine 0.1 mg/capsule) two times daily at 9:00 a.m. and 3:00 p.m.

The order of the administration of placebo or drug was counterbalanced over the six blocks and across subjects.

On days 4 and 5 of each block (choice trials), subjects were given the opportunity at each of the scheduled dosings (9:00 a.m. and 3:00 p.m.) to choose which of the two drugs they would receive and the number of capsules (1, 2, or 3 capsules) they would receive. Subjects were required to take at least one capsule of their choice at each drug administration. The opportunity to choose the number of capsules to take allowed subjects to titrate the drug effect.

Subjective and performance measures: Multiple subjective effect measures were recorded throughout the day. A physical symptoms questionnaire, developed in this laboratory to measure opioid withdrawal symptoms experienced over the previous 24 hours, was filled out by the subjects daily at 8:30 a.m. The questionnaire consisted of 60 items which the subjects rated on a 4-point scale from not at all (0) to severe (3). At 11:30 a.m. and 5:30 p.m., 2.5 hours after each adjunct medication administration, subjects filled out a visual-analog questionnaire which contained scales for strength of drug effects, drug liking, and "good" and "bad" drug effects. Also at 5:50 p.m. subjects performed two psychomotor performance tasks -- a computerized Digit Symbol Substitution Test (McLeod et al. 1982) and a test of hand-eye coordination (Saccadic Fixator and Sequence Rotator, Wayne Engineering).

Data were analyzed to assess: (1) comparative effects of active drug versus placebo on symptomatology, other subjective measures, drug preference and self-administration behavior, and psychomotor/cognitive performance; and (2) changes in these effects over the course of the methadone detoxification (i.e., over successive test blocks).

RESULTS AND DISCUSSION

Overall results have shown, at the doses tested, that: (1) neither drug showed significantly greater efficacy than placebo in relieving the discomfort associated with this gradual methadone detoxification; (2) both drugs showed considerable between-subject variability in free-choice self-administration, with some subjects consistently choosing each of the active drugs in preference to placebo; and (3) clonidine, to a greater extent than oxazepam, produced a significant disruption in psychomotor/cognitive performance.

Table 1 shows for successive test blocks (indicated by Roman numerals) the mean methadone doses dispensed, mean physical symptom scores for no-choice trials, and mean percent of choice trials in which active drug was chosen (parenthetical numbers are standard errors of the mean). For all subjects as a whole, symptom scores tended to increase as methadone dose decreased, peaked as the methadone dose reached zero (Block IV), and then declined. However, for neither oxazepam nor clonidine was there a signifi-

cant reduction in symptomatology relative to their respective placebo control conditions -- either for specific test blocks or for the detoxification as a whole. Mean preference for active drug over successive test blocks was fairly stable for the oxazepam subjects, but more variable for the clonidine subjects; in neither case was there a marked preference for the active drug and in neither case did preference appear to be related to position in the methadone dose reduction schedule or to reported withdrawal symptomatology

TABLE 1: *Symptom scores and drug preference during methadone detoxification.*

	Successive Test Blocks					
	I	II	III	IV	V	VI
$\bar{x}$ % Methadone Maintenance Dose	50	35	10	0	0	0
	Oxazepam/Placebo Group					
Physical Symptom Scores						
Oxazepam	11.7 (2.8)	10.0 (2.9)	20.2 (4.7)	26.7 (5.7)	20.3 (7.4)	18.0 (7.6)
Placebo	11.2 (3.1)	11.0 (3.8)	15.5 (2.8)	31.2 (6.6)	22.8 (8.7)	20.4 (7.1)
% Oxazepam Choices	67 (14)	67 (14)	67 (14)	67 (15)	70 (18)	40 (17)
	Clonidine/Placebo Group					
Physical Symptom Scores						
Clonidine	15.0 (3.2)	28.2 (10.8)	41.2 (10.3)	46.2 (3.7)	39.5 (6.4)	21.0 (10.6)
Placebo	18.5 (8.0)	24.5 (8.5)	42.2 (10.5)	50.6 (7.0)	40.2 (8.5)	21.6 (9.2)
% Clonidine Choices	54 (17)	58 (18)	75 (16)	60 (22)	38 (21)	50 (24)

Values shown are means; standard errors are in parentheses.

Examination of the choice-trial data for individual subjects revealed considerable between-subject variability in drug versus placebo preferences, both among oxazepam and among clonidine subjects. These data are shown in Table 2. While the group average data show no significant overall preferences, several individual subjects in each group did show consistent preferences. Three out of six subjects consistently chose oxazepam over placebe; one

subject appears to have avoided oxazepam, and two subjects showed no preference. Oxazepam was chosen in a mean of 65.9% (SE 13.4) of all choice trials, not significantly more than placebo. On trials in which oxazepam was chosen, subjects took an. average of 26.5 mg (or about 2½ capsules). Clonidine was chosen consistently by four of six subjects; the remaining two subjects appeared to avoid clonidine. Clonidine was chosen in a mean of 61.3%. (SE 15.3) of all choice opportunities, not significantly different from placebo. The average dose of clonidine was 0.24 mg (or about 2½ capsules).

TABLE 2: *% Active drug choices for individual subjects*

	Oxazepam (N=6)	Clonidine (N=6)
	100	100
	100	92
	87	81
	58	75
	38	20
	12.5	0
X % Choices	65.9	61.3
S.E.	13.4	15.3

Both oxazepam and clonidine produced deficits in psychomotor performance at the doses tested (Table 3). Both drugs significantly decreased performance on the hand-eye coordination task; between-drug comparison showed that the performance deficit produced, by clonidine was significantly greater than that produced by oxazepam ($p<0.05$; two tailed t-test). Only clonidine significantly decreased performance on the Digit Symbol Substitution Test.

TABLE 3: *Psychomotor performance decrements: Mean differences from placebo*

	Oxazepam	Clonidine
Digit Symbol Substitution Test	-0.76 (0.90)	-5.35" (1.12)
Hand- Eye Coordination Test	-3.41* (1.00)	-15.29” (2.01)

Standard errors are shown in parentheses.
** p<0.05, two tailed t-test.*

In addition to producing significant deficits in psychomotor performance, both drugs were reported on the visual analog scales to be significantly different from placebo for strength of drug effects and for good drug effects. Thus, the doses of clonidine and oxazepam were high enough to produce appreciable objective

effects and to be discriminated from placebo.

In summary, the results from clonidine, a proposed specific withdrawal treatment, and oxazepam, a more symptomatic treatment, were similar. Neither drug alleviated withdrawal symptoms as measured, nor were the patterns of self-administration of the agents related in any apparent way to the schedule of detoxification or pattern of withdrawal symptoms. Although some individual subjects consistently preferred each of the active drugs over placebo, the preferences between placebo and the active drugs in the overall group data were not significant. Two points, however, are noteworthy: (1) at least half of the subjects in each group consistently chose active drug over placebo in spite of the fact that these drugs did not appear to be better than placebo in alleviating withdrawal symptoms; and (2) clonidine produced substantial impairments in psychomotor performance, indicating a risk of behavioral toxicity associated with its use as a detoxification adjunct.

REFERENCES

Chamey, D.S., Steinberg, D.E., Kleber, H.D., Heninger, G.R., and Redmond, E. The clinical use of clonidine in abrupt withdrawal from methadone. Arch Gen Psychiatry, 38:1273-1277, 1981

Davis, W.M., and Smith, S.G. Catecholaminergic mechanisms of reinforcement: Direct assessment by drug self-administration. Life Sci, 20:483-492, 1977.

Gold, M.S., Pottash, A.C., Sweeney, D.R., and Kleber, H.D. Opiate withdrawal using clonidine. JAMA, 243:343-346, 1980.

Goldstein, A. Heroin addiction and the role of methadone in its treatment. Arch Gen Psychiatry, 25:291-297, 1972.

Kleber, H.D., and Gold, M.S. Use of psychotropic drugs in treatment of methadone maintained narcotic addicts. Ann NY Acad Sci, 311:81-98, 1978.

McLeod, D.R., Griffiths, R.R., Bigelow, G.E., and Yingling, J. An automated version of the digit symbol substitution test (DSST). Behav Res Methods Instrum, 14:463-466, 1982.

Shear-man, G., Hynes, M., Fielding, S., and Lal, H. Clonidine self-administration in the rat: A comparison with fentanyl self-administration. Pharmacologist, 19:171, 1977.

Stitzer, M.L., Griffiths, R.R., McLellan, A.T., Grabowski, J., and Hawthorne, J.W. Diazepam use among methadone maintenance patients: Patterns and dosages. Drug Alc Depend, 8:189-199, 1981.

Washton, A.M., and Resnick, R.B. Clonidine in opiate withdrawal: Review and appraisal of clinical findings. Pharmacother, 1: 140-146, 1981.

Woody, G.E., O'Brien, C.P., and Greenstein, R. Misuse and abuse of diazepam: An increasingly common medical problem. Int J Addict, 10:843-848, 1975.

Woolverton, W.L., Wessinger, W.D., and Balster, R.L. Reinforcing properties of clonidine in rhesus monkeys. Psychopharmacology, 77:17-23, 1982.

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Marijuana, Affect and Tolerance: A Study of Subchronic Self-Administration in Women

Thomas F. Babor, Barbara W. Lex, Jack H. Mendelson, and Nancy K. Mello

The study of differential tolerance development has important implications for evaluation of the abuse potential of a particular drug. Tolerance to the disruptive behavioral and physiological effects of marijuana could make it possible for experienced users to function at their "normal" level of efficiency, even after heavy, daily consumption. On the other hand, tolerance to the positive psychological effects that users variously describe as euphoric, pleasurable and consciousness-expanding, would diminish the reinforcing value of the drug at a given dose, and thereby increase the necessity of more intense or more frequent consumption to achieve previous levels of intoxication. In the light of evidence that chronic marijuana consumption may be related to impaired respiratory function and other health consequences, tolerance to these effects might prove detrimental to the user (Institute of Medicine, 1982).

To demonstrate that tolerance to THC leads to increased consumption, it would be necessary to establish that: 1) reliable changes are produced by acute doses of marijuana to psychological states, behavioral performance, or physiological function; 2) these effects diminish over a period of time when marijuana iS administered repeatedly; 3) marijuana self-administration increases progressively in those individuals who have developed tolerance. To date, several studies of subchronic self-administration conducted in controlled settings provide some evidence of the inadequacy of this tolerance model to fully explain the often noted tendency for regular users of marijuana to increase consumption over time (Mendelson, Meyer and Rossi 1976; Babor et al. 1975).

One limitation of these studies is that they have been conducted exclusively on male research subjects. In view of the growing concern over adverse effects of marijuana on female hormonal function (Institute of Medicine, 1982), it would seem important to investigate the determinants of marijuana consumption in women as well as men. Another limitation is that they have been restricted to only a few of the potentially relevant variables, specifically. pulse rate and subjective feelings of intoxication. Since affective states have often been implicated as possible reinforcers for marijuana consumption, it would also seem important to study tolerance development to these variables as well.

The present study was therefore designed to replicate previous research on tolerance development (Babor et al. 1975), using females with a previous history of either moderate or heavy marijuana consumption. Expanding the assessments employed in the previous investigation of males, this study included systematic measures of affective states. The relationship between tolerance development and marijuana consumption was evaluated by analysis of how the acute effects of marijuana on pulse rate, affective states, and subjective intoxication change during a 21-day period of free-choice marijuana self-administration.

METHODS

Subjects. Twenty-one adult female volunteers between the ages of 21 and 36 years were recruited through advertisements placed in local newspapers, On the basis of interviews and questionnaire data, subjects Were classified as either "moderate" or "heavy" users of marijuana. Moderate users were persons who smoked marijuana more than five times per month but less than daily during the previous year. Heavy users were persons who smoked marijuana between five and seven times per week during the previous year. All subjects had a history of at least two years marijuana use prior to admission to the study. The two groups did not differ significantly on any variable except prior marijuana consumption. Subjects tended to be lower middle or middle class single females in their late 20s with some college education. Experience with drugs other than marijuana and alcohol was infrequent, although all subjects indicated polydrug use patterns to some degree.

Experimental Design and Setting. The research was conducted within the context of an extensive multidisciplinary investigation of behavioral and biological concomitants of-free-choice marijuana smoking in women. Six identical studies were conducted, three of four-person groups and three of three-person groups. Each study lasted 35 days and consisted of three phases: a pre-drug baseline period lasting seven days; a 21-day drug acquisition period during which marijuana cigarettes could be purchased and smoked on a free-choice basis; and a seven-day post-drug period. During all three phases of the study, subjects had an opportunity to work at a simple operant task to earn points which were exchangeable for money. Money earned could be used to buy marijuana or could be retained by the subject at the Conclusion of the study. A full battery of assessments was performed daily to evaluate biochemical, physiological, and behavioral concomitants of subchronic marijuana smoking. The investigation took place on a closed research unit at McLean Hospital, Belmont, Massachusetts. Although 21 subjects eventually completed the research, only 18 were included in the present analyses for reasons described below.

Marijuana Dosage. Marijuana cigarettes were obtained from the National Institute on Drug Abuse in a lot standard dosage form. Each cigarette contained approximately one gram of marijuana with a THC content of 1.8 percent. During the 21-day drug acquisition period, subjects were free to determine frequency, amount, and duration of marijuana consumption. No attempt was made to standardize the ingestion procedure since the associated inconvenience might have inhibited free-Choice smoking behavior. However, a record was kept of the amount of unsmoked marijuana returned by each subject and this provided a basis for estimating amount consumed (and presumably, absorbed). Since it was important to control for cumulative and acute dosage in evaluating affective changes and the development of tolerance, only those subjects who self-administered marijuana on a regular schedule were included in the analysis. Using these criteria it was determined that nine "moderate" users and nine "heavy" users had administered relatively constant doses throughout the smoking period. On the average, the moderate smokers consumed more than 70 percent of each marijuana cigarette, while the heavy smokers consumed more than 84 percent. The differences in average dosage were statistically significant ($t=2.59$, $p < .05$). The three subjects omitted from this analysis consumed an average of only 50 percent of each marijuana cigarette.

Procedure. The major dependent variables selected for analysis were ratings of subjective intoxication level, readings of standing pulse rate, and ratings of an adjective checklist measuring eight dimensions of mood. Each assessment was conducted immediately before, immediately after, and 25 minutes after the first marijuana cigarette consumed each day. Ratings of subjective intoxication level were obtained by means of an 11-point scale based on the following question: "In comparison to the highest you've ever been on marijuana, rate below how high you feel now." The response categories ranged from (o) "No effect, not high at all" through (10) "Highest ever." Standardized mood reports were obtained by means of a 72-item version of the Profile of Mood States (POMS). Used widely as a measure of mood changes in drug evaluation research McNair, Lorr and Droppleman 1971). this simple adjective checklist provides interval measures of the following mood dimensions: tension, anger, depression, fatigue, confusion, elation, friendliness, and vigor.

To determine whether any of these assessments was sensitive to the acute effects of marijuana, comparisons were first made between ratings obtained before and after the first cigarette of the day. The first, cigarette of the day was used in these analyses in order to obtain response measures that were relatively independent of the previous acute dose, but nevertheless sensitive to the possible effects of cumulative doses on tolerance development. Those measures proving sensitive to the acute effect of marijuana were subjected to a correlational analysis, designed to measure the extent to which

the acute effect diminished with continued consumption. In this analysis, within-subjects correlation coefficients were first computed between the dependent measure obtained after each daily acute dose and the cumulative amount of marijuana consumed to that point. Cumulative dose was calculated by summing the amounts consumed over all previous doses, beginning on the first day of availability. To control for possible variations in self-determined dosage as well as in baseline levels of the dependent measure, these variables were partialled out of the resulting coefficient. Each subject's partial correlation coefficient was then transformed using Fisher's "z" transformation, and the transformed coefficients were averaged across subjects. To determine if the mean correlation was significantly different from zero, t values were computed by dividing the group mean by the standard error of the mean. To summarize the magnitude of the relationship, the average Fisher's z transformation was reconverted into a correlation coefficient, herein referred to as the average Pearson r.

RESULTS

The data were first analyzed to determine the extent to which acute effects of marijuana were manifested on the various dependent measures. Acute effects were evaluated immediately after and 25 minutes after marijuana smoking. In the first set of t-test comparisons, pulse rate, intoxication level and POMS scores recorded before the first marijuana cigarette smoked on the first day of availability are compared to corresponding values immediately after and 25 minutes after smoking. These comparisons, referred to as initial reactions, reflect the acute effects of marijuana after a period of at least seven days of total abstinence. The second set of comparisons was based on individual means of pulse rate, intoxication level, and POMS scores, averaged across all days that subjects self-administered marijuana. These comparisons reflect the consistency of the acute effect over the 21-day Self-administration period.

On average, moderate users chose to smoke marijuana on 18 of the 21 days of availability, while heavy users smoked on an average of 20 days. moderate users indicated initial reactions by significant increases in intoxication level, pulse rate, and in feelings of confusion and vigor after the first marijuana cigarette smoked. Intoxication level, pulse rate, vigor and confusion remained consistently elevated above pre-drug levels, as indicated in comparisons based on average values across all smoking days. In addition, increases in elation and friendliness were also manifested consistently during the entire smoking period.

On the first day of availability, heavy users showed a significant increase in pulse readings, intoxication level, and in feelings of elation and friendliness. Pulse, intoxication, and elation continued to be elevated over all subsequent trials as

well. In addition, heavy users showed a significant reduction in tension over all trials, and a significant increase in vigor.

Partial Correlation coefficients were next computed to determine the extent to which the acute effects of marijuana decline in relation to the cumulative amount smoked over the entire self-administration period. Moderate users showed a significant decline in level of intoxication, as well as significant reductions in feelings Of depression, fatigue, confusion, friendliness, and vigor. Heavy users indicated a progressive decline in intoxication level, tension, elation, and friendliness.

In summary, moderate users show evidence of tolerance to intoxication level, negative feeling states (depression, confusion), and several positive feeling states (vigor, friendliness): However, they do not indicate tolerance to elation. Heavy users whose acute effects were primarily in the areas of increased intoxication, tension reduction, elation, friendliness, and vigor, give evidence of tolerance to all of these effects except vigor: Neither group demonstrated tolerance to the pulse rate effect.

The final question addressed in this study pertains to the predicted association between tolerance development and increased marijuana consumption. To evaluate this hypothesis, the amount of change indicated on each variable after the first cigarette of the day was correlated with the amount of marijuana consumed in the subsequent 24-hour period. If tolerance development leads to increased marijuana consumption, then consumption should increase in direct relation to the decrease in acute effects over time. The results showed that on days when moderate smokers were feeling less elated, they tended to smoke more marijuana. These subjects also smoked more marijuana on days when, after the first cigarette smoked, they reported themselves more angry, depressed, and fatigued. Heavy users, on the other hand, showed no significant correlations between declining acute effects and Subsequent consumption.

DISCUSSION

The results of this study are consistent with several investigations on tolerance to marijuana in males (Babor et al. 1975; Mendelson, Meyer,and Rossi 1974). The findings suggest that tolerance develops differentially to a variety of acute effects in women, depending on previous drug history and cumulative dosage ingested over time. Further, the data suggest that the tendency to increase consumption over time may not be associated with tolerance to the acute effects of marijuana in women.

Acute effects after the first cigarette of the day were generally rated as pleasurable and stimulatory, although the less experienced moderate users also reported cognitive

confusion initially. Positive effects persisted over a three-week period of daily self-administration. Many of the subjective effects showed- a subtle but statistically significant decay over time, particularly the global rating of subjective intoxication.

Even though moderate users gave evidence of tolerance, there was no tendency for consumption to increase over the course Of the study. Although heavy users did increase consumption over time, further examination of the data provided no evidence to support the tolerance-consumption hypothesis. Nevertheless, the findings did show that moderate users tended to smoke more on days when anger or depression increased after the first dose of the day.

In conclusion, the results suggest that while tolerance development may be a necessary condition for increased marijuana use, it is by no means a powerful explanatory variable in accounting for individual differences in consumption, and for the tendency for consumption to increase over time. In the absence of a strong relation between tolerance development and consumption, it appears that a broader conceptualization of acquisition and dependence is necessary to account for chronic marijuana use as it occurs in the natural environment.

REFERENCES

Babor, T.F., Mendelson, J.H., Greenberg, I., Kuehnle, J.C. Marijuana consumption and tolerance to physiological and subjective effects. Arch. Gen. Psychiatry 32: 1548-1552, 1975.

Institute of Medicine. Marijuana and Health. Washington, D.C.: National Academy Press, 1982.

McNair, D.M.. Lorr, M., Droppleman, L.F. Profile of mood states (POMS). San Diego: Educational and Industrial Testing Service, 1971.

Mendelson, J.H., Rossi, A.M., Meyer, R.E. (Eds.) The Use of Marijuana: A Psychological and Physiological Inquiry. New York: Plenum Publishing Corp., 1974.

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Oral Fenoprofen Compared to Intramuscular Morphine and Oral Aspirin in Cancer Patients With Postoperative Pain

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The search for more effective non-narcotic analgesics has prompted the evaluation of selected nonsteroidal anti-inflammatory agents. Fenoprofen is one of several such drugs being evaluated for its analgesic effectiveness relative to narcotic analgesics in a variety of pathological pain models. These studies, however, have not been designed to provide estimates of relative analgesic potency compared to a "strong" narcotic analgesic such as intramuscular morphine. The primary objective of this study is to determine the potency of oral fenoprofen relative to intramuscular morphine. Secondary objectives are to determine: the effectiveness of fenoprofen relative to aspirin, 650 mg; the relative occurrence and spectrum of side effects; whether there is a relationship between age and relative analgesic potency; and the relationship between analgesia and mood for fenoprofen as compared to morphine.

METHODS

The assay consists of three equi-log-spaced doses of oral fenoprofen (50, 100 and 206 mg) and intramuscular morphine (4, 8 and 16 mg). Each patient receives two study medications on separate days: a lower dose of one drug and an upper dose of the other, or the middle doses of each drug on a double-blind, randomized basis, and balanced for order. Aspirin (656 mg) is substituted for half of the 8 mg morphine doses to provide for a second standard of comparison. The design incorporates a series of blocks of six patients each. Patients are assigned to a particular series of blocks according to three age groups: 18-35, 36-64 and 65 years and older.

The methodology adheres to the principles of clinical design and study previously employed and reported (Houde et al. 1960; Wallenstein and Houde 1975). Adult cancer patients with moderate to severe postoperative pain are seen hourly by an analgesic nurse observer who obtains the patient's subjective reports of pain intensity, pain relief and mood, employing both categorical and visual analog scales. Volunteered and observed side effects are also recorded. Observations are continued for either six hours or until pain returns to the premedication level, at which time the patient's routine analgesic is administered.

RESULTS

This assay remains in progress and, to date, 50 patients have received fenoprofen and morphine within balanced blocks, and 10 patients have received both fenoprofen and aspirin. There is, however, considerable data from yet uncompleted blocks and, also, from patients who had not completed the crossover.

Relative Potency

Estimates of relative potency were calculated using both the data from the twin-crossover comparisons within balanced blocks and the data from all first doses. Table 1 details the results.

TABLE 1. *Relative analgesic potency of oral fenoprofen and intramuscular morphine in cancer patients with moderate to severe postoperative pain.*

	Twin-crossover (50 patients, 100 obs.)			First dose (89 patients, 89 obs.)		
Variable[1]	ø[2]	95% C.I.[3]	λ[4]	ø	95% C.I.	λ
Total pain relief	0.11	(0.07–0.28)	0.88	0.10	(0.08–0.20)	0.66
Total pain decrease ...	0.10	(0.03–0.61)	1.12	0.05	(0.03–0.08)	0.77
Total mood improvement.	0.04	-	-	0.06	(0.03–0.14)	1.02
Peak pain relief	0.14	(0.07–1.19)	1.02	0.08	(0.07–0.14)	0.62
Peak pain decrease	0.09	-	-	0.08	(0.03–1.98)	1.23
Peak mood improvement .	0.04	-	-	0.06	-	-
Hours to remedication .	0.11	(0.08–0.17)	0.65	0.10	(0.08–0.20)	0.66

[1]Sum of hourly visual analog scale scores (total) or peak score.

[2]Ratio of morphine to fenoprofen doses consistent with equi-analgesic effect.

[3]The 95% confidence interval for the estimate of relative potency.

[4]An estimate of assay precision, the common standard error divided by the common slope; inversely related to precision.

To date, there is generally good agreement between the relative potency estimates using crossover and first dose data. Oral fenoprofen is about one-tenth as potent as intramuscular morphine in terms of analgesia. Comparisons of peak, total and duration estimates indicate little differences. While slopes do not significantly deviate from parallelism, dose-response curves for fenoprofen are consistently more shallow than those-for morphine, especially between- upper doses. While most potency estimates for mood are not yet significant, they are lower than those for analgesia.

Side Effects

Side effect data is based on all patients who had received a study medication, irrespective of whether or not they had completed the crossover, or whether or not they are within balanced blocks. Since analgesia following all doses of fenoprofen combined was comparable to that following all doses of morphine combined, we can be confident that we are comparing side effect data at equianalgesic doses, overall. The analgesic effect of aspirin, however, was significantly less.

TABLE 2. *Occurrence of side effects (SE) following intramuscular morphine, oral fenoprophen and aspirin in cancer patients (Pts) with postoperative pain.*

Treatment	IM morphine			PO fenoprofen			PO aspirin
Dose (mg)	4	8	16	50	100	200	650
No. of Pts	34	14	25	28	23	31	14
No. with SE ...	12	8	14	7	9	10	1
% with SE	35%	57%	56%	25%	39%	32%	7%

Table 2 details the occurrence of side effects following each of the study treatments. The overall occurrence of side effects following morphine was about 50% greater than that following fenoprofen at combined doses providing equianalgesic effects. While the occurrence of side effects was considerably less following aspirin, as noted above, aspirin provided considerably less pain relief.

Table 3 details side effect data in terms of the particular side effects and their distribution among treatments. Each side effect is following by a number indicating its occurrence.

TAELE 3. *Side effect spectrum of intramuscular morphine (M), oral fenoprofen (F) and aspirin (ASA) in postoperative cancer patients.*

Exclusive to morphine	Common to morphine and fenoprofen	M	F	ASA	Exclusive to fenoprofen
Dry mouth .. 4	Dizzy	2	1		Flushed ... 1
Groggy 2	Weak	3	2	1	Dyspnea ... 1
Vomiting ... 1	Sleepy	22	16	1	
Depressed .. 1					
	Lightheaded .	2	2		
	Nausea	1	1		
	Relaxed	1	1		
	Crying	1	1		
	Headache	1	1		
	Shaky	1	1		
	Sweating	3	6		
	Hot	1	2		

Most side effects were common to fenoprofen and morphine, the most common being "sleepiness." The next most. common for morphine was "dry mouth" and for fenoprofen, "sweating." These, however, had a considerably lower occurrence relative to "sleepiness." If "sweating", "hot" and "flushed" can be considered symptoms of a common drug effect, then this effect tends to be greater with fenoprofen than with morphine.

Age: Relative Effectiveness and Potency

Table 4 shows the results of the regression of total pain relief (visual analog scale) on patient's ages. Significant positive linear regression correlation coefficients were obtained only for morphine doses. To date there have been too few patients entered

into the study who are at the extremes of adult age in order to provide for valid relative potency estimates in these stratified patient groups. Nevertheless, preliminary calculations demonstrate a trend toward age-related differences in relative analgesic potency. For example, in terms of duration of analgesia, fenoprofen appears to be approximately one-third as potent as morphine in the youngest group (18-35 yr), one-tenth as potent in the middle-aged group (36-64 yr) and one-thirtieth as potent in the oldest group (65 yr and older). The regression analyses (Table 4) indicate that these apparent differences in relative analgesic potency are due to an age-related increase in the effectiveness of morphine, rather than to any age-related change in the effectiveness of fenoprofen.

TABLE 4 *Regression analyses of total pain relief*[1] *on age*

Treatment	IM morphine			PO fenoprofen		
Dose (mg)	4	8	16	50	100	200
Correlation (r)	0.42	0.26	0.45	-0.13	0.21	0.12
N	34	14	25	28	23	31
P <	0.02	NS	0.05	NS	NS	NS

[1]Sum of hourly visual analog scale pain relief scores.

Analgesia and Mood

While estimates of relative analgesic Potency were approximately one-tenth, estimates of relative potency in terms of mood improvement were approximately one-twentieth (Table 1). Regression analyses were carried out in order to determine whether there were differences between fenoprofen and morphine in terns of the degree of of Mood improvement provided at comparable degrees of analgesia (Table 5).

Table 5. *Regression of total mood improvement on total pain decrease for morphine and fenoprophen in cancer patients with postoperative pain.*[1]

Treatment	IM morphine	PO fenoprofen
Correlation (r)	0.44	-0.26
N	73	82
P <	0.001	NS

[1]Sum of hourly visual analog scale scores for IECKI improvement and pain intensity decrease.

A significant positive linear correlation coefficient was obtained with the morphine data, but not with the fenoprofen data: while increasing analgesia is accompanied by significant mood improvement following morphine, mood improvement and analgesia do not go hand-in-hand following fenoprofen administration.

DISCUSSION

This assay is continuing. The data presented here represents just more than half that which we expect to obtain at the completion of the assay.

Analgesic Effectiveness and Relative Potency

Preliminary data indicate that oral fenoprofen, 50 to 200 mg, is as effective as intramuscular morphine, 4 to 16 mg, in the dose range commonly employed in postoperative patients with moderate to severe pain. While definitive estimates of relative analgesic potency remain to be obtained, preliminary data indicate that oral fenoprofen is approximately one-tenth as potent as intramuscular morphine. While there is a consistent trend toward shallower dose-response slopes for fenoprofen relative to morphine, these do not significantly deviate from parallelism in our analyses. It is likely, however, that these slopes do, in fact, differ, as has been previously reported in terms of a shallower dose-response slope for fenoprofen at higher doses, an indication of a "ceiling" for fenoprofen analgesia (Sunshine et al. 1978).

Side Effects

Side effect evaluation is difficult in our patients population, primarily due to the many effects unrelated to study drug that may be observed or reported. Nevertheless, the difference in the occurrence of side effects is substantial, 50% greater following morphine than following fenoprofen.

Age: Relative Effectiveness and Potency

It has been reported that the disposition of oral fenoprofen in the plasma of geriatric patients differs from that in the plasma of young normal volunteers only in terms of a slightly slower rate of absorption, with no differences in the area under the plasma concentration - time curves (Kamal and Koch 1981). In contrast, it has been reported that the elimination half-life of morphine increases (Kaiko et al. 1978) and the total body clearance decreases (Kaiko et al. 1982) in relation to the age of the subject. There is also ample evidence that aging is accompanied by an increased analgesic response to intramuscularly administered morphine (Bellville et al. 1971; Kaiko 1980; Kaiko et al. 1983).

While our preliminary data shows a trend toward progressively lower relative analgesic potency values for fenoprofen compared to morphine with patient groups of increasing age, it is likely that this results primarily from a greater effectiveness of morphine with increasing age, as indicated by the results of the regression analyses . In the clinical setting, the use of an overall estimate of relative analgesic potency of one-tenth in the substitution of intramuscular morphine for oral fenoprofen in an elderly patient would likely result in a greater than predicted analgesic effect.

Estimates of relative analgesic potency have traditionally been established without regard for differences that might exist as a function of differences in patient's age. The results of this and other of our current studies in which we are examining the data for such differences suggest that age-related differences in relative analgesic potency can be quite significant, especially if the two analgesics differ in terms of their pharmacological class, or mechanism of action, or in terms of whether or not biotransformation processes produce active or inactive metabolites.

Analgesia and Mood

While narcotic analgesic drugs exert their analgesic effect via interaction with receptors within the central nervous system, non-steroidal anti-inflammatory agents are primarily acting on the peripheral nervous system. Consonant with these differences is the concept that narcotic analgesics alter not only the sensation of pain, but one's reaction to pain. Narcotic-induced analgesia has both a sensory and an affective component. Furthermore, the effects of centrally acting narcotic analgesics on mood are thought to be reinforcing factors, and involved in these drugs' high abuse liability.

Our preliminary data indicates that mood improvement is less after fenoprofen than after morphine administration at doses which provide comparable analgesia. Fenoprofen is approximately one-tenth as potent as morphine in terns of analgesia, but only in the range of one-twentieth as potent as morphine in terms of mood improvement. While morphine-induced mood improvement parallels pain decrease, no such relationship is apparent as yet for fenoprofen-induced mood improvement and pain decrease. The present study does not directly address the issues of whether or not the mood improvement observed with morphine is either clinically significant or desirable.

CONCLUSIONS

On the basis of the preliminary data presented here we may tentatively provide the following conclusions.

Oral fenoprofen, in the dose range employed, is approximately one-tenth as potent as intramuscular morphine in terms of analgesia in cancer patients with moderate to severe postoperative pain.

Fenoprofen is as effective as commonly used doses of morphine.

The higher doses of fenoprofen appear to be more effective than aspirin, 650 mg.

Side effect occurrence is less following fenoprofen than following morphine at doses providing comparable analgesia.

The potency of morphine relative to fenoprofen increases with increasing patient age.

Increasing analgesia following fenoprofen is not accompanied by increasing mood improvement as observed with morphine.

REFERENCES

Bellville, J.W., Forrest, W.H., Jr., Miller, E., and Brown, B.W., Jr. Influence of age on pain relief from analgesics. J Am Med Assoc, 217:1835-1841, 1971.

Houde, R.W., Wallenstein, S.L., and Rogers, A. Clinical pharmacology of analgesics: 1. A method of assaying analgesic effect. Clin Pharmacol Ther, 1:163-174, 1960.

Kaiko, R.F. Age and morphine analgesia in cancer patients with postoperative pain. Clin Pharmacol Ther, 28:823-826, 1980.

Kaiko, R.F., Houde, R.W., Rogers, A., Inturrisi, C.E., Wallenstein, S.L., Grabinski, P., and Foley, K.M. Annual report of the Memorial Sloan-Kettering Cancer Center, Analgesic Studies Section: Disposition and action of narcotic analgesics. 40th Annual Scientific Meeting of the Committee on Problems of Drug Dependence, Inc., Proceedings of, 1978. pp. 183-193.

Kaiko, R.F., Wallenstein, S.L., Rogers, A.G., Grabinski, P.Y., and Houde, R.W. Narcotics in the elderly. Med Clin N Amer 66:1079-1089, 1982.

Kaiko, R.F., Wallenstein, S.L., Rogers, A.G., and Houde, R.W. Sources of variation in analgesic responses in cancer patients with chronic pain receiving morphine. Pain, 15:191-200, 1983.

Kamal, A., and Koch, I.M. Plasma profiles of two differing doses of fenoprofen in geriatric patients. Phamatherapeutica, 2:552-557, 1981.

Sunshine, A., Slafta, J., and Gruber, C. A comparative analgesic study of propoxyphene, fenoprofen, the combination of propoxyphene and fenoprofen, aspirin, and placebo. J Clin Pharmacol, 18:556-563, 1978.

Wallenstein, S.L., and Houde, R.W. The clinical evaluation of analgesic effectiveness. In: Ehrenpreis, S., and, Neidle, A., eds. Methods in Narcotic Research. New York: Marcel Dekker, Inc., 1975. pp.127-145.

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Measurement and Extinction of Conditioned Withdrawal-Like Responses in Opiate-Dependent Patients

Anna Rose Childress, A. Thomas McLellan, and Charles P. O'Brien

INTRODUCTION

As O'Brien has reviewed elsewhere in this volume (O'Brien et al. 1983), there has been much experimental work on opiates and Pavlovian conditioning processes since Wikler's original observations of withdrawal-like responses in drug-free patients (Wikler 1948). Several studies have found evidence of conditioned withdrawal-like and opiate-like responses in rats, monkeys, and humans (Eikelboom and Stewart 1979; Grabowski and O'Brien 1980). Addict patients viewing slides or videotapes of drug-related stimuli (Teasdale 1973; Sideroff and Jarvik 1980) or handling drug objects in a preparation ritual (Ternes et al. 1979) experience subjective craving and withdrawal-like changes in physiological measures of skin temperature, heart rate, pupillary dilation, etc. Research from our own laboratory has demonstrated that opiate withdrawal-like responses in humans can be conditioned to an arbitrary conditioned stimulus (O'Brien et al. 1977).

These studies leave little doubt that conditioned withdrawal-like phenomena exist and can be both reliably elicited and measured. They do not, however, address the clinical significance of these responses. Though Wikler (1948) proposed conditioned withdrawal as the primary cause of relapse in drug-free patients, this link has not been clinically tested and is still controversial. Based on interviews with Baltimore street addicts, McAuliffe (1982) had recently suggested that conditioned withdrawal-like phenomena are relatively infrequent and rarely trigger opiate use.

RATIONALE

Though interesting, the McAuliffe interview data cannot substitute for research to empirically determine 1) the actual incidence of conditioned withdrawal-like phenomena, and 2) their role in clinical outcome, including relapse. We are currently conducting a large-scale treatment-outcome study which directly addresses both these issues. In this study, each patient's conditioned with-

drawal-like responses are first measured in the laboratory, Following measurement, one group of patients is given repeated, non-reinforced exposure to drug-related stimuli in an attempt to extinguish the conditioned withdrawal-like responses. If conditioned withdrawal-like responses trigger drug use/relapse, then reducing or removing these responses through extinction should have a beneficial effect on clinical outcome.

The present paper will present the methodology and early results of our attempts to measure and to extinguish conditioned withdrawal-like responses in opiate-dependent patients. Clinical outcome data concerning the possible benefits of extinction are currently being collected and will be presented in future papers.

METHODOLOGY

Subjects - The subjects for this ongoing study are male veteran methadone patients from the Drug Dependence Treatment Unit of the Philadelphia VA Medical Center. Patient volunteers are recruited through direct contact or referral from their drug counselor. All patients are clinically screened to rule out diagnoses of major thought disorders (schizophrenia) or organic brain syndrome.

Design Considerations - In an earlier protocol (O'Brien et al. 1979) we attempted extinction of conditioned withdrawal-like responses by asking patients maintained on an opiate antagonist (naltrexone) to undergo double-blind cook-up and unreinforced self-injection rituals. In this procedure, opiate administration was either omitted (saline trials) or pharmacologically blocked due to the antagonist treatment. After a few initial trials, most patients experienced such strong dysphoria, withdrawal and craving that they refused to participate in further extinction sessions. Though no subject completed extinction, there was some suggestion that patients who completed more trials had somewhat better outcomes at six-month follow-up than other non-extinction naltrexone patients (O'Brien et al. 1979).

In the current study, the extinction procedure was modified in two ways designed to increase patient comfort and compliance: 1) patients were given early trials with a graded hierarchy or drug-related stimuli as a prelude to the highly-evocative cook- up/ self-injection ritual; 2) each extinction trial was followed by 15-20 minutes of deep relaxation training to allow the patient to "wind down" from any discomfort or craving stirred by exposure to the drug-related stimuli.

As a final consideration, we recognized that an extinction procedure--even if well-tolerated by patients--would address only the conditioned factors of their disorder. If the significant psychological, social and vocational components of the addiction were left untreated, the possible clinical benefits of the extinction could be overshadowed and perhaps not even measurable. With this in mind, we decided to integrate our laboratory-derived extinction procedure with professional psychotherapy, a clinical treatment

which had previously produced pervasive therapeutic benefits for our clinical population (Woody et al. 1981).

Procedure - Patients eligible for the study are randomly assigned to one of three treatment groups. The clinical outcome of patients receiving cognitive-behavioral (CB) psychotherapy, extinction, and relaxation will be compared against two control groups: one group receiving CB therapy and relaxation (but no extinction) and a standard treatment control group which receives extra drug counseling and educational/control materials. Professional attention, session length, and small payments contingent upon session attendance are equivalent for all treatment groups.

Measurement of Conditioned Withdrawal-Like Responses - Prior to treatment, and at the end of treatment, and at 1 and 6 month follow-up points; each patient's conditioned withdrawal-like responses are assessed in laboratory measurement sessions. All laboratory sessions are conducted in an environmentally controlled, electrically shielded recording chamber. Physiological measures include skin temperature, galvanic skin resistance (GSR. a qeneral arousal index), heart rate; respiration and blood pressure. These physiological measures (except blood pressure) are continuously recorded on a polygraph and then converted to computer storage for later analysis.

In addition to the physiological measures, patients are asked to rate the degree of subjective high, carving or withdrawal they experience in response to test stimuli.

Both physiological and subjective responses are measured under two types of stimulus conditions: Neutral and Drug-Related. Each patient experiences both conditions, acting as his own control. For either stimulus condition, the following sequence obtains, lasting approximately one hour: 1) Resting Baseline; 2) Videotape (Neutral or Drug-Related); 3) Baseline; 4) Activity (Neutral or Drug-Related); and 5) Baseline.

The neutral videotape features a travelogue; the neutral (non-drug-related) activity allows patients to play a computerized "pong" game. The drug-related videotape features a cook-up-shoot-up ritual; the drug-related activity requires patients to go through a mock cook-up and tie-off, with optional self-injection of saline. Previous research in our center has shown pre-injection (drug preparation and cook-up) stimuli to be powerful elicitors of conditioned withdrawal-like responses (Ternes et al. 1979).

Extinction - Each hour-long treatment session for patients in the extinction group begins with 30 minutes of psychotherapy. followed by approximately 10 minutes of exposure to extinction stimuli. Each session ends with 15-20 minutes of relaxation, guided by audio cassette. Extinction stimuli include self- produced verbal imagery ("drug stories"), audiotapes of drug talk, color slides of cook-up-shoot-up rituals, videotape of drug purchase, cook-up and injection, and finally, handling of drug objects in a mock cook-up/tie-

off procedure. Saline self-injection, the final member of the extinction series, is encouraged but optional. For each patient; the ordering of extinction stimuli across sessions is the same, and we now employ a fixed trials procedure which determines the number of exposures to each stimulus category.

Data for the extinction trials is currently based on the Within-Session Rating Scale (1982), a quantified subjective report listing 24 withdrawal-like and 24 high-like symptoms. The WSRS is administered before and immediately after exposure to the extinction stimuli. We have also recently begun to record GSR and skin temperature during treatment sessions, allowing us to track the course of the extinction across sessions and to compare subjective with physiological responses.

Extinction sessions for outpatient subjects are conducted three times weekly, with 35 sessions comprising a complete course of treatment. We have recently initiated the same study with inpatients undergoing gradual methadone detoxification over a four-week period. For these inpatients, extinction trials are conducted five times weekly, for a total of 22 treatment sessions.

Daily methadone is administered immediately after measurement or extinction sessions so that its onset effects not interfere with physiological or subjective measures.

RESULTS

Pretreatment Measurement of Conditioned Withdrawal - We have now obtained laboratory measurement of responses to drug-related stimuli for more than 35 patients. In the measurement sessions, patients respond to drug-related stimuli with a variety of physiological responses, including an increase in arousal (a decrease in GSR) and transient charges in heart rate and respiratory patterns. Of all the physiological measures recorded, a time-linked decrease in skin temperature has usually provided the most reliable and specific index of a conditioned withdrawal-like response.

Figure I.

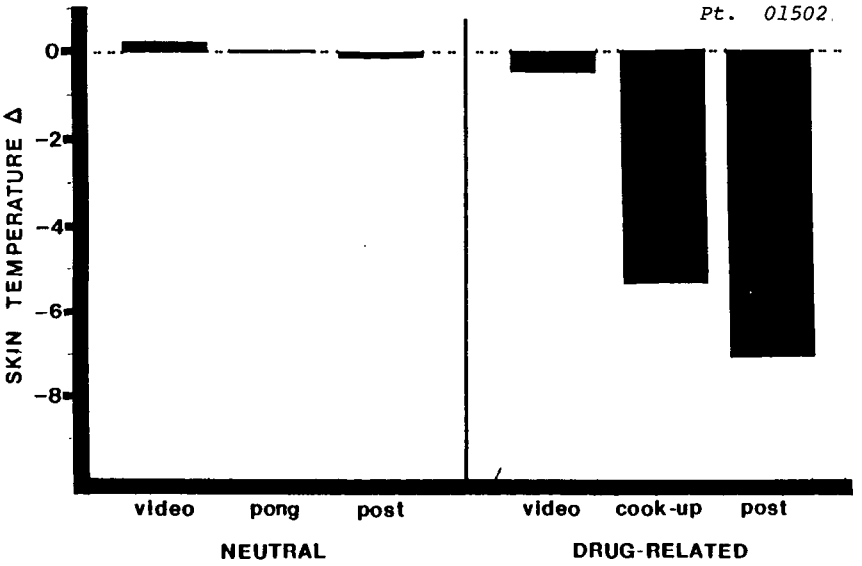

Figure 1. Skin temperature change as a function of exposure to neutral or drug-related stimuli.

Thus far, 35 to 40% of the patients tested exhibit a withdrawal-like decrease in skin temperature which is specific to drug-related stimuli. Temperature change data from a representative 'responder' is presented in Figure 1. In this figure, the vertical bars represent temperature difference scores obtained by subtracting the mean skin temperature (°F) for a 4-minute baseline period (immediately preceding the neutral or drug-related video) from the mean skin temperature for an equivalent stimulus period (video, activity, post-activity). As shown in the right half of the graph, a decrease in skin temperature begins to develop during the drug video presentation and becomes quite pronounced during the cook-up ritual, persisting into the post-cook-up interval. Temperature response to the neutral stimuli (left half of Figure 1) is negligible or shows no consistent pattern.

The average decrease in skin temperature to drug-related stimuli for clear-cut 'responders' is nearly 7°F. Some responders have experienced drops in skin temperature exceeding 12°F in a 15-20 minute period. Recovery time of skin temperature back to baseline level is usually roughly proportionate to the degree of temperature decrease, and usually occurs within 10 to 15 minutes of its nadir.

As many as one-third of the patients tested can be characterized as non-responders - they show no withdrawal-like temperature response to the drug-related stimuli. The remaining patients are more difficult to characterize, but several fall into the category of 'non-specific arousers', showing mild arousal patterns to both neutral

and drug-related activities, but no differential response in skin temperature or the other physiological measures.

More than a third of the patients report increases in subjective craving and withdrawal following exposure to the drug-related test stimuli. Interestingly, though subjective intensity of craving/withdrawal is roughly correlated with the degree of physiological response, patients sometimes show a withdrawal-like decrease in skin temperature without reporting an increase in subjective withdrawal or craving. The reverse situation, in which a patient reports increased subjective craving but exhibits no profound change in physiological response also occurs, but is less common.

Extinction - Within extinction sessions, from one-half to two-thirds of the patients report increases in subjective craving and/or withdrawal following exposure to the drug-related stimuli: In the early extinction trials, a few patients have also reported an increase in high-like symptoms, but these responses usually fade quickly and are replaced by relatively persistent withdrawal-like symptoms. Withdrawal symptoms elicited by the drug-related stimuli usually subside by the end of the 15-minute relaxation period.

Of patients who reliably respond to extinction stimuli with an increase in withdrawal-like symptoms, over half show a reduction in subjective response across the 35 sessions, suggestive of extinction. For at least two pilot patients the withdrawal-like responses persisted beyond 50 extinction trials.

Post-Treatment Measurement of Conditioned Withdrawal - Though the number of 'responder' patients who have completed this measurement phase is still relatively small, we do have early encouragement that extinction trials may reduce conditioned withdrawal-like responses. In general physiological 'responders' who undergo extinction tend to show a diminution of the temperature response to drug-related stimuli in post-treatment testing. 'Responder' patients in the non-extinction groups (therapy or extra counseling) do not exhibit this trend; and the temperature response is often similar to that at the outset of treatment.

SUMMARY

Data from laboratory measurement sessions indicate that a substantial proportion--at least 40%--of opiate-dependent patients show physiological evidence of conditioned withdrawal in response to drug-related stimuli. The index response, a time-linked decrease in skin temperature, is often accompanied by increases in subjective craving and withdrawal. In extinction sessions, up to two-thirds of the patients tested respond with an increase in subjective craving and/or withdrawal to drug-related stimuli. Preliminary extinction data suggest that conditioned withdrawal-like responses, though relatively persistent, may be reduced with sufficient trials. Post-treatment laboratory measurements indicate that the extinction procedure may attenuate the withdrawal-like reduction in skin temperature.

Up to one-third of our patient population can be characterized as 'non-responders '--they fail to show physiological and/or subjective withdrawal-like responses to the drug-related stimuli used in our procedure. This lack of response is particularly intriguing since many of these 'non-responders' have had extensive drug use histories which should have allowed ample opportunity for conditioning to occur. We are currently exploring the possibility that certain emotional states (anxiety, etc.) may have become an integral part of the conditioned stimulus complex which elicits craving/withdrawal (Poulos et al. 1981), such that drug-related stimuli alone-unaccompanied by the mood state--are insufficient to elicit the conditioned response.

Although the clinical impact of our integrated treatment package awaits determination from outcome data, the presence of conditioned withdrawal-like responses in a significant proportion of our patient population suggests its possible benefit. The modified extinction procedures have been successfully integrated with psychotherapeutic techniques to produce a treatment with retention rates approaching 70%. We are optimistic that this combined methodology will finally allow evaluation of the role of conditioned factors in opiate use and relapse.

REFERENCES

Eikelboom, R., Stewart, J. Conditioned temperature effects using morphine as the unconditioned stimulus. Psychopharmacology, 61:31-38. 1979.

Grabowski, J.C. and O'Brien, C.P. Conditioning factors in opiate use. Advances in Substance Abuse, Vol. 11, Edited by N.K. Mello. Greenwich, CT: JAI Press, 1980.

McAuliffe, W.E. A test of Wikler's theory of relapse due to conditioned withdrawal sickness. International Journal of the Addictions, 17(1):19-33, 1982.

O'Brien, C.P., Testa, T. O'Brien, T.J. Brady, J.P. and Wells, B. Conditioned narcotic abstinence in humans. Science, 195:1000-1001, 1977.

O'Brien, C.P., Greenstein, R., Ternes, J., McLellan; T. and Grabowski, J. Unreinforced self-injections: Effects of rituals and outcomes in heroin addicts. Problems of Drug Dependence 1979. CPDD Proceedings. NIDA Research Monograph 27. Washington D.C.: supt. of Docs., U.S. Govt. Print. Off., 1980.

O'Brien, C.P., Ternes, J.W. and Ehrman, R.N. Classical conditioning in opiate dependence. Plenary session. Proceedings of the 45th Annual Meeting, Committee on Problems of Drug Dependence, Lexington, Kentucky, 1983.

Poulos, C.X., Hinson, R.E. and Siegel, S. The role of Pavlovian processes in drug tolerance and dependence: Implications for treatment. Addictive Behaviors, 6:205-211, 1981.

Sideroff, S. and Jarvik, M.E. Conditioned responses to a videotape showing heroin-related stimuli. International Journal of the Addictions, 15(4):529-536, 1980.

Teasdale, J Conditioned abstinence in narcotic addicts. International Journal of the Addictions, 8:273-292, 1973.

Ternes, J., O'Brien, C.P., Grabowski, J., Wellerstein, H. and Jordan-Hayes, F. Conditioned drug responses to naturalistic stimuli. Proceedings of the 41st Annual Meeting, Committee on Problems of Drug Dependence, NIDA Research Monograph, #27, 1979.

The Within-Session Rating Scale (for opiate withdrawal-like and high-like symptoms). Drug Dependence Treatment Unit, Philadelphia VA Medical Center, Philadelphia, PA, 1982.

Woody, G.E., O'Brien, C.P., McLellan, A.T., Lucorsky, L. and Mintz, J. Psychotherapy for opiate addiction: Some preliminary results. Annals of the New York Academy of Sciences, 362:91-100, 1981.

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Perinatal Addiction: The Effects of Maternal Narcotic and Nonnarcotic Substance Abuse on the Fetus and Neonate

Ira J. Chasnoff, William J. Burns, and Sidney H. Schnoll

Although a great deal of research has been devoted to the evaluation of the effects of intrauterine exposure to heroin and methadone on the fetus and neonate, information regarding the outcome of nonnarcotic-exposed neonates is sparse and consists primarily of case reports (Rementeria and Bhatt 1977; Mazzi 1977, Hill et al. 1977, Mangurten and Benwara 1980, Golden et al. 1980). At the Perinatal Addiction Project of Northwestern Memorial Hospital's Institute of Psychiatry and Prentice Women's Hospital and Maternity Center, the number of pregnant women using and abusing nonnarcotic substances has increased dramatically in the last few years. This paper is a review of all infants delivered to women enrolled in our program since 1976 and will review and compare the intrauterine growth and neonatal neurobehavior of these infants.

SUBJECTS AND METHODS

From April 1976 to December 1982, 95 infants were born to mothers enrolled in the Perinatal Addiction Project. All of the women were enrolled in the first or early second trimester of pregnancy and completed a course of intensive prenatal care. Maternal urine samples were obtained regularly in order to screen for illicit drug use. The 95 infants were divided according to the type of primary maternal addiction: heroin/methadone (N=51), mixed sedative/stimulant (N=22), pentazocine/tripelennamine (N=13) and phencyclidine (N=9). Women with a history of heavy alcohol use were enrolled in a different section of the program and thus were not included in this study.

Mothers in Group I conceived while on heroin.. Forty-seven of these women were abusing heroin alone while the remaining four women abused either one or two nonnarcotic drugs in addition. Upon admission to the program, each woman was placed on a variable initial daily dose of methadone. This dosage was steadily decreased to the lowest level which would prevent craving or withdrawal in the mother. By the beginning of the third trimester, each woman was on a maintenance dose of methadone which ranged from 5 to 40 mg daily (mean=15.9, S.D.=10.4). This dose was

held at the same level for the rest of the pregnancy, and no woman was completely withdrawn during pregnancy. On daily urine screens the women, with three exceptions, remained clean of narcotic and nonnarcotic drugs other than the prescribed methadone.

Mothers in Group II were addicted to multiple licit or illicit non-narcotic drugs. Each woman used two to five of the following drugs in various combinations before and during pregnancy: phenobarbital, diazepam, marijuana, codeine and cocaine. These women received the same regimen of prenatal care as Group I except that they did not receive methadone. Although abstinence was the objective for this group, only five of the women remained clean of drug use throughout the third trimester of pregnancy.

Thirteen infants were delivered to women who abused a combination of pentazocine and tripelennamine (T's and blues) during pregnancy (Group III). All of the women in this group sporadically used other, nonnarcotic drugs, but T's and blues were the only drugs consistently used throughout pregnancy. Although abstinence was the objective of the program, none remained clean of T's and blues during the third trimester of' pregnancy.

Group IV infants were delivered to nine women whose primary drug of abuse throughout pregnancy was phencyclidine hydrochloride (PCP). All of the women had positive urine screens which demonstrated sporadic use of other nonnarcotic drugs in addition to the PCP, but PCP was the only substance used heavily throughout the third trimester.

Three of the Group II women sporadically used T's and blues or PCP during pregnancy, but this use was very limited and did not occur in the third trimester; hence these three women were included in Group II based on their primary abuse of various sedative and stimulant substances throughout pregnancy.

A group of drug-free mothers was selected in the order they presented for prenatal care to the clinic of Prentice Women's Hospital and Maternity Center (Group V, N=27). These women had no history or evidence of drug or alcohol abuse, and management of prenatal care and nutrition was similar to the four drug-abusing groups of women.

All groups were evaluated for maternal factors which might affect neonatal outcome: race, maternal age, education, gravidity, prenatal care, nutrition, cigarette smoking, alcohol ingestion, and drug use. Analysis of variance and Chi square analysis were utilized for statistical analysis of these parameters. All neonates were examined at birth when weight, crown-to-heel length and fronto-occipital head circumference were recorded. The Brazelton Neonatal Behavioral Assessment Scale (Brazelton 1968) (BNBAS) was administered at two days of age by trained examiners who were blinded to the infants' prenatal history. Results were analyzed utilizing analysis of variance. For those items which reached statistical significance (< .05), 'the Fischer's LSD was utilized to identify homogenous subsets.

RESULTS

Demographic data for the five groups of women was similar as was the frequency of cigarette smoking in each of the groups (Table I).

TABLE I. Maternal demographic data

	I Methadone	II Sedative/ Stimulant	III T & B	IV PCP	V Drug-free
N	51	22	13	9	27
Age (mean)	23.0	22.4	23.0	22.0	22.2
Education (mean)	10.7	11.5	11.4	11.1	10.7
Gravidity	2.8	2.5	3.3	2.0	2.2
Cigarettes (% users)	60	69	62	57	59
Weight gain in pregnancy (kg)	10.5	10.2	9.8	10.6	11.1

Mean weight gain during pregnancy for all five groups of women was similar. However, racial distribution varied between the groups (Table II). Thus, for analysis of data, race was controlled through covariate analysis when each drug-using group was compared to the Group V (drug-free) mothers and infants.

TABLE II. Racial distribution

	I		II		III		IV		V	
	N	%	N	%	N	%	N	%	N	%
White	33	65	10	45	1	8	8	89	7	26
Black	16	32	11	50	12	92	1	11	12	44
Hispanic	2	3	1	5	0	-	0	-	7	26
Oriental	0	-	0	-	0	-	0	-	1	4

All infants were delivered at term gestation as determined by the criteria of Ballard et al. (1977). There was an even distribution of infants by sex in each group. Apgar scores in the five groups were similar, and no significant perinatal complications occurred in any group. Two infants delivered to mothers on methadone maintenance (Group I) had meconium aspiration syndrome and one had seizures on the second day of life controlled with phenobarbital. Twelve infants in Group I required therapy for significant withdrawal based on clinical criteria of marked irritability, poor feeding and/or excessive weight loss. No infant in the other drug groups required therapy for withdrawal.

Somatic measures. Infants delivered to mothers in Group I and in Group III had a significantly lower weight and length than control (Group V) infants (Table III). These Group I and Group III infants in addition had a significantly smaller head circumference than both the control infants and those in Groups II and IV.

TABLE III. Somatic growth parameters

	I Methadone		II Sedative/ Stimulant		III T & B		IV PCP		V Drug-free	
	X	S.D.	X	S.D.	X	S.D.	X	S.D.	X	S.D.
Weight (gm)	2840*	600	3165	560	2799*	430	3201	440	3479	623
Length (cm)	48.2*	3.5	50.0	3.1	48.1*	1.8	49.3	2.6	51.1	2.8
Head circumference (cm)	32.2*	2.4	33.9	1.5	32.9*	1.2	33.7	2.0	34.7	1.7

*ANOVA (Specific Drug Group x Group V), $p < .01$

†Significant difference from Groups II and IV (Multiple Range Test)

These differences remained when race was statistically controlled. The birth weights, lengths and head circumferences of the sedative/stimulant and PCP-exposed infants were not significantly different from those of the control infants.

Neonatal behavior. Means and standard deviations for those BNBAS items for which statistically significant differences were obtained are listed in Table IV. Significant differences were obtained in items related to interactive ability, motor. maturity and state control. Items related to visual and auditory orientation and motor maturity differentiated the methadone-dependent group from both the control and all other drug groups (Fischer's LSD). All four groups of drug-exposed neonates showed deficits in state control with an abnormal predominant state, an increased lability of state and poor consolability. In addition, PCP-exposed infants (Group IV) showed significantly increased lability of states and poorer consolability when compared to all other drug groups (Fischer's LSD).

DISCUSSION

With the increasing frequency of nonnarcotic substance use and abuse by pregnant women, it is incumbent upon physicians to be able to recognize and evaluate the neonate passively exposed to these substances. In the present study, newborns delivered to women addicted to sedative/stimulants or PCP were found to demonstrate marked deficits in neonatal behavior. These two groups of infants showed significantly poorer state organization and consolability than the drug-free controls. However, they did not manifest significant differences from normals in somatic growth measures, orientation or motor maturity responses. Deficits in intrauterine growth appeared to affect mainly narcotic-exposed infants, especially in relation to poor head growth. T's and blues-exposed infants showed significantly lower somatic growth rates and poorer state control than the drug-free controls, similar to methadone-exposed neonates. In addition, the methadone-addicted infants could be further differentiated by poorer visual and auditory orientation responses and poor motor control.

From the data presented, it is evident that the neurobehavioral risks of perinatal addiction extend to nonnarcotic- as well as narcotic-addicted infants. These behavioral risks identified at birth with the Brazelton scale will have repercussions for later development, since infants who have deficits in their ability to maintain alertness and to interact do not respond appropriately to caretaker attempts at comforting and nurturing. Consequently, the withdrawing, irritable infant enters into a pattern of behavior which increases maternal frustration and anxiety. resulting in a greater likelihood of maternal rejection. Professional intervention at this point is necessary if this cycle of infant passivity and maternal rejection is to be interrupted.

REFERENCES

Ballard, J.L., Dazmaier, K., and Driver, M. A simplified assessment of gestational age. Pediatr Res, 11:372, 1977.

TABLE IV. BNBAS items which discriminated between the groups*

	I Methadone		II Sedative/ Stimulant		III T & B		IV PCP -		V Drug-free	
	X	S.D.	X	S.D.	X	S.D.	X	S.D.	X	S.D.
Interactive										
Inanimate Visual Orientation	3.3*	2.2	5.7	2.1	5.2	2.7	6.0	2.0	5.6	1.9
Inanimate Auditory Orientation	3.4*	1.2	5.6	1.4	5.4	1.7	4.3	2.0	5.3	2.3
Animate Visual Orientation	3.9*	1.7	4.9	2.1	4.5	1.9	4.5	1.3	5.7	1.9
Animate Auditory Orientation	3.9*	1.6	5.2	1.6	4.3	.5	4.5	1.0	5.2	2.4
Consolability	4.4*	2.4	3.7*	2.2	4.2*	2.2	2.5*	1.0	6.3	1.5
Motoric										
Motor Maturity	3.3*	1.4	4.5	1.4	4.7	2.4	5.0	2.2	4.8	1.6
Organization, State										
Predominant State	4.1*	1.4	4.8*	.4	4.5*	1.9	4.8*	.5	3.9	1.0
Lability of State	3.2*	1.6	3.7*	1.9	3.3*	1.5	5.0*	1.9	1.6	1.1

*ANOVA (Specific Drug Group x Group V), $p < .01$

Brazelton, T. B. National Behavioral Assessment Scale. Philadelphia, Spastics International Medical Publications, 1968. pp. 63-64.

Golden, N .L., Sokol, R. J., and Rubin, I.L. Angel dust: possible effects on the fetus. Pediatrics, 65 : 18-20, 1980.

Hill, R.M., Verniaud. W.M., Morgan, N.F., Nowlin, J., Glazener, L.J., and Horning, M.G. Urinary excretion of phenobarbital in a neonate having withdrawal symptoms. Am J Dis Child, 131:546-550, 1977.

Mangurten, H.H., and Benwara, R. Neonatal codeine withdrawal in infants of nonaddicted mothers. Pediatrics, 65:159-160, 1980.

Mazzi, E. Possible neonatal diazepam withdrawal : a case report. Am J Obstet Gynecol, 121:586-587, 1977.

Rementeria, J.L.. and Bhatt, K. Withdrawal symptoms in neonates from intrauterine exposure to diazepam. J Pediatr, 90:123-126, 1977.

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Drug Preference in Humans: Lorazepam

Harriet de Wit, C. E. Johanson, and E. H. Uhlenhuth

A drug's capacity to reinforce behavior in a laboratory setting usually correlates with its dependence potential in the general population. In laboratory tests, diazepam is not an effective positive reinforcer, either in laboratory animals using drug self-administration tests (Griffiths and Ator, 1980) or in normal human volunteer subjects using a choice test (Johanson and Uhlenhuth, 1980; de Wit et al., 1983). The failure to find evidence for a positive reinforcing effect of diazepam in these experimental tests is inconsistent with clinical reports that diazepam is used excessively by some people.

The failure to demonstrate the positive reinforcing efficacy of diazepam in an experimental situation may be due in part to the drug's long duration of action (half-life = 24 - 48 hours; Hillestad, 1974). In animal self-administration studies that test the reinforcing efficacy of drugs, it has been found that benzodiazepines that have shorter durations of action are also more effective reinforcers (Griffiths et al., 1981). In the present study, human subjects were tested for preference for lorazepam, a benzodiazepine with effects similar to diazepam but with a shorter half-life than diazepam (half-life = 12-15 hours; Greenblatt et. al., 1979; Ameer and Greenblatt, 1981).

METHOD

Subjects. Twelve normal healthy volunteers, aged 21 to 27 (4 males, 8 females) participated in this study. They were recruited using advertisements in the local student newspaper, notices posted on the University campus, and word-of-mouth referrals. Prior to acceptance, subjects were interviewed to explain the nature of the study and to ascertain their medical, psychiatric and drug use histories. Subjects were accepted if they were considered normal and healthy on the basis of this interview and a subsequent EKG. Most subjects had some experience with psychotropic drugs but none had a history of any type of drug abuse.

Subjects signed a consent form prior to participation which outlined the study in detail and indicated all possible side effects of any drug they might be given. They were informed that they would not be told what drug they ingested at the time, except that it would either be a

psychomotor stimulant, minor tranquilizer, or a placebo, and that the dose would be within the daily therapeutic range. Bach subject also agreed not to take other drugs, except their normal amounts of coffee and cigarettes, 12 hours before and 6 hours after taking a capsule. Except for the actual drug ingested, subjects were completely informed of all other procedural details as outlined below.

Procedure: All subjects participated in each of four experiments, presented in counterbalanced order. The procedure for each experiment was identical except for the drugs available, which were as follows:

Experiment 1: lorazepam, 0.5 mg versus placebo
Experiment 2: lorazepam, 1.0 mg versus placebo
Experiment 3: lorazepam, 2.0 mg versus placebo
Experiment 4: lorazepam, 1.0 mg. versus diazepam, 5 mg

The doses of lorazepam are within the therapeutic range for the drug's anxiolytic effect, and the doses of lorazepam and diazepam tested in Expt. 4 are considered therapeutically equipotent (e.g., Yalarino and Perez-Lopez, 1976).

Every experiment consisted of three sessions per week over a 3-week period, resulting in a total of nine sessions. During the first four sessions, the subject reported to the experimental room between 9 and 10 a.m. At that time, he/she filled out mood forms (see below) and received a colored capsule for immediate ingestion. Approximately half of the subjects received drug during sessions 1 and 3 and placebo (or diazepam in Expt. 4) during sessions 2 and 4. The order was reversed for the other half. For each subject, each drug was dispensed in a capsule of a consistent and distinctive color in order to facilitate identification. Capsule colors were assigned randomly across subjects to avoid the influence of color preference. Each subject was instructed during the initial four sessions to note the capsule. colors, and to try to associate each of the two colors with the effects of the substances contained in them. After ingesting the capsule, subjects were free to leave. They took three additional mood forms with them, which they were to fill out 1, 3, and 6 hr later. In addition, subjects filled out a questionnaire at hour 6, indicating whether they liked the drug (from "disliked a lot" to "liked a lot"), what they thought it was (stimulant, tranquilizer and placebo), and whether they had experienced any unusual reactions. During the last five sessions, the procedure was identical in every respect except that the subjects were given a choice of the two colored capsules to ingest.

Subjective Effects. The scales used to assess mood were an experimental version of the Profile of Mood States (POMS) and a shortened version of the Addiction Research Center Inventory (ARCI). Both have been shown to be sensitive to the effects of psychotropic drugs (Johanson and Uhlenhuth 1960; Haertzen, 1966). The POMS consists of 72 adjectives commonly used to describe momentary mood states. Subjects indicate how they feel at the moment in relation to each of the 72 adjectives on a 5-point scale ranging from "not at all" (0) to "extremely" (4). There are eight clusters of items (subscales) which have been separated empirically using factor analysis (Anxiety, Depression, Anger, Vigor, Fatigue, Confusion, Friendliness, and Elation). The value of each subscale is determined by adding the numbers checked for each adjective in the cluster and dividing the total by the number of adjectives. Two additional subscales, Arousal and Positive Mood, were derived from the other

subscales as follows: Arousal = (Anxiety + Vigor) - (Fatigue + Confusion), Positive Mood = Elation - Depression. The ARCI consists of 49 true/false items which have been separated into 5 clusters described as measuring typical drug effects such as stimulant-like (A and BG), euphoric (MBG), sedative (PCAG) and dysphoric (LSD).

For each experiment the ten POMS scores were averaged across sessions for each subject separately for drug and placebo at each of the four time periods. A two-way analysis of variance (drug x hour) was performed separately for each factor. If a significant ($P < 0.05$) drug x hour interaction was found, further statistical tests were conducted to determine at which hours the scores for the two drugs were significantly different.

Drug Preparation. Drug tablets (Ativan, Wyeth Laboratories; Valium, Hoffman-LaRoche) of the required dose were placed in opaque gelatin capsules (size 00) which then were filled with dextrose powder. Placebo capsules were identical in size and contained dextrose powder alone.

Results. The number of drug choices in each of the four experiments is illustrated in Figure 1. The mean number of lorazepam choices out of 5 were as follows: 0.5 mg dose: 2.5 (49%); 1.0 mg: 1.6 (32%); and 2.0 mg 0.6 (16%). Only the- proportion of choices for 2.0 mg lorazepam over placebo differed significantly from chance (one-sample t-test, $t = 4.2$, $df = 11$, $p < .01$) but a trend of decreased preference related to dose is apparent. Subjects showed no preference for either drug in the comparison between lorazepam and diazepam (46% lorazepam choice). The POMS and ARCI scores reflected lorazepam's known anxiolytic and sedative effects. At the 0.5 mg dose only Anxiety scores (POMS) were decreased at hour 6. At 1.0 mg the drug decreased Arousal and increased Fatigue and Confusion on the POMS. At 2.0 mg, Anxiety, Vigor and

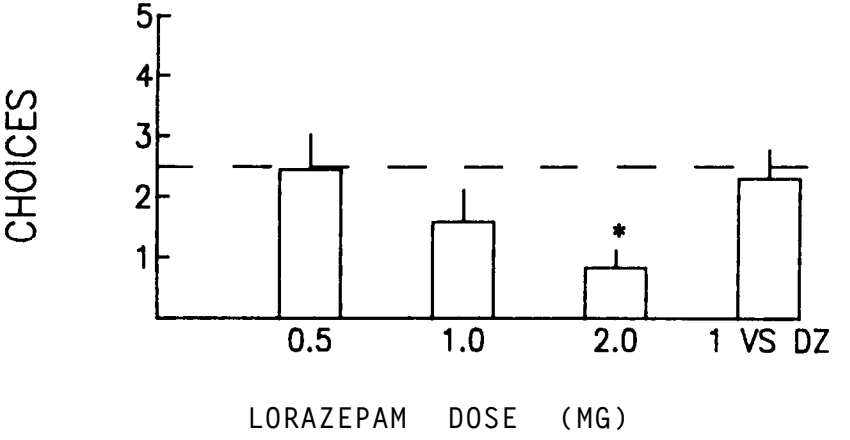

Figure 1. Mean (and S.E.M.) number of lorazepam choices (out of 5) in comparisons between lorazepam (0.5, 1.0 or 2.0 mg) and placebo, and lorazepam (1.0 mg) and diazepam (5.0 mg). Dashed line indicates level of choice that would be expected by chance; asterisk indicates significant differences from chance.

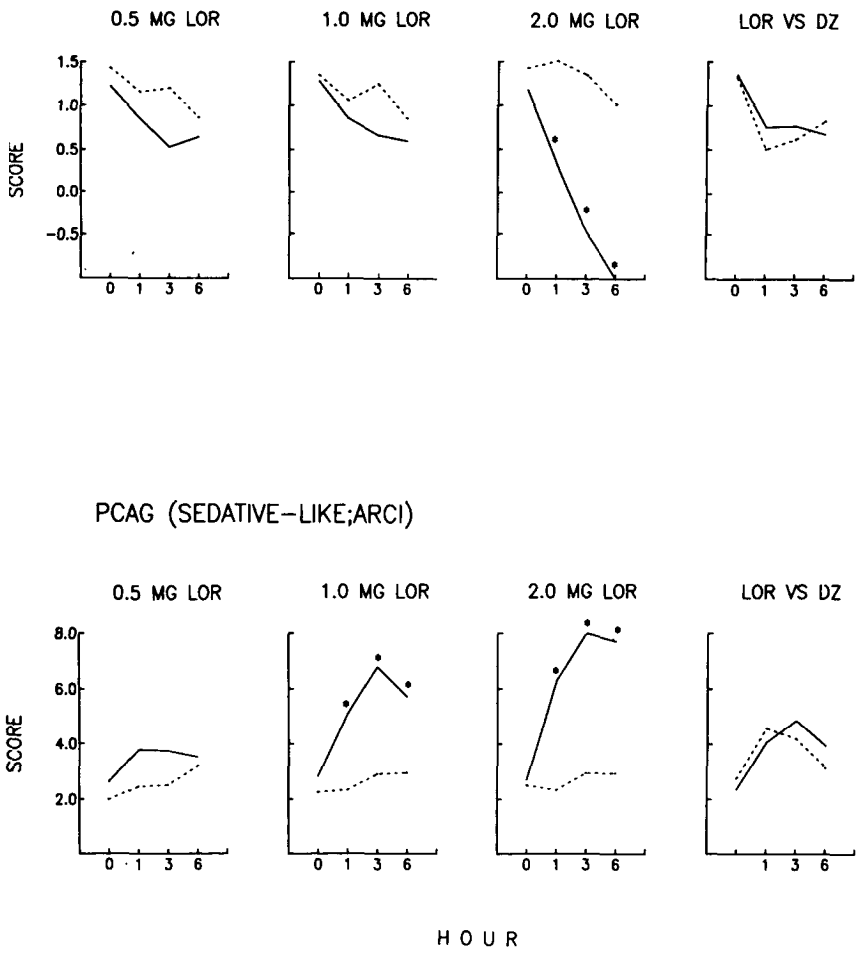

Figure 2: Examples of POMS (Arousal) and ARCI (Sedation) subscales showing subjective effects of lorazepam (solid lines) versus placebo (broken lines) or diazepam (dashed line, last panel) over a 6-hour period. Asterisks indicate significant ($p < .05$) differences between drug and placebo (Fisher's LSD post hoc test).

Arousal were decreased, and Confusion increased. On the ARCI, PCAG scores (sedative effects) were increased and BG (stimulant effects) scores were decreased at 1.0 mg and 2.0 mg. LSD scores were elevated at the 2.0 mg dose. There were no significant differences in the comparison between lorazepam and diazepam. For purposes of illustration, the drug's effects on one POMS (Arousal) and one ARCI (PCAG) subscale are presented in Fig. 2 (a and b).

Although the data are not shown here, the results from the post-session questionnaires on liking and drug identification were consistent with the choice and subjective effects results already described. Accuracy in labelling lorazepam as a tranquilizer increased as the dose increased, and mean ratings of drug liking were in agreement with mean drug choices for the three doses, that is, both measures decreased with increasing doses. The degree of concordance between the liking rating on Sessions 1 - 4 and the number of times the drug was subsequently selected was determined by calculating Pearson's r between these two measures for each of the 3 experiments. Only in the 2.0 mg comparison did this correlation reach significance ($r = 0.798$, $df = 10$, $p < .01$).

Discussion

These experiments showed that normal volunteer subjects did not prefer lorazepam over placebo in a laboratory test of choice. At the lowest dose tested (0.5 mg), subjects appeared to be indifferent to the drug whereas at the highest dose (2.0 mg) there was a clear preference for the placebo. When therapeutically equipotent doses of lorazepam and diazepam were compared, subjects showed no preference for either drug.

The subjective effects of lorazepam were in general consistent with the drug's known sedative properties, and, with some exceptions (see below), the same as the effects of diazepam. The most notable and surprising finding from the point of view of the purpose of this study was the relatively long duration of effect of this drug. On each of the subscales where there was a significant drug x hour interaction, the difference between drug and placebo was present 6 hours after drug ingestion (as measured by Fisher's LSD post-hoc tests). In some cases the drug's effect was also evident at hours 1 and 3, but in no case did the effects peak before hour 3 or disappear by hour 6. These results are in marked contrast to previous findings with 5 and 10 mg diazepam (Johanson and Uhlenhuth, 1980; de Wit et al., 1983), when the drug effects peaked at hour 1 and had largely dissipated by the 6th hour.

While it seems paradoxical that a drug (lorazepam) whose half-life is shorter than another (diazepam) should have a longer duration of effects, closer examination of the pharmacokinetic properties of these two drugs provides an explanation. After oral administration, peak plasma concentrations of lorazepam are reached at about 2 hours (Greenblatt et al., 1976) whereas for diazepam peak levels are reached 30 minutes after administration (Hillestad et al., 1974). In both cases, the peak clinical effects of the drugs correspond to the peak plasma levels. In addition, lorazepam crosses the blood-brain barrier less readily because of its lower lipophilicity. The drugs also differ in the extent to which they are distributed to tissue. Diazepam is more readily absorbed by peripheral tissue, decreasing plasma levels somewhat but delaying total

elimination time. Thus, plasma levels of lorazepam remain higher after peak levels are reached, but are then also more rapidly eliminated.

Lorazepam's effects on mood are very similar to the effects of diazepam. In the experiment comparing equipotent doses of the two drugs (1 mg lorazepam versus 5 mg diazepam), there were no differences on the POMS or ARCI subscales. Furthermore, the results of the three dose comparisons with placebo can be compared to previous findings with diazepam versus placebo. Both drugs produced dose-dependent increases in Fatigue and Confusion, and decreases in Vigor and Arousal (Johanson and Uhlenhuth, 1980; de Wit et al., 1983). While direct comparisons cannot be made across experiments, casual inspection of the data indicate that the direction and magnitude of the effects of the two drugs are similar, although the time course of effects is notably different, as described above. Anxiety scores on the POMS were decreased after lorazepam, an effect reported with diazepam in one study (de Wit et al., 1983) but not in another (Johanson and Uhlenhuth, 1980). The drugs appear to have similar effects on the PCAG and BG subscales of. the ARCI, but the increased LSD scores observed after the highest dose of lorazepam have never been reported with diazepam.

It is notable that decreased Anxiety was the only measurable mood effect after 0.5 mg lorazepam, and that it occurred in the absence of any measurable sedative-like effects. This dissociation of effects has implications both for the drug's usefulness in treatment, and for our understanding of the mechanisms of action of anxiolytic drugs.

In conclusion, these experiments provide data on the reinforcing property of another benzodiazepine, lorazepam, in normal volunteer subjects. The results with lorazepam are in agreement with previous findings using diazepam, both in the drug's sedative-like effects on mood, and the notable absence of a preference for the drug over placebo. The absence of preference for these drugs suggests that in this subject population their dependence potential is low. The hypothesis that a shorter-acting benzodiazepine is more reinforcing could not be tested, due to the unexpectedly long duration of lorazepam's effects.

REFERENCES AVAILABLE ON REQUEST

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Ultradian Consummatory Cycles and Smoking

Judith Green and Walter N. Tapp

INTRODUCTION

Smoking is a major preventable cause of life-threatening disease. However, we succeed poorly in preventing disease due to smoking because most intervention efforts exe relatively ineffective and are marked by high rates of recidivism (Jaffe 1980; Russell and Feyerabend 1978). One major obstacle to the effective treatment of smoking is the failure to unravel the complex tangle of physiological, pharmacological, psychological and social factors that seem to play a role in initiating and sustaining compulsive smoking behavior. In this study we examined cyclic consummatory behavior, a manifestation of a physiological process that may either predispose people to become habitual smokers or may later maintain their smoking behavior. Cyclic consummatory behavior encompasses not only conventional meal patterns, but shorter cyclic patterns of ingestion known as ultradian consummatory rhythms. These rhythms occur in people and nonhuman primates with periods that typically range from 60-150 minutes, involving eating and drinking (Natelson and Bonbright 1978; Bowden et al. 1978) and such nonnutritive oral activities as smoking (Friedman and Fisher 1967; Friedman 1972; Oswald et al. 1970). While ultradian cyclicity is but one of a host of biological rhythms that modulate physiological and behavioral fluctuations, the ultradian consummatory rhythm seems to reflect an underlying rhythm of oral drive (Friedman and Fisher 1967; Friedman 1972; Oswald et al. 1970). In this study, smokers and exsmokers exhibited significantly shorter cycles of ingestive activity (eating and drinking) than nonsmokers, suggesting that shorter physiological cycles, I.e., more frequent peaks of oral drive contribute to the impulse to smoke.

METHODS

Three groups of subjects between the ages of 18 and 39 participated in the study. Subject groups were equivalent in age, sex, and height-weight index (ratio of observed pounds of body weight per inch of height to ideal pounds per inch; ideal weights from Fishbein 1970). There were 11 nonsmokers, 11 smokers, and eight exsmokers. The smokers' mean current cigarette use was 18.0 + 5.5 SEM (range = 3 to 70) cigarettes per day, with an average of 7.2 ± 1.7 SEM years as a smoker. Exsmokers' prior mean cigarette use was 17.9 ± 4.3 SEM (range = 3 to 42) cigarettes per day for an average of 8.1 ± 2.1 SEM years followed by an average of 3.8 + 1.9 SEM years of abstinence from tobacco. Nonsmokers reported never having used tobacco.

Each subject was observed individually through one-way glass for a period of six or seven hours beginning at 9 A.M. The subject's room was ccmfortably furnished and had a refrigerator stocked with beverages, cheeses, and fruit. Snacks and hot drinks were located on a table. Subjects were told that they were participating in a study concerned with the ways in which people structure time in an environment without time cues. Subjects brought work, leisure reading, and hobby materials and were instructed to follow their inclinations as though spending a quiet day at home.

Consummatory activities were recorded as they occurred in each five-minute interval of the observation period. Number of sips, bites, and puffs, as well as total amounts of items consumed, were noted. Patterns of sips + bites were treated as the dependent variable in the time series analysis of the data and in the computation of consummatory episodes. Puff patterns were not included in the present analysis because there was no comparable measure across all three groups.

A time series technique, spectrum analysis, was used to identify and evaluate ultradian rhythms in consummatory data. Spectrum analysis decomposes a waveform into its component sine and cosine waves, providing information on the relative contributions of a spectrum of underlying frequencies to the total variance of the series. Each frequency component of a time series is associated with a value that represents the percentage of the total power of the spectrum contributed by that frequency. A scheme that was developed by Tapp et al. (1981) to evaluate short time series similar to those of the present study was used to compute each subject's individual spectrum.

RESULTS

The time series analysis revealed that ultradian rhythms in eating and drinking occurred in all groups of subjects (table 1). Moreover, smokers and exsmokers had similar cycles which in both cases were significantly shorter (that is, of higher peak frequencies) than those of nonsmokers. The spectrum values in table 1 were derived by computing a mean within each group from the peak f're-quency in each subject's spectrum. The peak spectrum frequency is the frequency with the greatest power; i.e, the one that most contributed to the variance of the subject's time-related pattern of data. Spectrum frequencies are expressed in radians; for convenience, the corresponding period lengths (minutes) have been listed.

It is important to note that smokers and exsmokers did not eat or drink more on the whole than nonsmokers, either in terms of sips + bites, or in terms of estimated calories. Rather, it was differences in the distribution of intake that produced higher frequency consummatory patterns in those with histories of smoking. There were no significant differences between the mean frequencies of smokers and exsmokers.

Examination of the mean power distributions of the spectra of the three groups of subjects for sip + bite patterns clearly reveals

TABLE 1. *Comparison of peek spectrum values of consummatory (sip + bite) activity, frequency of consummatory episodes (CE), number of sips + bites (S+B) and caloric intake in nonsmokers (Nsmok), smokers (Smok) and exsmokers (Xsmok). Values are the mean ± the standard error of the mean of each variable.*

	Spectrum Power Peaks				
	Cycle length (min.)	Cycle frequency (radians)	No. CE per hr.	No. S+B per hr.	No. calories per hr.
Nsmok	95.45 ± 9.91	0.3681 ±0.0411	0.588 ±0.043	24.6 ±3.5	172 ± 27
Smok	72.20 ± 5.91	0.4633* ±0.0356	0.785+ ±0.050	21.6 ±2.7	153 ± 22
Xsmok	63.02 ±5.87	0.5323** ±0.0543	0.730** ±0.053	19.5 ±2.9	108 ± 15

*Significantly greater then nonsmokers, $p < 0.05$ by Student's t-test for unpaired values; **$p < 0.025$; +$p < 0.005$.

the higher frequencies observed in smokers and exsmokers (figure 1). Compared with nonsmokers, the mean spectrum power peaks of smokers and exsmokers are shifted to the right (to higher frequencies). Smokers and exsmokers had average cycle lengths 20 to 30 minutes shorter than nonsmokers.

Corroborating the spectrum analysis findings, smokers and exsmokers also engaged in significantly more frequent consummatory episodes (table 1). A measure of ingestive tendency, consummatory episodes were defined as eating, drinking or same combination of these, exceeding a total of three bites or sips, which began 15 or more minutes after a prior episode and which ended at least 15 minutes before the onset of a subsequent episode. On an hourly basis nonsmokers engaged in 0.59 ± 0.04 SEM consummatory episodes, while smokers engaged in 0.78 ± 0.05 episodes ($p < 0.005$) and exsmokers engaged in 0.73 ± 0.05 ($p<0.025$).

DISCUSSION

'The present findings suggest that shorter ultradian consummatory periods contribute to the impulse to smoke. Both smokers and exsmokers showed significantly shorter consummatory periods than nonsmokers, resulting in more frequent peaks of oral activity (eating and drinking). Clearly these rhythms of consummatory activity are independent of smoking, since nonsmokers, exsmokers and smokers all manifested rhythms in the ultradian frequency range. Previous studies have focused only on smokers (Friedman and Fisher 1967; Friedman 1972; Oswald et al. 1970). This finding supports prior evidence that ultradian consummatory cycles represent

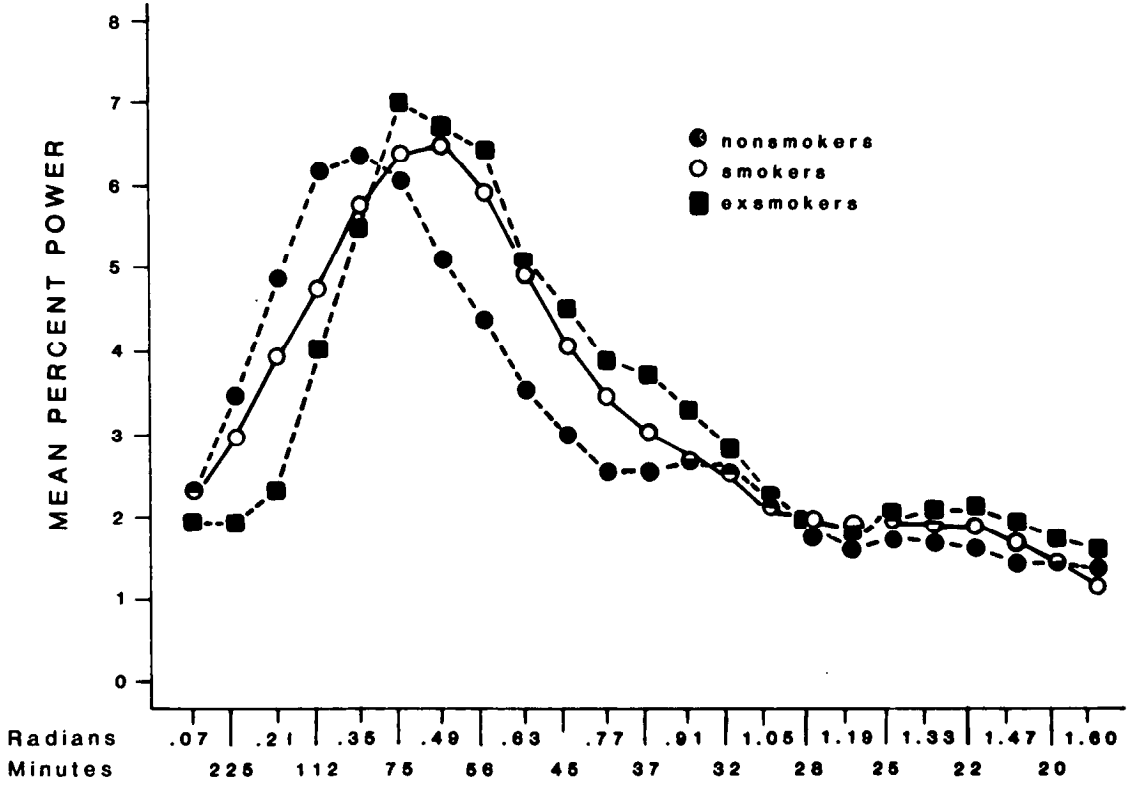

FIGURE 1. Mean percent spectral power in 23 spectral frequencies for sip + bite patterns of smokers, exsmokers, and nonsmokers. The X-axis shows frequency (in radians); every other frequency has been labeled with its corresponding period, or cycle length (in minutes) to aid interpretation.

endogenous physiological rhythms (Natelson and Bonbright 1978; Bowden et al. 1978; Emde et al. 1975; Kleitman 1969). The similar length of consummatory periods of smokers and exsmokers indicate that the more frequent oral impulses are not a pharmacological effect of nicotine or a result of other aspects of tobacco use. Instead, short consummatory cycles seem to be characteristic of people who have histories of smoking and are apparent even after individuals cease to smoke.

At present we cannot determine whether the short ultradian periods precede the smoking habit or whether habitual smoking shortens the period of the ultradian rhythm. If the period is fixed before a person begins smoking, then it would appear that short cycles of oral activity predispose toward cigarette use. Alternatively, if smoking shortens the ultradian period, then an important consequence of the smoking habit would be a relatively permanent increase in the frequency of oral activity, since short cycles were observed in exsmokers after several years of abstinence. In either event, the relationship between between smoking behavior and the length of the ultradian consummatory period appears to be important in understanding the nature of compulsive smoking. However it is established, the shorter period of a smoker's ultradian rhythm may contribute greatly to the persistence of the smoking habit or to the degree of difficulty in quitting or remaining abstinent. In the future it may be possible to develop empirically based intervention strategies that target the short consummatory cycles of smokers and thus alleviate or permit better management of the heightened oral drive.

REFERENCES

Bowden, D.M., Kripke, D.F., and Wyborney, V.G. Ultradian rhythms in waking behavior of rhesus monkeys. Physiol Behav, 21:929-933, 1978.

Emde, R.N., Swedberg, J., and Suzuki, B. Human wakefulness and biological rhythms after birth. Arch Gen Psychiat, 32:780-783, 1975.

Fishbein, M., ed. The New Illustrated Medical and Health Encyclopedia. New York: Stuttman, 1970. p. 1346.

Friedman, S., and Fisher, C. On the presence of a rhythmic, diurnal oral instinctual drive cycle in man. J Am Psychiat Assoc, 15:317-343, 1967.

Friedman, S. On the presence of a variant form of instinctual regression: oral drive cycles in obesity-bulimia. Psychoanal Quart, 41:364-383, 1972.

Jaffe, J.H. Drug addiction and drug abuse. In: Gilman, A.G., Goodman, L.S. and Gilman, A., eds. The Pharmacological Basis of Therapeutics. New York; Macmillan, 1980. pp. 535-584.

Kleitman, N. Basic rest-activity cycle in relation to sleep and wakefulness. In: Kales, A., ed. Sleep. Physiology and Pathology. Philadelphia: Lippincott, 1969. pp. 33-38.

Natelson, B.H., and Bonbright, J.C. Patterns of eating and drinking in monkeys when food and water are free and when they are earned. Physiol Behav, 21:201-213, 1978.

Oswald, I., Merrington, J., and Lewis, H. Cyclical "On demand" oral activity by adults. Nature, 225:959-960, 1970.

Russell, M.A.H., and Feyerabend, C. Cigarette smoking: a dependence on high-nicotine boli. Drug Metab Rev, 8:29-57, 1978.

Tapp, W.N., Levin, B.E., and Natelson, B.H. Ultradian rhythm of plasma norepinephrine in rats. Endocrinology, 109:1781-1783, 1981.

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Clinical Evaluation of Mecamylamine for Withdrawal From Nicotine Dependence

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ABSTRACT

Mecamylamine (MCL) has been shown to extinguish nicotine dependence in rats and monkeys. MCL was administered to fourteen nicotine-dependent persons to determine if it may be effective in withdrawing nicotine-dependent humans at doses which have acceptable toxicity. Subjects smoked 20 to 60 cigarettes per day for a mean of 2.4 years, and none had been nicotine abstinent for as much as one day for at least one year. MCL was started in a dose of 5 to 10 mg per day and progressively raised until the subject experienced nicotine blockage and/or toxic effects. During MCL administration, 7 of 14 (50%) totally ceased smoking within the first 11 days'of treatment, and an additional 4 (28.6%) subjects reduced cigarette consumption to less than five per day by the end of three weeks. Thirteen of 14 (92.9%) subjects stated that MCL blocks nicotine, reduces nicotine craving, and "works." At least some minor side-effects of MCL were observed in every subject. The most intolerable side-effects were constipation, urinary retention, abdominal cramps, and weakness, and these were responsible for drop-out of 5 (35.7%) subjects. Although there is a high prevalence of side-effects, MCL is probably a viable withdrawal treatment for some cases of recalcitrant nicotine dependence.

INTRODUCTION

Nicotine dependence is widely recognized as a problem which needs specific pharmacologic treatment.[1,2] Recent studies in rats and monkeys revealed that it is possible to extinquish nicotine dependence with the ganglionic blocker, mecamylamine (MCL).[3,4] When given to humans MCL will block the acute physiologic effects of nicotine. This study was undertaken to determine if MCL may be effective in the clinical withdrawal of nicotine-dependent humans at doses which have acceptable toxicity.

METHODS

Fourteen nicotine-dependent volunteers were selected for study. Criteria for selection were as follows: smoked at least 20 (one pack) cigarettes per day; had not been nicotine abstinent for more than one day for at least one year; no history of hypertension, pregnancy, cardiovascular disease, prostate enlargement, glaucoma, or dependence upon alcohol or other drugs. Subjects also had to have the perception that they were "addicted" to nicotine (Table One). Following informed consent, subjects underwent physical examination, blood pressure recording, pulmonary function testing, $_{6}$and urine analysis for the qualitative presence of nicotine.

Subjects were instructed to attend the clinic daily for five consecutive days, and every other day for two more weeks to make a maximum study period of 21 days. The first day's dose of MCL was 2.5 mg given two or three times for a 24-hour total of 5 to 7.5 mg. Beginning on day two, doses of MCL were raised by 2.5 to 5 mg per day until the subject perceived nicotine blockade and/or experienced significant side-effects. When significant side-effects occurred, the dosage of MCL was either reduced or held constant. If a subject stopped smoking cigarettes, he or she was allowed to continue MCL up to a maximum of 21 days.

During each clinic visit, the subject's blood pressure was recorded in the sitting and standing positions, and physical assessment was done for sedation, tremor, and motor impairment. A nicotine withdrawal score was determined on each day of attendance by assigning a numerical score of 0 for absent; +1 for mild; +2 for moderate; and +3 for severe to each of the following eight symptoms: agitation; depression; energy; myalgia/arthralgia; chills; nausea-vomiting; insomnia and anorexia. The maximum daily withdrawal score was 24. A daily check list of 24 side effects was assessed, and the list included abdominal cramps, blurred vision, constipation, dizziness, drowsiness, dry mouth, dysphoria, headache, irritable, lethargy, palpitation, photophobia, tremor, urinary hesitancy, urinary retention, and weakness. Subjects were specifically asked on each day of attendance if MCL blocked the effects of nicotine, if it "works," helps reduce nicotine craving, and if they wanted to continue MCL.

RESULTS

Subjects were a very experienced and dependent group of nicotine smokers (Table One). They had smoked a mean of 24.4 years and had not been nicotine-abstinent for even one day for periods ranging from one to 36 years (mean of 10.2 years). Half (7 of 14: 50%) reported they had to awaken at night to smoke. Thirteen of 14 (92.9%) subjects reported that MCL at least partially blocked the effects of nicotine, reduced nicotine craving, and that MCL "works." Seven (50%) subjects completely stopped smoking between the fourth and eleventh day of the study after progressively reducing, on a daily basis, their cigarette intake

(Table Two). The mean 24-hour dose of MCL on the day that each stopped smoking was 26.7 mg. Four additional subjects progressively reduced their cigarette intake to less than five per day, but never totally stopped. Ten of 14 (71.4%) converted their urine from nicotine positive to nicotine negative during the study (Table Three).

Very little nicotine withdrawal was observed in or reported by these subjects. The highest withdrawal score for any subject was 5 out of a maximum possible score of 24. Subjects appeared to definitely perceive MCL's ability to block nicotine's effects. This perception was described as cigarettes tasting bad, "wasting time," loss of craving, or smoking a low-nicotine cigarette. Statements relayed to us were quite descriptive, and some actual quotes are given here (Table Four). Side-effects were very common and severe enough to cause drop-outs in five (35.7%) subjects (Table Five). Urinary retention occurred in two subjects. One of these was a 25-year-old female who weighed 55.9 kg and who received 12.5 mg of MCL on the second day of the study, which produced the urinary retention. The other subject in whom this occurred was a 56-year-old male who received 40 mg of MCL on the day of urinary retention. The other side-effects which were most intolerable to subjects and caused study drop-outs were constipation, abdominal cramps, and weakness. Blood pressure dropped slightly in only one subject (110/80 mm Hg to 100/60 mm Hg).

After cessation of cigarettes by the seven successful subjects, each challenged MCL by smoking at least one cigarette on two different days (Table Five). Each expressed the view that they were "wasting time" and had no further challenges during the three week trial.

DISCUSSION

A number of pharmacologic treatments have been unsuccessfully attempted for nicotine dependence.[4,6-10] Schuster and co-workers were unable to alter smoking behavior in normal human subjects with d-amphetamine, meprobamate, and lobeline and Hanson and co-workers were unable to extinguish nicotine dependence in rats with pentolinium, amphetamine sulfate, caffeine, ethanol, chlordiazepoxide, phenobarbital, diphenylhydantoin, and chlorpromazine. Rosecrans has extensively studied rats who were trained to discriminate between nicotine and saline and found that arecoline, hexamethonium, atropine, dibenamine, and propanolol would not antagonize nicotine. Nicotine gum may assist smoking cessation by preventing abstinence symptoms, but its clinical usefulness is still uncertain.[2,8,9] MCL was first observed by Stone et al. in 1958 to antagonize nicotine-induced convulsions. Dominio, in 1967, showed that nicotine's arousal effects could be antagonized by MCL.[12] Rosecrans confirmed these observations. Henningfeld and co-workers demonstrated that MCL will selectively block at least some of nicotine's acute behavorial effects in humans.[5]

The dose of MCL used to extinguish nicotine self-administration in rats and monkeys was 1 to 4 mg/kg.[3,4] This dose is considerably higher than the daily dose of MCL normally used for treatment of hypertension, so a first consideration in testing MCL was to determine if toxicity may be too great to use in clinical treatment. Although side-effects were severe enough to cause 5 of 14 (35%) to drop out of treatment, the majority tolerated MCL well despite our attempt to progressively and rapidly increase the daily dose to reach blockade or toxic effect. Side-effects could possibly be lessened by slower induction of MCL, and it could also be taken over a longer period than 21 days. An initial first-day dose of 10 mg appeared to be tolerated well by all subjects. Hypotension was not a limiting factor in these subjects, and urinary retention was the most serious side-effect. Even though the majority of subjects tolerated MCL, the high prevalence of toxic side-effects observed with a nicotine blocking dose is such that MCL should probably not be used for nicotine dependence treatment unless all other methods have failed.

MCL's nicotine blocking effects were impressive as indicated by subjects' quotes and perceptions. Thirteen of 14 (92.9%) subjects, including subjects who dropped out, perceived that MCL blocked the effects of nicotine. The seven subjects who totally stopped smoking in the three-week study period progressively reduced cigarette intake each day until all smoking stopped. They also challenged MCL by smoking one or more cigarettes after they had gone a full 24 hours without smoking. Challenge during antagonist treatment is apparently to be expected, since it commonly occurs during naltrexone treatment for opioid addiction.[13] Subjects surprisingly reported that MCL reduced nicotine craving. Little nicotine withdrawal was documented by the method used here, but it was difficult to separate side-effects from nicotine withdrawal symptoms.

Subjects in this pilot study were very experienced smokers who smoked a mean of 24.4 years and who had not been nicotine abstinent for as much as a day in over a year. Seven of the 14 ceased nicotine during the 21-day study period and five of these relapsed within 30 days after stopping MCL. Therapeutic outcome may have been better if a less dependent group of subjects had been selected. Whether these persons ceased or reduced nicotine consumption due to MCL or placebo effect is unknown, since placebo and psychologic treatments may be effective in treating many nicotine-dependent persons.[9,14] Further clinical trials and double-blind, placebo-controlled studies will be needed to determine this. Regardless, preliminary observations of subjects studied here indicate that the clinical efficacy and safety of MCL should be studied more extensively, since MCL may be useful treatment in recalcitrant nicotine dependence.

TABLE ONE
CHARACTERISTICS OF SUBJECTS
N=14

Males	6 (42.9%)
Females	8 (57.1%)
Age Range (Yrs.)	24-61
Mean Age (Yrs.)	39.7
Weight Range (kg) Females	51.8-85.9
Mean Weight (kg) Females	62.1
Weight Range (kg) Males	66.3-103.6
Mean Weight (kg) Males	90.4
Range Nicotine Use (Yrs.)	10-44
Mean Nicotine Use (Yrs.)	24.4
Range Cigarettes Per Day	20-60
Mean Cigarettes Per Day	43.9
Mean Time Between Cigarettesin Daytime (Mins.)	18
Number Who Perceived "Addicted"	14 (100.0%)
Awake at Night to Smoke	7 (50.0%)
Failed Previous Attempts to Stop	14 (100.0%)

TABLE TWO
RESULTS AND OUTCOME

Range Days MCL Administration	2-21
Mean Days MCL Administration	13.5
No. Ceased Smoking	7 (50.0%)
No. Decreased Cigarettes to Less Than 5 Per Day	4 (28.6%)
Highest Withdrawal Score Observed	5
No. Who Converted Urine to Nicotine Negative	10
No. Dropped Out Due to Side-Effects	5 (35.7%)
No. Dropped Out Due to Non-Effectiveness	2 (14.3%)

TABLE THREE
DATA ON SUCCESSFUL SUBJECTS
N=7

Stopped on Which Days of Study	4-11
Range Dose of MCL Which Produced Cessation (mg)	7.5-50
Mean Dose of MCL Which Produced Cessation (mg)	26.7
Relapsed Within 30 days	5 (71.4%)
Females	4 (57.1%)
Males	3 (42.9%)
No. Who Smoked at Night	4 (57.1%)

TABLE FOUR
SOME ACTUAL QUOTES OF SUBJECTS CONCERNING
MECAMYLAMINE'S EFFECTS ON SMOKING

I FOUND OUT I'M WASTING TIME SMOKING

HARDLY THINK ABOUT IT

AFRAID TO NOT TAKE MEDICINE

DIDN'T DESIRE TO SMOKE WHEN I GOT UP

THEY TASTE TERRIBLE

LOTS OF ENERGY

JUST DON'T WANT THEM

DON'T SMOKE WHOLE CIGARETTE

TASTE LINGERS IN MY MOUTH

MOST OF TIME HALF THE CIGARETTE IS SMOKED

HORRIBLE TASTE

HAVE A RANK TASTE

LIKE SMOKING A LOW TAR CIGARETTE - LOTS OF AIR

NAUSEA WHEN I SMOKE

ONLY TWO PUFFS OF CIGARETTES AND PUT REST OUT

TABLE FIVE
SIDE-EFFECTS OBSERVED IN SUBJECTED
N=14

SIDE EFFECT	NUMBER
ABDOMINAL CRAMPS	8 (57.1%)
BLURRED VISION	3 (21.4%)
CONSTIPATION	12 (85.7%)
DIZZINESS	6 (42.9%)
DROWSINESS	8 (57.1%)
DRY MOUTH	11 (78.6%)
DYSPHORIA	2 (14.3%)
HEADACHE	1 (7.1%)
IRRITABLE	5 (35.7%)
LETHARGY	2 (14.3%)
PALPITATION	1 (7.1%)
PHOTOPHOBIA	1 (7.1%)
TREMOR	1 (7.1%)
URINARY HESITANCY	2 (14.3%)
URINARY RETENTION	2 (14.3%)
WEAKNESS	3 (21.4%)

REFERENCES

1. Friedman, G.D., Petitti, D.B., Barval, R.D., et al. Mortality in cigarette smokers and quitters: Effect of base-line differences; N Engl J Med, 304: 1407-1414, 1981.
2. Jaffee, J.H., Kanzler, M. Smoking as an addictive disorder. In Krasnegor, N.A. (ed). Cigarette Smoking as a Dependence Process. National Institute on Drug Abuse Research Monograph 23. DHEW Pub. No. (ADM)79-800. Washington, D.C.: Supt. of Docs., U.S. Govt. Print. Off., 1979. pp. 4-23.
3. Goldberg, S.R., Spealman, R.D., Goldberg, D.M. Persistent behavior at high rates maintained by intravenous self-administration of nicotine. Science, 214:573-575, 1981.
4. Hanson, H.M., Ivester, C.A., Morton, B.R. Nicotine self-administration in rats. In Krasnegor, N.A. (ed). Cigarette Smoking as a Dependence Process. National Institute on Drug Abuse Research Monograph 23. DHEW Pub. No. (ADM)79-800. Washington, D.C.: Supt. of Docs., U.S. Govt. Print. Off., 1979. pp. 70-90.

5. Henningfield, J.H., Katsumasa, M., Johnson, R.E., et al. Rapid physiologic effects of nicotine in humans and selective blockade of behavioral effefts by mecamylamine. In Harris, L.S. (ed.). Problems of Drug Dependence, 1982. DHHS Pub. No. (ADM)83-1264. Washington, D.C.: Supt. of Docs., U.S. Govt. Print. Off., 1983. pp. 259-265.
6. Mule, S.J. Identification of narcotics, barbiturates, amphetamines, tranquilizers and psychotomimetics in human urine. J. Chromatogr, 39:302-311,. 1969.
7. Schuster, C.R., Lucchesi, B.R., Emley, G.S. The effects of d-amphetamine, meprobamate, and lobeline on the cigarette smoking behavior of normal human subjects. In Krasnegor, N.A. (ed). Cigarette Smoking as a Dependence Process. National Institute on Drug Abuse Research Monograph 23. DHEW Pub. No. (ADM)79-800. Washington, D.C.: Supt. of Docs., U.S. Govt. Print Off., 1979. pp. 91-99.
8. Schneider, N.G., Popek, P., Jarvik, M.E., et al. The use of nicotine gum during cessation of smoking. Am J Psychiatry, 134:439-440, 1979.
9. Raw, M., Marvis, M.J., Feyerabend, C., et al. Comparison of nicotine chewing gum and psychological treatments for dependent smokers. Br Med J, 281:481-482, 1980.
10. Rosecrans, J.S. Nicotine as a discriminative stimulus to behavior: Its characterization and relevance to smoking behavior. In Krasnegor, N.A. (ed). Cigarette Smoking as a Dependence Process. National Institute on Drug Abuse Research Monograph 23. DHEW Pub. No. (ADM)79-800. Washington, D.C.: Supt. of Docs., U.S. Govt. Print. Off., 1979. pp. 58-69.
11. Stone, C.A., Meckelnberg, K.L., Torchiana, M.L. Antagonism of nicotine-induced convulsions by ganglionic blocking agents. Arch Int Pharmacodyn Ther, 117:419-434, 1958.
12. Domino, E.F. Electroencephalographic and behavioral arousal effects of small doses of nicotine: A neuropsychological study. Ann NY Sci, 142:216-244, 1967.
13. Judson, B.A., Carney, T.M., Goldstein, A. Naltrexone treatment of heroin addiction: Efficacy and safety in a double-blind dosage comparison. Drug and Alcohol Dependence, 7:325-346, 1981.
14. Puska, P., Bjorkquist, S., Koskela, K. Nicotine-containing chewing gum in smoking cessation: A double bline trial with half year follow-up. Addict Behav, 4:141-146, 1979.

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Adverse Effects of Cocaine Abuse

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ABSTRACT

Specific, consequences of cocaine abuse on health and psychosocial functioning were assessed in 55 cocaine-abusing subjects who called a telephone "helpline." Results showed a high incidence and wide range of adverse consequences including: (a) impairment of job functioning, interpersonal relationships, and financial status; (b) disturbances of mood and cognitive functioning; (c) psychiatric symptoms of depression, paranoia, and increased suicidal/violent tendencies; and (d) physical symptoms of exhaustion, weight loss, sleep problems, and seizures. Cocaine-related automobile accidents, suicide attempts, and violent acts, including a cocaine-related homicide, were also reported. Intranasal users reported no fewer and no less severe adverse consequences than free-base smokers or intravenous users. Our findings challenge popular notions that cocaine is a benign "recreational" drug and that the intranasal route of administration guarantees protection against addictive patterns of use and adverse effects.

INTRODUCTION

Cocaine use has escalated to epidemic proportions in the U.S. in recent years. Nationwide surveys estimate that over 22 million American have used cocaine and the numbers continue to soar at an alarming rate. Moreover, cocaine use has spread considerably into the middle-class and working-class segments of American society. It is no longer a drug used only by the wealthy or elite.

Health consequences of escalating cocaine use are reflected in figures showing more than a 200% increase in cocaine-related deaths and emergency room visits and more than a 500% increase in cocaine-related admissions to federally funded treatment programs between 1976 and 1981. Despite these alarming trends, the

popular belief that cocaine is a benign "recreational" drug continues to be perpetuated. Recent studies of cocaine use have focused primarily on "social-recreational" users (Seigel, 1977) or on individual case reports of dysfunctional use (Wesson and Smith, 1977). Such reports tend to underestimate the prevalence of dysfunctional cocaine use and resulting adverse consequences. This is especially true for intranasal use which is commonly regarded as much safer than free-base smoking or intravenous use.

We now report data on specific adverse effects of cocaine use on health and psychosocial functioning in a sample of intranasal, free-base, and intravenous users.

SUBJECTS AND METHODS

A telephone "helpline" for cocaine abusers was established at out facility in February 1983. Local television and radio stations publicized the helpline by informing cocaine users that they would call anonymously for information, advice or referral to treatment. We conducted a 20-30 minute telephone interview and administered an extensive questionnaire to the callers to obtain data on demographic variables, drug use, psychiatric history, and particularly on consequences of cocaine use within specific areas of physical health and psychosocial functioning. Consequences were grouped into several categories each containing a checklist of specific items, as follows: (1) Physical Consequences - nausea/vomiting, sweating, nasal sores/bleeding, headaches, persistent cough/sore throat, feel run down and Weak, sleep problems, chills, hands tremble, double vision, seizures/loss of consciousness; (2) "Major" Psychological or Behavioral Consequences - paranoid ideation, physically injured someone, hallucinations; (3) "Minor" Psychological or Behavioral Consequences - irritable, short-tempered, depressed, anxious, lazy, low on energy, difficulty concentrating, confused thoughts, memory problems, loss of sex drive; (4) Vocational Consequences - lateness, absence, reduced productivity at Work; (5) Interpersonal Consequences - increased discord with spouse/mate, spouse leaves or threatens to leave, impairment of social life and friendships, impaired sexual relationship; (6) Legal, Consequences - arrests for possesion or sale of cocaine or related crimes; (7) Financial Consequences - depleted bank acounts, unable to keep up with bills, no extra money, accumulated debts; (8) Automobile Accidents - any cocaine-related auto accident involving damage to property and/or persons.

RESULTS

Over 2,000 calls Were received on the helpline during its first eight weeks of operation and calls continue to be received at a rate of 25-100 per day. Approximately 70% of the calls are from cocaine abusers themselves and the remainder from concerned

family members, friends, or professionals. Data presented here are fran the first 55 cocaine abusers who were interviewed on the helpline.

Our sample was: 78% male, 22% female, ages 22-59 years (X=33 yrs); 56% were white, 35% black, 9% Hispanic. Mean level of education was 14.1 years, range 9-18 years. Forty-nine percent had annual incomes over $25,000; 53% had occupations in the categories of white collar, professional, or self-employed business owner. Preferred route of cocaine administration was: intranasal (IN) 51%; free-base smoking (FB) 22%; and intravenous (IV) 27%. Estimates of weekly cocaine use ranged from 1-32 gram/week with a mean of 8.2 grams. Forty-eight Percent used 6 grams or more per week. Frequency of cocaine use averaged 5.7 days/week; 56% used at least 5 days/week. At prices of $lOO-$125 per gram, the average amount of money spent per week on cocaine was over $800 and ranged fran $109 to $3,150. No differences were found between the IN, FB, and IV groups with regard to weekly dose estimates or frequency of cocaine use.

Percentages who responded "yes" to each of the following statements about their cocaine use were: (a) psychologically addicted to cocaine - 92%; (b) have lost control over cocaine use - 91%; (c) crave cocaine and feel a compulsion to use it - 81%; (d) want to stop using cocaine - 80%; and (e) feel unable to stop using cocaine without help - 75%. Sixty-four percent reported no concurrent regular use of drugs other than cocaine; the remaining 36% reported using tranquilizers, marijuana, alcohol, or heroin to reduce the stimulant effects of cocaine or to relieve the dysphoric "crash" when cocaine effects wore off.

A high incidence and wide range of adverse consequences of cocaine use were reported. The percentage of subjects reporting at least one, but usually several consequences within each category were as follows: (a) Physical Health - 98%; (b) Interpersonal - 93%; (c) "Minor" Psychological - 93%; (d) "Major" Psychological - 56%; (e) Vocational - 64%; (f) Financial - 84%; (g) Legal - 13%; and, (h) Auto Accident - 6%. A characteristic pattern of disruptive functioning was reported by most subjects, consisting of: (a) absenteeisn and reduced effectiveness at work; (b) increased discord with spouse/mate leading to actual separation or threat to separate; (c) diminished or exhausted financial resources and accumulation of debts; and, (d) feeling depressed, anxious, irritable, and overwhelmed with problems. The incidence of specific consequences within areas of physical health and psychological functioning is shown in Table 1.

TABLE1
INCIDENCE OF REPORTED CONSEQUENCES OF COCAINE USE
(N=55)

	# S's	% S's
PHYSICAL HEALTH		
Exhausted and weak	33	60%
Sleep difficulties	32	58%
Nasal sores/bleeding	18	33%
Hands tremble	18	33%
Significant weight loss	18	33%
Headaches	16	29%
Nausea/vomiting	14	25%
Cough/sore throat	12	22%
Double vision	8	15%
Seizure and loss of consciousness	6	11%
"MAJOR" PSYCHOLOGICAL/BEHAVIORAL		
Paranoid ideation	26	47%
Panic attacks	11	20%
Feel violent	10	18%
Hallucinations	9	16%
Physically injured someone	6	11%
Suicidal ideation	4	7%
Attempted suicide	4	7%
"MINOR" PSYCHOLOGICAL/BEHAVIOR		
Irritable, short-tempered	37	67%
Depressed, bad mood	36	65%
Nervous, anxious	35	64%
Lazy, unmotivated	30	55%
Loss of sex drive	24	44%
Difficulty concentrating	20	36%
Confused thoughts	19	35%
Memory problems	18	33%

The most common physical complaints were exhaustion and excessive sleeping following a binge of cocaine use. Nasal problems and headaches were characteristic of IN users with one reporting a perforated nasal septum. Persistent cough and sore throat was characteristic of FB smokers. The most disturbing physical consequence was seizure with loss of consciousness which reportedly occurred on at least several occasions of high-dose cocaine use in some of our subjects. Reported "minor" psychological consequences included mood disturbances, loss of sex drive, and impaired cognitive functioning. Among "major" psychological consequences, the most commonly reported was paranoid ideation consisting of exaggerated concerns about intruders as well as extreme mistrust of family members, friends, and neighbors. Two

subjects reported symptoms of cocaine-induced psychosis characterized by eleaborate paranoid delusions with ideas of reference and persecution, as well as auditory and visual hallucinations. Increased violent and suicidal feelings or behaviors were reported in some cases. One subject reported committing a murder while high on cocaine. No consistent relationship was found between the incidence of reported consequences and either dose or frequency of cocaine use. A substantial number of consequences were consistently reported across a Wide range of doses and frequencies of cocaine use. Also, the IN, FB, and IV groups did not differ with regard to the incidence or type of reported consequences. Contrary to expectation, IN users reported no fewer and no less severe consequences than FB or IV users. The psychoactive, mood-altering effects of cocaine considered most desirable by our subjects included: feelings of elation, mastery, confidence, self-control, and sexual arousal; increased talkativeness, physical energy, and contentment; and, elimination of boredom, fatigue, and stress. However, almost without exception these desirable effects diminished or disappeared entirely With continued, chronic use and were eventually replaced by an increasing number of adverse effects such as depression, irritability, loss of sex drive, and intense guilt. In a futile attempt to recapture the desirable effects and to Ward off the unpleasant "crash," behavior Was driven toward continued and intensified patterns of cocaine use. Our subjects found themselves progressively drawn into a powerful, vicious cycle of obtaining, using, and recuperating from cocaine. The accumulated adverse consequences of cocaine use and resulting psychological distress are what ultimately led them to call our helpline for assistance.

DISCUSSION

The large volume of calls to our helpline appear to reflect the increasing prevalence of cocaine dependence in the U.S., especially among White, middle-class males who are otherwise not heavily involved in drugs. Not only are more people using cocaine, but increasing numbers are developing addictive patterns of use and suffering serious disruption to their functioning. The popular belief that cocaine is a non-addictive, social drug, With low abuse potential, especially if used by the intranasal route, is challenged by our findings that all three routes of administration (snorting, smoking, injecting) were associated With compulsive use patterns characterized by loss of control over use, persistent craving and compulsion to use cocaine, and continued use despite serious adverse consequences. Although addictive patterns might develop more rapidly with free-base smoking or intravenous use, our findings and others (Helfrich et al. 1983) demonstrate that intranasal users are not exempt from uncontrollable use or from adverse consequences. Recent reports of death from intranasal use (Wetli and Wright, 1979) underscore the fact that toxic blood levels of cocaine can be achieved by this route. Individuals With epilepsy, hypertension, or cardiovascular disease might be especially prone to fatal reactions.

Admittedly, our sample was biased since all subjects were problematic users as self-defined by their calling the helpline. Moderate users, by definition, tend not to suffer medical or psychological consequences. Nonetheless, it cannot be concluded that even moderate levels of intranasal use guarantee protection against eventual dependence and other adverse effects. Most of our subjects began with occasional use and were rather surprised at how quickly and intensely their use escalated to compulsive patterns especially because they had thought cocaine was non-addictive. The question of why some users escalate to addictive patterns is of substantial clinical importance, but remains largely unanswered at present. Comparison of social-recreational users with compulsive users might elucidate some important individual differences.

Although psychological dependence on cocaine does occur, whether cocaine produces true physical dependence remains a question. After cessation of chronic cocaine use, the user usually does not experience a clearly definable withdrawal syndrome as with heroin or barbiturates. However, the generalized dysphoria and feelings of malaise following cocaine use may be viewed as a withdrawal state especially since they are associated with drug craving and drug-seeking behavior and are relieved by resumption of cocaine use. Furthermore, our findings suggest that with chronic use of cocaine tolerance develops to many of its effects.

The large volume of anonymous calls to our helpline suggests that a substantial portion of cocaine abuse is otherwise hidden from scientific or public analysis. Estimates of the prevalence and consequences of cocaine abuse based on death rates, emergency-room visits, and treatment admissions may grossly underestimate the current extent of the problem. Nearly all our subjects had no history of treatment for any aspect of drug abuse, especially the more middle-class abusers who had dismissed the idea of seeking help in the usual type of drug abuse clinic that is known to treat mainly heroin addicts. There is currently no specific treatment for cocaine abuse with demonstrated efficacy. A number of pharmacologic agents (e.g., desipramine, methylphenidate, lithium carbonate) are being evaluated in the treatment of cocaine abusers at our outpatient facility and elsewhere (Rawson et al. 1983; Khantzian, 1983). Preliminary findings suggest the potential usefulness of these medications in selected patients when combined with frequent contact and supportive counseling or psychotherapy. Some cocaine abusers require hospitalization because of psychosis, acting-out behavior, or inability to refrain from cocaine use.

It is likely that adverse consequences of cocaine use will become increasingly prevalent and visible during the next few years. Cocaine's reputation as a relatively safe recreational drug has already contributed to more widespread abuse. If

cocaine were more readily available and at a substantially lower cost, or if social sanctions and scientific information failed to caution against intensified use, even larger numbers Would suffer serious consequences from this very seductive drug. It is imperative, therefore, that accurate information be gathered and disseminated about specific consequences of cocaine abuse.

There is a pressing need for expanded research and treatment efforts to combat the current epidemic of cocaine abuse in the U.S. and prevent it from escalating further.

REFERENCES

Center for Disease Control. National surveillance of cocaine use and related health consequences. Morbidity and Mortality Report, 1982 May 28: 265-276. U.S. Dept. of Health and Human Services, Washington, D.C.

Helfrich, A.A., Crowley, T.J., Atkinson, C.A., and Post, R.D. A clinical profile of 136 cocaine abusers. In Harris, L.S. ed. Problems of Drug Dependence, 1982. National Institute on Drug Abuse Research Monograph. DHHS Pub. No. (ADM) 83-1264. Washington, D.C., Supt. of Docs., U.S. Government. Print. Off, 1983 pp. 343-350.

Khantzian, E.J., Garvin, F., Kleber, H.D., and Riordan, C.E. Methylphenidate treatment of cocaine dependence: a preliminary report. Am J. Psychiatry, in press.

National Institute on Drug Abuse, National Household Survey on Drug Abuse, 1982. National Clearinghouse for Drug Abuse Information, Rockville, Maryland.

Rawson, R.A., Tennant, F.S., and McCann, M.J. Withdrawal from phencyclidine, amphetamine, and cocaine dependence With dispramine. In Harris, L.S., ed. Problems of Drug Dependence, 1982. National Institute on Drug Abuse Research Monograph. DHHA Pub. No. (ADM) 83-1264. Washington, D.C., Supt. of Docs., U.S. Govt. Print. Off., 1983. pp. 351-355.

Siegal, R.K. Cocaine: recreational use intoxication. In Peterson, R.C., and Stillman, R.C., eds. Cocaine: 1977. National Institute on Drug Abuse Research Monograph 13. DHEW Pub. No. (ADM) 79471. Washington, D.C., Supt. of Docs., U.S. Govt. Print. Off., 1977. pp. 119-136.

Wesson, D., and Smith, D. Cocaine: its use for central nervous system stimulation including recreational and medical uses. In Peterson, R.C., and Stillman, R.C., eds. Cocaine: 1977. National Institute on Drug: Abuse Research Monograph 13. DHEW Pub. No. (ADM) 79471. Washington, D.C., Supt.of Docs., U.S. Govt. Print. Off., 1977. pp. 137-152.

Wetli, C.V., and Wright, R.K. Death caused by recreational cocaine use. JAMA, 241(23): 2519-2522, 1979.

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Comparison of a Behavioral and a Pharmacological Treatment for Reduction of Illicit Opiate Use

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Although many methadone maintenance patients eliminate their use of illicit opiate drugs after enrolling in treatment, continuing use of heroin or other opiates is commonly observed in methadone maintenance clinics. Typically, 20 - 40 percent of methadone maintenance patients show at least an occasional opiate-positive urine test over a period of several months of urinalysis screening (Bigelow et al. 1980; Ling et al. 1978; Newman and Whitehill 1979; Stitzer et al. 1980), while a smaller subset of these patients show persistent illicit opiate use during methadone maintenance treatment. Recent studies have suggested that patients maintained on higher methadone doses show lower rates of illicit drug use than patients maintained on relatively lower methadone doses (Ling et al. 1978; McGlothlin and Anglin 1981). This observation suggests that raising the methadone dose for individual drug-abusing patients may decrease supplementation with illicit opiate drugs, and in fact raising the dose is a common strategy employed by drug abuse clinics for patients who engage in persistent opiate supplementation. However, the effects of methadone dose increases on rates of illicit drug supplementation have not been systematically evaluated. The present study will provide such an evaluation.

Another line of research has suggested that contingency management procedures can effectively reduce illicit drug supplementation among methadone maintenance patients. In previous studies, money and take-home privileges have been offered to chronic opiate supplementers for providing opiate-free urine samples (Hall et al. 1979; McCaul et al. 1983; Stitzer et al. 1980). A methadone dose increase might also serve as a reinforcer for maintenance patients since these patients claim that dose increases are desirable (Stitzer and Bigelow 1978) and reliably self-administer such increases when given the opportunity (Stitzer et al. 1983). In the present study, dose increases were offered contingent upon provision of opiate-free urine samples. If dose increases serve as reinforcers, this contingent arrangement should increase the number of drug-free urines delivered. The study hypothesis is that a contingent methadone dose increase may be more effective

than a noncontingent increase for influencing supplemental opiate drug use since the contingent procedure should specifically motivate behavior change in addition to providing a beneficial pharmacological intervention.

The present study, then, is a comparison between two procedures, both of which were expected to have a beneficial impact on chronic opiate supplementation. One procedure was purely pharmacological, consisting of a blind methadone dose increase. The second procedure was a behavioral intervention which involved patient-controlled dose increases available contingent upon opiate-free urinalysis test results.

METHODS

Participants were seven male methadone maintenance patients, selected because of persistent opiate-positive urine test results during routine clinic urinalysis screening. Five subjects were black and two were white. Average age was 33.4 years (range 29 - 40 years). Subjects reported an average of 11.4 years of continuous opiate use and had been enrolled in this clinic for an average of 4.3 months prior to study participation (range 2 - 7 months).

All subjects received their regular 50 mg/day methadone dose throughout this study and provided urine samples routinely. Urine samples were tested for presence of opiates using an on-site EMIT system (Syva Corp.). Urines were also tested for a variety of additional drugs of abuse (including opioids, benzodiazepines, sedatives, and stimulants) by an outside urinalysis laboratory employing thin layer chromatography. Urine samples were collected twice weekly (Mon and Fri) during the 6 - 8 week baseline evaluation period which preceded each intervention and during the pharmacological intervention; urines were collected three times weekly during the behavioral intervention.

The pharmacological intervention consisted of an abrupt blind methadone dose increase from 50 mg to 75 mg/day. The dose was held at this level for six weeks then gradually decreased back to its original stable level over a 2 - 3 week period. Neither subjects nor counseling staff were aware of the dose alterations.

During the behavioral intervention, dose alteration opportunities were based on urinalysis test results. If a given urine sample was opiate-positive, no dose change was allowed. If the sample was opiate-free, the subject could increase his stable dose by a maximum of 25 mg per day. The dose could be increased each day, with the size of the increase selected daily until the next urinalysis test day. At this point, the procedure in effect was again determined by the urinalysis test result. That is, if the urine was opiate-positive, no dose increase was allowed; while if the urine was opiate-free, the patient could continue to request dose increases. The contingent dose increase procedure WAS described to subjects 3 - 4 days prior to their first dose in-

crease opportunity and was in effect for six weeks. At the end of this time, the opportunity to increase dose was no longer available, resulting in an abrupt withdrawal of extra methadone and a return of dose to the original maintenance level.

Three subjects have been exposed to both treatment conditions in a cross-over design while four subjects have been exposed to only one study intervention. Cross-overs have not been possible to run with all subjects either because the subject left the clinic or because illicit drug use did not return following exposure to the first treatment intervention.

Subjects completed a 60-item symptomatology report form twice weekly on Monday and Friday on which they rated the extent of each symptom on a scale of 0 (not present) to 3 (severe). Symptoms included those typical of opiate withdrawal and opiate intoxication as well as a variety of miscellaneous complaints. Changes in symptomatology related to the implementation and withdrawal of study procedures were assessed by subtracting each subject's average baseline symptom score from the average score obtained during and after each intervention; average scores were generally based on data collected during 6-week blocks of time.

RESULTS

Figure 1 summarizes urinalysis results as percent of opiate-free urine samples collected for successive two-week time periods before (open bars) and during (closed bars) the two study interventions. During pre-intervention baseline periods, only about 20 percent of urine samples were opiate-free for the group and no subject delivered more than 50 percent opiate-free urines during any given two-week time period.

Overall, the behavioral intervention produced a greater and more immediate increase in opiate-free urines than the blind dose increase procedure. Following the blind dose increase, 48 percent of urines were opiate-free, a nonsignificant increase from baseline. Two of the four subjects studied showed marked increases in opiate-free urines following the blind dose increase. However, increases in drug-free urines generally were not apparent until the third week after initiation of the blind dose change.

In contrast, 74 percent of urines were opiate free during the contingent dose increase procedure. This is a significant increase from baseline levels in a test of differences between proportions ($p < 0.01$). Four of six subjects showed marked increases in opiate-free urines during the behavioral procedure. Furthermore, the effect of the behavioral procedure was generally immediate.

Six patients exposed to the contingent methadone dose increase procedure amassed a total of 211 opportunities to raise their methadone dose. The dose increase opportunity was never refused, but neither did patients invariably choose the maximum allowable

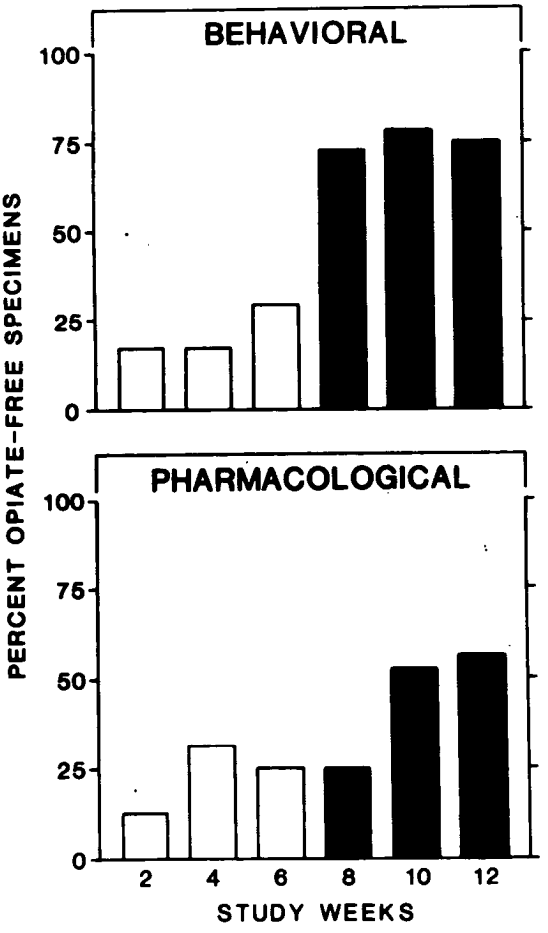

FIGURE 1. *Percent opiate-free urine specimens are shown during successive two-week blocks of baseline evaluation (open bars) and study intervention (filled bars). Shown in the upper panel are data from an intervention in which methadone dose increases were available contingent upon opiate-free urine specimens (N=6). Shown in the lower panel are data from an intervention in which blind methadone dose increases were given independent of urine test results (N=4).*

increase. The maximum allowable dose increase was selected on 65.4 percent of opportunities, while the remaining choices were about equally distributed between doses of 10, 15, and 20 mg.

Symptom reports were examined for evidence of withdrawal complaints or opiate intoxication during and after each of the study procedures. No consistent changes in symptom scores were observed during either the behavioral or the pharmacological intervention. Nor, surprisingly, did symptom complaints increase following withdrawal of the blind 25 mg methadone dose increase. The one consistent effect noted in the four subjects who responded to the behavioral procedure and received dose increases was an elevation of symptom scores following termination of the contingent dose increase procedure.

Examination of full urine screens revealed that these subjects were primarily heroin abusers. Only three of the subjects ever showed drug positives other than morphine, and the use of other drugs was not consistently related to study conditions.

DISCUSSION

This preliminary study conducted with a small number of subjects has shown that a blind methadone dose increase can reduce opiate drug supplementation in some methadone maintenance patients. Improvement was noted in 2 of the 4 patients assigned to this procedure; onset of improvement was delayed by about two weeks after the dose increase was initiated. On the other hand, a behavioral procedure which involved the opportunity for subjects to raise their own dose if they provided a drug-free urine specimen was effective more consistently across subjects, produced a larger overall decrement in opiate drug supplementation, `and had a more immediate impact on drug use than the blind increase procedure. Although these results must be considered tentative at this point, they suggest that the effectiveness of methadone dose increases for reducing illicit opiate drug supplementation can be enhanced by scheduling dose increases contingent on urinalysis test results.

It should be noted that patients were actually receiving more total mg of methadone during the pharmacological than during the behavioral dose increase procedure. This suggests that the motivational aspect of the contingent procedure, which targets a specific behavior change in patients, is a more important element of the intervention than the magnitude of the dosage increase which patients receive.

The clinical utility of dose increase procedures rests on their safety as well as their efficacy. It could be the case that fluctuations in the methadone dose during the contingent procedure or after dose alteration procedures were withdrawn, caused patient discomfort which could potentially contribute to renewed drug use. However, the dose increase procedures appeared to be safe as well as effective. No increases in symptomatic complaints were noted

while either intervention was in effect. Although there were increased complaints observed after withdrawal of the behavioral procedure, these appeared to be influenced by suggestibility factors since subjects knew that extra methadone would no longer be available and similar complaints did not occur following the blind dosing procedure. Another indication of the safety of these procedures is that no increase in the use of other drugs was reliably observed during periods of time when illicit opiate drug use was suppressed. This lack of "symptom substitution" is consistent with results of previous studies which have used contingent reinforcement procedures to reduce illicit drug use (Stitzer et al. 1980, 1982).

The present study suggests that the way in which methadone dose increases are scheduled may be important for determining the efficacy of the procedure in suppressing illicit opiate use among methadone maintenance patients. Specifically, the contingent dose increase procedure described in the present study may be more effective than the noncontingent dose increase procedure which is commonly employed. However, contingent procedures are suitable for application only in clinics where on-site urinalysis testing equipment is available. In other clinics, increasing the methadone dose noncontingently is still a useful procedure for reducing supplementation with illicit opiate drugs.

REFERENCES

Bigelow, G.E., Stitzer, M.L., Lawrence, C., Krasnegor, N., D'Lugoff, B., and Hawthorne, J. Narcotics addiction treatment: Behavioral methods concurrent with methadone maintenance. Int J Addict, 15:427-437, 1980.

Hall, S.M., Cooper, J.L., Burmaster, S., and Polk, A. Contingency contracting as a therapeutic tool with methadone maintenance clients: Six single subject studies. Behav Res Ther, 15:438-441, 1977.

Hall, S.M., Bass, A., Hargreaves, W.A., and Loeb, P. Contingency management and information feedback in outpatient heroin detoxification. Behav Ther, 10:443-451, 1979.

Ling, W., Klett, J., and Gillis, R.D. A cooperative clinical study of methadyl acetate. Arch Gen Psychiatry, 35:345-353, 1978.

McCaul, M.E., Stitzer, M.L., Bigelow, G.E., and Liebson, I. Contingency management interventions: Effects on treatment outcome during methadone detoxification. J Appl Behav Anal, in press, 1983.

McGlothlin, W.H., and Anglin, M.D. Long-term follow-up of clients of high- and low-dose methadone programs. Arch Gen Psychiatry, 38:1055-1063, 1981.

Newman, R.G., Whitehill, W.B. Double-blind comparison of methadone and placebo maintenance treatments of narcotic addicts in Hong Kong. Lancet, September 8, 1979, 485-488.

Stitzer, M., and Bigelow, G. Contingency management in a methadone maintenance program: Availability of reinforcers. Int J Addict, 13:737-746, 1978.

Stitzer, M.L., Bigelow, G.E., and Liebson, I. Reducing drug use among methadone maintenance clients: Contingent reinforcement for morphine-free urines. Addict Behav, 5:333-340, 1980.

Stitzer, M.L., Bigelow, G.E., and Liebson, I. Contingent reinforcement for benzodiazepine-free urines: Evaluation of a drug abuse treatment intervention. J Appl Behav Anal, 15:493-503, 1982.

Stitzer, M.L., McCaul, M.E., Bigelow, G.E., and Liebson, I.A. Human oral methadone self-administration: Effects of dose and alternative reinforcers. Clin Pharmacol Ther, in press, 1983.

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Methadone Plasma Levels and Persistent Drug Abuse in High Dose Maintenance Patients

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ABSTRACT

Methadone maintenance patients who maintained on a high daily dose were divided into good performers and performers based on whether they demonstrated persistent use of heroin, non-prescription diazepam, and/or excessive alcohol consumption. Mean methadone plasma levels 24 l-ours after an oral dose of 80 mg were found to be 410.4 ng/ml in good performers compared to 101.8 ng/ml in poor performers (P<.O5). Seven of nine (77.8%) poor compared to two of 15 (13.3%) good performers had 24-hour methadone plasma levels under 50 ng/ml (P<.01). High dose methadone patients who show evidence of persistent drug or alcohol abuse should have their 24-hour methadone plasma level determined to help assess whether the patient should receive more methadone or find an alternative treatment.

INTRODUCTION

Methadone maintenance (MM) has been the principal treatment for recalcitrant heroin dependence for over a decade (4). A major unresolved problem in MM treatment is the patient who persistently abuses drugs and/or alcohol despite high daily doses of methadone (4, 5, 16). This study was done to determine if some persistent drug use may be related to methadone metabolism and to develop a useful clinical technique to identify patients in whom methadone will not provide maintenance for a full 24 hours.

METHODS

A 200-person MM program that allowed patients to maintain on a maximum of 8O mg/day was surveyed. Out of this patient group, 24 (12.0%) persons who had been on MM for over three months and who had voluntarily chosen to raise their MM dose to 80 mg/day were selected for study. For one month, each subject was tested weekly by urine analysis for morphine, amphetamines, codeine, barbiturates, and diazepam (15). A physical

examination for fresh needle marks and a breath alcohol level were also done each week (17). At the conclusion of the study period, each subject was given a written questionnaire which asked if the subject had used heroin or other non-prescribed drugs, and if they drank more than two alcoholic drinks per day. Also asked was whether they perceived themselves to be doing "very well," "fair," or "not well" on the program, and if their daily dose of methadone "holds" for 24 hours. Patients were considered to be poor performers on MM if they demonstrated, during the study month, abuse of alcohol or persistent use of unprescribed diazepam or heroin. At some point in the study period, a methadone plasma level was taken 24 hours after the subject was observed to take an oral dose of 80 mg of methadone. Analysis was done by use of gas liquid chromatography and each plasma sample was analyzed twice (9). Alcohol abuse was documented by at least two separate, positive breath alcohol tests during daytime clinic l-ours, plus admission on the questionnaire that more than two alcoholic drinks were consumed each day. Persistent use of non-prescription diazepam and heroin was documented by at least two urine tests which showed the presence of these drugs. Confirmation was obtained by the written questionnaire or, for heroin, the presence of fresh needle marks. The 24-hour methadone plasma levels were compared in the poor and good performers.

RESULTS

Table One shows drug use and demographic characteristics of subjects. There was no statistically significant difference in mean weight, total years of heroin use, or length of time on MM (Table One). Poor performers were older ($t = 2.0$; $P<.05$). Nine of 24 (37.5%) subjects were classified as poor performers. Seven of the 9 (77.8%) regularly used heroin. Three of nine (33.3%) also regularly used non-prescribed diazepam. No good performer had a urine test that contained drugs other than methadone; presence of fresh needle n-arks: or had a positive breath alcohol test during the study period. The mean methadone plasma level at the end of 24 hours was different in the poor and good performing groups. (Poor: 101.8 ng/ml; Good: 410.4 ng/ml; P .05) (Table Two). Two patients in the poor group and one in the good group had no detectible methadone levels, and the highest level was 607 ng/ml in the poor group. Seven of 9 (77.8%) poor compared to only 2 of 15 (13.3%) good performers had blood levels under 50 ng/ml ($P<.01$). Three of 9 (33.3%) poor compared to 11 of 15 (66.7%) good performers stated that their daily methadone dose of 80 mg "holds" for 24 hours (PNS). The two good performing patients who had blood levels under 50 ng/ml stated that methadone did not "hold" for 24 hours.

COMMENT

Studies of small numbers of subjects have shown methadone plasma levels obtained 24 hours after a high, oral dose of methadone to

be very similar to those found in our good performing patients (1,7,8,10,12,18). Twenty-four (24) hours after a 100 mg dose in nine patients, Kreek found a mean plasma level of 480 ng/ml (12). Verebey et al. found 420± 97 ng/ml after a dosage of 80 n-g in six patients, and Inturrisi and Verebey found in five patients a mean plasma level of 460 ng/ml following a 100 mg dose (10,18). The mean plasma level 24 hours after an 80 mg dose in our good performing patients was 410 ng/ml. In Sweden, Holmstrand et al. attempted to determine if plasma levels showed correlation with therapeutic outcome. Regardless ofwhether the patients received a low (30 mg) or high (60 mg) daily methadone dose, a plasma level of over 200 ng/ml was associated with fewer urines which contained illicit drugs. Poor performing patients here had a mean plasma level of only 101.8 ng/ml, and only 1 of 9 (11.1%) had a plasma level of over 200 ng/ml.

Horns, Radol and Goldstein found that methadone plasma levels and symptom complaints, including estimate of the degree of "sickness" had no correlation (8). In general, patients at low dosages had low mean plasma levels, and those at high dosage had high mean plasma levels, although plasma levels varied over a range of twofold to threefold. Blake and Distocio found that high methadone plasma levels tended to correlate with reduced anxiety levels, although plasma methadone levels varied as much as tenfold with different oral doses (1). It is interesting to note, however, that 5 of 15 (33.3%) good performers who had high 24-hour plasma levels subjectively felt their methadone was not holding for 24 hours and 4 of 15 (26.7%) perceived they were not doing "very well" on the program. It, therefore, appears that subjective symptoms and perception have little correlation with plasma levels, although ancillary drug and alcohol use appear to be associated with low plasma methadone levels (6,7).

There have been conflicting reports as to whether the levels of daily methadone dosage influence goals and outcomes of treatment (2,3,6,7,13,14). All reports beginning with Jaffe et al. in 1970 (11), with the exception of Goldstein et al. in 1971 (6), indicate that programs which are flexible and allow daily methadone doses up to 80 mg per day retain patients longer, and that high dosage patients have less drug use, higher employment rates, and less criminal behavior (2,3,13,14). The peculiar tendency of some programs to restrict daily methadone dosages to under 50 mg doesn't appear rational in view of these reports. Data presented here provides a logical explanation as to why some patients must have a high daily methadone dose to adequately maintain a satisfactory blood level for 24 hours. This study does not, however, provide an explanation for the low plasma levels observed, and it may be due to absorption, degradation, or disposition of methadone. Patients who showed low plasma levels were older (38.5 years versus 32.9 years; $P<.05$), so it is possible that age may affect methadone metabolism.

The majority of MM patients on a high dose of 80 mg/day who persistently used non-prescription diazepam, heroin, and/or excessively consumed alcohol had low 24-hour methadone plasma levels compared to patients on the same dose who showed no drug or alcohol abuse. The abuse of opioids, diazepam, and alcohol have emerged, as major problems in MM patients (4,5,16), and these were the three problems identified most praninently in patients who had 24-hour plasma levels below 50 ng/ml. At present, it is not definitely known whether methadone dosages higher than 80 mg/day can correct the problem of inadequate methadone plasma levels over a 24-hour period, but several studies now show that MM programs that allow high doses have superior treatment outcomes (2,3,13,14). If a higher daily methadone dose is not utilized in patients with a plasm level below 50 ng/ml, it appears particularly appropriate to switch to a longer-acting opioid, such as levo-alpha-acetylmethadol (LAAM) (11). Since MM patients attend a clinic almost daily, it is feasible and practical to collect a methadone plasma specimen 24 hours after the last oral dose. This procedure should particularly be utilized in MM patients who demonstrate persistent use of diazepam, heroin, or excessive alcohol consumption.

TABLE I

Demographic And Drug Use Characteristics
of Good and Poor Performers*

	Good N=15	Poor N=9	Statistical Significance
Males	12(80.0%)	8 (88.9%)	PNS
Mean Age (yrs.)	32.8	38.8	(t=2.10;P<.05)
Weight (Kgs.)	70.2	73.6	PNS
Number Years Used Heroin	13.8	15.8	PNS
Length Time on Methadone	15.8	22.0	PNS

*Poor performers demonstrated persistent use of non-prescription diazepam, heroin, and/or excessive alcohol consumption at the time this study was conducted.

TABLE II

Methadone Blood Levels After 24 Hours
Patients Perception In Good And poor Performers*

	Good N=15	Poor N=9	Statistical Significance
Mean Methadone Blood Level (ng/ml)	410.4	101.8	(t=1.99;p<.05)
Range Methadone Blood Levels	0-1300 ng/ml	0-607 ng/ml	
Blood Levels Below 50 ng/ml	2 (13.3%)	7 (77.8%)	(x^2=9.98;P<.01)
Blood Levels Above 200 ng/ml	8 (53.3%)	1 (11.1%)	(x^2=4.62;P<.05)
Doing "Very Well" in Program	11 (73.3%)	3(33.3%)	PNS
Methadone "Holds" 24 Hours	10 (66.7%)	4 (44.4%)	PNS

*Poor performers demonstrated persistent use of non-prescription diazepam, heroin, and/or excessive alcohol consumption at the time this study was conducted.

REFERENCES

1. Blake, DA, Distasio C. A Comparison of Levels of Anxiety, Depression, and Hostility With Methadone Plasma Concentration in Opioid-Dependent Patients Receiving Methadone on a Maintenance Dosage Schedule: in, Proceedings of the Fifth National Conference on Methadone Treatment. National Association Prevention of Addiction to Narcotics, New York, pp 1308-1326, 1973.

2. Brow-n, BS, Watters, JK, Inglehart, AS, et al; Methadone Maintenance Dosage Levels and Program Retention. Am J Drug Alcohol Abuse, 9: 129-139, 1982.

3. Craig, RS. Effectiveness of Low Dose Methadone Maintenance for the Treatment of Inner City Heroin Addicts. Int J Addict, 15:701-710, 1980.

4. Dole, VP, Nyswander, ME. Methadone Maintenance Treatment: A Ten-Year Perspective, JAMA, 235;2117-2119, 1976.

5. Gelb, AM Richman, BL, Peyser, NP. Alcohol Use in Methadone Maintenance Clinics. AM J Drug Alcohol Abuse, 6:367-373, 1979.

6. Goldstein A. Blind Dosage Comparison and other Studies in a Large Methadone Program. , J Psychedelic Druqs, 4:177-181,1971.

7. Holmstrand, J, Anggard, E, Gunne, LM. Methadone Maintenance: Plasm Levels and Therapeutic Outcome. Clin Pharmacol Ther, 23: 175-180, 1978.

8. Horns, WH, Rado M, Goldstein, A. Plasma Levels and Symptom Complaints in Patients Maintained on Daily Dosage of Methadone Hydrochloride. Clin Pharmacol Ther, 17: 636-649, 1975.

9. Inturrisi, CE, Verebey, K. A Gas-Liquid Chromatographic Method for the Quantitative Determination of Methadone in Plasma and Urine. J Chromatogr, 65:361-369,1972.

10. Inturrisi, CE, Verebey, K: The Levels of Methadone in the Plasm in Methadone Maintenance. Clin Pharmacol Ther, 13:633-637, 1972.

11. Jaffe, JH, Schuster, CR, Smith, BB, et al. Comparison of Acetylmethadol and Methadone in the Treatment of Long-Term Heroin Users. JAMA, 211: 1834-1836, 1970.

12. Kreek, MJ. Plasma and Urine Levels of Methadone. NY State J Med, 73:2773-2777, 1973.

13. Ling, W, Blakis, M, Holmes, Ed.et al. Restabilization on Methadone After Methadyl Acetate Maintenance. Arch Gen Psychiat, 37:194-196, 1980.

14. McGlothin, WH, Anglin, MD: Long-Term Follow-Up of High and Low Dose Methadone Programs. Arch Gen Psychiat, 38:1055-1063, 1981.

15. Mule, SJ. Identification of Narcotics, Barbiturates, Amphetamines, Tranquilizers, and Psychotomimetics in Human Urine. J Chromatogr, 39: 302-311, 1969.

16. Stitzer, ML,, Griffiths, RR, McLellan, AT, et al. Diazepam Use Among Methadone Maintenance Patients: Patterns and Dosages. Am J Drug Alcohol Abuse, 8: 189-199, 1981.

17. Tennant, FS Jr; Use of a Pocket-Size Breath Analyzer in the Detection and Treatment of Alcohol Misuse. J Stud Alcohol, 40: 518-521, 1979.

18. Verebey, K, Volavka, J, Mule, S, et al. Methadone in Man: Pharmacokinetic and Excretion Studies in Acute and Chronic Treatment. Clin Pharmacol Ther, 18: 180-190, 1975.

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Methadone Detoxification: Effects of Methadone Dose Versus Time in Treatment

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Outpatient methadone detoxification is a frequently used treatment modality for opiate dependence; unfortunately such detoxifications are generally unsuccessful. Clients typically drop out prior to treatment completion or relapse to illicit opiate use while still receiving methadone at the clinic (Canada 1972; Wilson et al. 1974). Treatment failures have generally been attributed to the dosing regimen used for detoxification. However, it is also possible that outcome measures deteriorate over time because of the cyclic relapsing nature of drug abuse disorders.

The present study attempted to dissociate methadone dose and time in treatment as contributing factors to detoxification treatment outcome. Three groups of subjects received different dose reduction protocols during a 6-week period in the detox. One group received a rapid dose reduction to 10 mg at the beginning of the 6-week period and was then maintained at this low dose. A second group was maintained-on a constant moderate 20 mg dose of methadone throughout the 6-week period. In previous studies, 10 mg to 15 mg of methadone had been suggested as a critical dose or a "breaking point" in the detox above which patients report few withdrawal symptoms and generally do not supplement their methadone dose with street drugs, but below which discomfort appears and relapse to drug use increases. Thus, methadone dose during the 6-week study period was stabilized above this theorized critical dose for the 20 mg group and below the critical dose for the 10 mg group. The third group in the present study received a gradual dose reduction of 2 mg per week during the study weeks; this group provided a comparison, employing the type of gradual dose reduction schedule most commonly employed in clinic-controlled detoxes. Illicit opiate and nonopiate drug use, clinic attendance and withdrawal symptomatology were assessed throughout the 13-week detoxification protocol.

METHODS

Subjects. Approximately 100 subjects dependent on illicit opiates and not currently participating in treatment were enrolled in a

90-day or 13-week detoxification. During the first three weeks of the program, urine specimens were collected twice weekly on Mondays and Fridays and analyzed on an EMIT system for the presence of opiates. Clients were selected for the present study if at least three of these six specimens were opiate free. Sixty-four subjects met this criterion; 34 clients either dropped out of treatment prior to study assignment or were excluded from further participation in this study because of their high initial levels of drug use. Illicit drug use was used as a selection criterion in this study since the effects of the pharmacological intervention would be obscured if subjects were frequently supplementing their methadone dose with additional opiate drugs.

Subjects were eligible for detox on the basis of a self-reported history of opiate use and urinalysis evidence of current opiate use. Sixty percent of the subjects were black, 40 percent were white . Subjects' average age was 29 years and they reported an average of 8 - 10 years of continuous opiate use prior to this treatment enrollment.

Procedures. All doses of methadone were mixed with a cherry syrup vehicle and administered under double-blind conditions by clinic nursing staff. All clients were stabilized on 30 mg per day of methadone during the first three weeks of the detox. In week 4, the methadone dose was decreased abruptly to 20 mg per day for all clients. From weeks 5 through 10, dosing regimens differed for the three groups. For subjects in the 10 mg group, the methadone dose decreased to 10 mg in week 5 and was then stabilized at this level during weeks 6 through lb. For subjects in the 20 mg group, the dose was held constant at 20 mg during weeks 5 through 10. For subjects in the gradual dose reduction group, methadone decreased 2 mg per week during weeks 5 through 9 and remained at 10 mg during week 10. All subjects received 10 mg/day methadone in week 11, 5 mg/day in week 12 and then vehicle only in week 13. This protocol provided a 6-week period during weeks 5 through 10 for assessing the impact of methadone dose on a variety of outcome measures.

At the beginning of week 4, all subjects were enrolled in an incentive procedure which was in effect for the rest of the detox. Subjects could earn a take-home dose of methadone and $5.00 for each opiate-free specimen provided on Monday, Wednesday and Friday; they forfeited these incentives on days when the specimen was opiate-positive. This incentive procedure was used in an effort to maximize the impact of the pharmacological manipulation by decreasing illicit opiate use and increasing length of enrollment (McCaul et al. in press).

Dependent measures. The outcome variables examined in the present Study included: treatment retention -- the number of days subjects remained active in the clinic without missing three consecutive days; clinic attendance -- the percentage of missed clinic days out of the total number of opportunities to attend the clinic; illicit opiate use -- the mean percentage of opiate-

positive specimens out of the thrice weekly samples tested on the EMIT system; and withdrawal symptomatology -- the change in score on a 60-item self-report questionnaire in which symptoms were rated on a 4-point scale of increasing severity.

RESULTS.

Treatment retention was similar in the three study groups; mean number of days in treatment was 72.6 for the 10 mg group, 73.4 for the 20 mg group, and 74.7 for the gradual dose reduction group. Five to seven subjects dropped out of each treatment group for unidentified reasons prior to week 10 in the detox protocol. These subjects had slightly poorer attendance and slightly more illicit opiate use than those subjects who remained in treatment; however, overall there were few differences between those who remained in treatment and those who dropped out. Since the frequency of dropout was similar across groups, dropouts were excluded from further analyses; the results for clinic attendance, illicit opiate use and symptomatology include only those subjects who completed the dose manipulation procedures and remained at the clinic for at least ten weeks.

Clinic attendance was similar in the three study groups during the stabilization and dose manipulation periods of the detox. During weeks 2 and 3 of the stabilization period, subjects in all three groups missed fewer than 5 percent of their clinic doses. The percentage of missed clinic days then increased gradually throughout the detox. During week 10, at the end of the dose manipulation period, all subjects were missing approximately 10 to 15 percent of daily clinic visits.

Figure 1 summarizes the mean percentage of opiate-positive specimens for each treatment group throughout the detox. Less than 20 percent of specimens for all three groups were opiate positive during weeks 2 and 3 of the stabilization period; this low level of initial opiate use resulted in part from the subject selection criteria used in this study. Urine-positive rates increased in a similar fashion for the three study groups during weeks 6 to 10 such that approximately 40 percent of the specimens in the three treatment groups were opiate positive by the end of the dose manipulation period. Rates of urine-positive tests generally continued to rise during the final three study weeks. During the last two weeks in the detox, more than half of all specimens were opiate positive in the 10 mg and 20 mg treatment groups, although no further increase in urine-positive rates was seen in the gradual detox group.

There were no between-group differences on symptom reports during weeks 4 through 10 of the detox, while clients were exposed to different dose reduction schedules. During the final two study weeks, only the 20 mg group showed a gradual increase in symptom scores. This might be expected, since this group was experiencing a more precipitous dose reduction at this point than the other two groups.

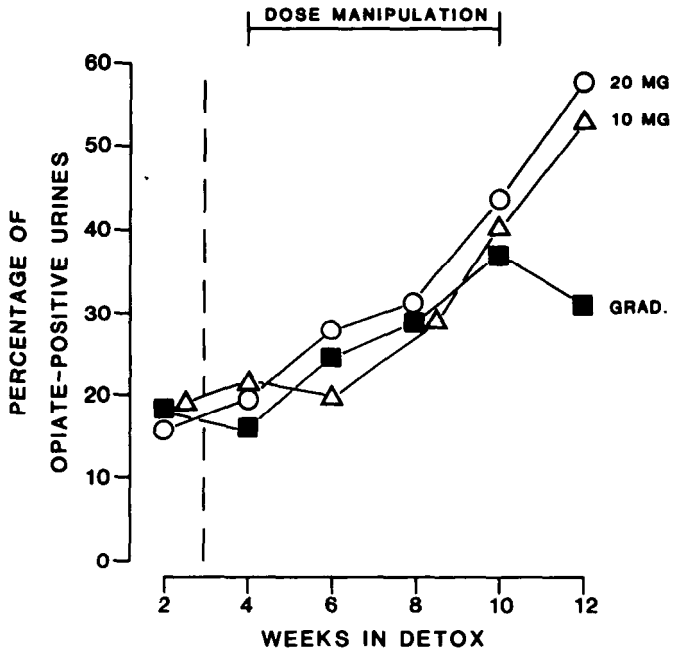

FIGURE 1. *The percentage of opiate-positive EMIT urine tests for the gradual dose reduction (■), 20 mg (O), and 10 mg (Δ) groups throughout the detox. Data points represent the total number of opiate-positive specimens provided during each two-week period divided by the total number of specimens possible during that period (3 specimens per week X 2 weeks X N). A missed specimen was treated as an opiate-positive result. The broken line following weeks 2 and 3 represents the introduction of the dose manipulation procedure.*

DISCUSSION

The present study attempted to dissociate the effects of methadone dose reduction schedule and time in treatment on outcome during opiate detoxification. Treatment retention, clinic attendance, illicit opiate use, and symptomatology were similar for subjects assigned to three different dosing regimens in the present study. These results suggest that treatment outcome is to some extent independent of the particular dosing protocol used in detoxification. If dose per se were critical, we would predict poorer performance in the 10 mg group than in the other two groups -- this clearly did not happen. Specifically, a rapid decrease in methadone dose from 30 mg to 10 mg did not increase the frequency of opiate-positive specimens when it occurred early in the detox and, therefore, was probably unexpected by the subjects. In contrast, subjects in the 20 mg group provided twice as many opiate-positive specimens in week 10 as in week 4, although their methadone dose remained constant throughout this period. such results suggest that the "threshold" or 'breaking point" postulated at 10 mg to 15 mg methadone may be more a function of time in treatment rather than dose per se.

Self-reports of withdrawal symptomatology also demonstrate the interaction of subjects' expectations and the methadone dose regimen. There was little withdrawal discomfort reported by subjects in the 10 mg group when their dose was decreased from 20 mg to 10 mg in week 5 of the 13 week detox; however, when a comparable dose reduction occurred in week 11 for subjects in the 20 mg group, withdrawal symptom scores were elevated.

Finally, although there were few complaints of withdrawal symptomatology in any of the treatment groups during weeks 4 through 10 of the detox, illicit opiate use increased steadily throughout this period. This finding indicates that relapse to illicit drugs is not necessarily precipitated by withdrawal sickness and suggests that treatment interventions must specifically target decreased drug use as a therapeutic goal rather than assuming that if withdrawal distress is minimized, relapse will be avoided.

REFERENCES

Canada, A.T. Methadone in a 30-day detoxification program for narcotic addicts : A critical review. Int J Addict, 7:613-617, 1972.

McCaul, M.E., Stitzer, M.L., Bigelow, G.E., and Liebson, I.A. Contingency management interventions: Effects on treatment outcome during methadone detoxification. J Appl Behav Anal, in press.

Wilson, B.K., Elms, R.R., and Thomson, C.P. Low-dosage use of methadone in extended detoxification. Arch Gen Psychiatry, 31: 233-236, 1974.

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Abstinence Treatments for Opiate Addicts: Therapeutic Community or Naltrexone?

Isabelle Arndt, A. Thomas McLellan, and
Charles P. O'Brien

INTRODUCTION

While naltrexone has been shown to be an effective narcotic antagonist and has been in use for about ten years, the clinical utility of naltrexone in the treatment of opiate-dependent individuals is still under evaluation. The delay is probably related to technical difficulties in studying this medication. For example, in studies which use a double-blind, placebo-controlled trial of naltrexone, the code can be broken by any patient who uses opiates while on study medication. In addition, most naltrexone studies are plagued by extremely high drop-out rates. Therefore, more indirect methods of investigation are necessary. In an effort to enhance the understanding of the clinical utility of naltrexone, we felt it would also be advantageous to compare naltrexone to another abstinence-oriented treatment modality, the therapeutic community. To this end, we evaluated "matched" groups of patients at the point of starting either naltrexone, or TC therapy and then six months later. We looked for changes with treatment and differences in outcome between the two groups. We would like to make the point at the onset that we do not feel we are measuring "which therapy is better" but rather investigating the outcomes of two groups of comparable patients, treated in two different abstinence-oriented modalities.

METHODS

Subjects

All subjects were male veterans admitted for drug dependence treatment to either the inpatient, drug-free, therapeutic community (TC) program at the Coatesville VA Medical Center, or to the outpatient opiate antagonist (Naltrexone) program at the Philadelphia VA Medical Center. All subjects had been detoxified from opiates prior to their admission to these rehabilitation programs and all subjects could have been admittedtoeither program.

Programs

Therapeutic Community - The TC is an intensive, 60-day, 30-bed program involving both personal and social treatment. Individual and group (encounter) therapies in addition to the

social structure of a self-governing community are the primary therapeutic tools. Family therapy, as well as educational and vocational counseling, is also offered. The program is directed by a social worker and includes a staff of two psychologists, two social workers, two nurses, and four rehabilitation technicians. Limited use of psychotropic medications is permitted when indicated by the attending physician.

b. Naltrexone - The naltrexone program is primarily an outpatient treatment modality. The program is open to any male veteran who abuses opiates in the absence of major medical problems or organic brain syndrome. Naltrexone induction is available in an inpatient or outpatient setting. The naltrexone program requires weekly counseling sessions with a drug rehabilitation technician and weekly visits with the nurse practitioner for recording vital signs, filling out study questionnaires, and promoting general health management. Naltrexone is dispensed three times weekly by the pharmacist, nurse practitioner, or physician. Psychotherapy is strongly recommended and available but remains at the patient's discretion.

Procedure

a. Subject Selection and Matching - All subjects were selected retrospectively from the pool of patients in each program who had been evaluated at admission to treatment and again at six month follow-up during 1982 (see Data Collection, below). Since it was not possible to randomly assign patients to the two programs, we elected to equate the two groups by matching them on a range of pretreatment characteristics that have historically been important in determining treatment outcome. In fact, since we had a much& smaller pool of naltrexone subjects, we selected subjects from the TC program to match each of the naltrexone patients. It is important to note that only the pretreatment data from these patients was considered in the matching procedure and no outcome information was used. A full table of matching variables is available from the authors.

The groups did not differ significantly in terms of age, race, years of education, years employed full time, or in the percent with a skill or trade.

The groups were also similar with respect to years of alcohol use, years of opiate use, years of depressant use, years of stimulant abuse, prior drug abuse treatments and months of longest abstinence ($p > .10$). However, the naltrexone sample did have significantly more years of methadone use (3.5 vs. 1: $p < .05$).

In legal status, the TC sample had a significantly greater history of criminal charges (8 vs. 4; $p < .05$), but the groups did not differ with respect to convictions, months incarcerated, percent court stipulated, or the percent on probation or parole ($p > .10$).

In comparisons of the two groups on variables associated with family relations, there were no significant differences in the percent married or remarried, the percent divorced or separated, and finally, the percent living alone (p> -10). Groups were also matched in length of treatment.

In summary, the naltrexone group had a significantly (p< .05) greater history of methadone use (3.5 vs. 1 year) than the TC subjects, while the TC group had a significantly greater history of criminal charges (8 vs. 4). However, the remaining 18 matching variables were not significantly different (p > -10) between the two groups and we conclude that the 27 pairs of matched subjects were at least roughly comparable in terms of background and pre-treatment history at the start of treatment.

b. Data Collection - All data collected during the study were obtained using the Addiction Severity Index (ASI) (McLellan et al. 1980) at admission to treatment and again at follow-up, six months later. The ASI is a structured, 30-40 minute, clinical research interview designed to assess problem severity in areas of life function commonly affected by addiction. Problem severity in each area is assessed independently and six areas are included: medical, legal, substance abuse, employment , family, and psychiatric problems. In each of the areas, objective questions are asked measuring the number, extent, and duration of problem symptoms in the patient's lifetime and in the past 30 days. The patient also supplies a subjective report of the recent (past 30 days) severity and importance of the problem area.

All ASI follow-up interviews were done six months following treatment admission by an independent research technician, either in person or over the phone. No information from secondary sources was used and all data were closely monitored to preserve confidentiality. The validity of the follow-up data was maintained through built-in consistency checks within the ASI and through spot checks on subsamples of the population assessing the ASI data against urinalysis, pharmacy, and criminal justice system records.

C. Outcome Criteria - It was important for the aims of this study to have general measures of treatment outcome, since single item measures can be inherently unreliable (Nunnally, 1967). We therefore constructed criterion composites or factors from sets of single items within each of the ASI problem areas. Several items from each problem area were intercorrelated to exclude those which were unrelated, and the remaining items were standardized and tested for conjoint reliability using Cronbach's formula (Cronbach & Furby 1970). Four to six items from each ASI problem area were selected using this procedure and each set of items showed a standardized reliability coefficient of .73 or higher. Seven composites or factors (medical problems, employment, drug use, alcohol use, legal status, family problems, and psychiatric function) were

constructed in this manner and scores on each were calculated for all patients at admission and follow-up.

Higher scores on these composite measures indicate greater severity, so improvement is shown through reduction in the composite scores. While these composite scores do offer reliable general estimates of patient status in each area they have no literal meaning. For this reason we have included additional items offering *more concrete* examples of patient functioning during the 30 days prior to treatment admission and 30 days prior to six-month follow-up.

Analysis of Outcome

Paired t-test analyses were used to assess the nature and extent of admission to six-month change in both groups. In addition we examined differences between the two groups in the six-month outcome status. For evaluation of out&me-we applied analysis of covariance (ANCOVA) to the six-month outcome variables using the admission values as covariates. This procedure adjusts the outcome measures for differences between the groups in their admission values (Cohen & Cohen 1975), permitting a fairer comparison of outcome status.

RESULTS

The results are shown in Table I. Neither group changed significantly in reported medical problems. In employment both groups showed positive change, significantly so for the TC group in the factor score and in days worked: and for the naltrexone group in days worked and earned income ($p<.05$). However, there was no significant difference between the treatment groups at six-month follow-up on any of the employment measures ($p> .10$). In the alcohol composite measure, the TC group improved significantly ($p< .05$) while the naltrexone group stayed about the same. However, the alcohol problems in the TC were more severe at the outset of treatment. Consequently, results of the ANCOVA on the alcohol composite measure indicated no significant between-group differences, although the TC group improved more than the naltrexone group in the number of days intoxicated ($p<.05$).

In the comparison of drug use, again both groups improved significantly ($p< .01$), particularly in the days of opiate use. The naltrexone group also showed significant improvement *in* days of depressant use ($p< .01$).

Both groups improved in legal status, with significantly less reported illegal income ($p < .05$). There were no significant differences between groups ($p > .10$). Similarly, in the psychiatric factor, both groups improved, but while the naltrexone group improved significantly ($p< .05$) this was not significantly more than the TC group (ANCOVA; $p>.10$).

TABLE I

COMPARISON OF IMPROVEMENTS AND SIX-MONTH OUTCOMES
IN 27 PAIRS OF MATCHED MALE DRUG ABUSE PATIENTS
TREATED IN THERAPEUTIC COMMUNITY OR NALTREXONE

	THERAPEUTIC COMMUNITY			NALTREXONE			POST ANCOVA[2]
	PRE		POST	Pre		Post	
MEDICAL FACTOR[1]	327		264	285		315	N.S.
Days of Med Problems	7		5	7		7	N.S.
EMPLOYMENT FACTOR	228	*	168	205		170	N.S.
Days Worked	5	**	9	7	*	11	N.S.
Earned Income	292		330	320	*	421	N.S.
ALCOHOL USE FACTOR	236	**	94	110		90	N.S.
Days Drinking	9		5	6		5	N.S.
Days Intoxicated	5	.07	3	2		1	*
DRUG USE FACTOR	346	**	109	361	**	151	N.S.
Days Opiates	16	**	5	18	**	10	N.S.
Days Depressants	2		1	7	*	2	*
Days Stimulants	6		2	2		1	N.S.
LEGAL FACTOR	271	.07	180	186		153	N.S.
Crime Days	6		4	4		3	N.S.
Illegal Income	650	*	195	451	*	169	N.S.
PSYCHIATRIC FACTOR	289		244	330	*	248	N.S.
Days Psych Symptoms	8		6	12		7	N.S.
Days Fam/Soc. Probs.	2		2	4	.08	1	N.S.

* = $p < .05$
** = $p < .01$

[1] All criteria were measured during the 30 days prior to treatment start and prior to 6-mo. follow-up. Larger factor scores equal worse status.

[2] Covariate was the pre-treatment criterion score.

In short, both treatments seemed effective and improvement was noted in several areas in most patients. However, neither treatment group had a clear advantage over the other at six-month follow-up.

DISCUSSION

Prior to discussion of the observed results it is important to consider the limitations of the data. First, all the data were self-reported and are therefore subject to potential discrepancy. Two points are relevant here. First, we have attended to the accuracy of these data through built-in validation checks within the ASI and through spot checks of patient reports against urinalysis, court records and employment records. We and other workers using similar data (Sale et al. 1977) have been satisfied that systematic distortion is minimal. Second, regardless of the degree to which these data are subject to intentional or unintentional distortion, there is no reason to believe that the subjects in the two groups differed in their degree of distortion. Thus, the between groups comparisons are appropriate.

A second potential problem with the presented data is that it results from self-selected participation in treatment rather than random assignment to the programs. It is possible that the self-selection process by which patients applied for the therapeutic community and the naltrexone programs produced samples which were different on some unrecognized quality which was important to their outcome. We attempted to reduce this possibility through our matching procedure and while the Table I data indicate considerable comparability between the samples, the possibility of unrecognized but important differences betweenthe samples must be recognized.

Again we would like to stress that we are not trying to assess which therapy is better. We have selected a subsample of the TC population which is comparable to our naltrexone population and looked at how the two groups did following treatment.

It should be noted also that early dropouts are included in the analysis of both the naltrexone and therapeutic Community samples. Since the two samples were matched with regard to treatment duration there were no differences between the two groups in drop-out rates. Gains might be greater if drop-outs were excluded, but including the drop-outs more accurately reflects treatment effectiveness.

It could be argued that the longer history of methadone use in the naltrexone group might indicate this would be a more refractory group. However, several investigators have noted that patients with longer histories of opiate use did better with naltrexone and additional years of methadone use probably means additional years of contact with therapy for drug abuse; therefore the naltrexone group may have a slight advantage in terms of outcome.

With these limitations in mind, we will reconsider the results of the present study. Within-group measures of change showed that both groups improved in employment, drug use, and legal status. In addition the naltrexone group improved in psychiatric status. The TC group showed a change in alcohol use.

Between-groups comparisons showed the TC superior to naltrexone in improving alcohol problems; however, the pretreatment alcohol status was more severe in the TC group. Both groups actually ended up at about the sane level of alcohol consumption.

Naltrexone seemed superior to the TC in reducing depressant use, but it should be noted that this could be due to the frequent use of ancillary medication (benzodiazepines), during detoxification at the Philadelphia VA Medical Center. This would cause an inflated self-report of sedative use during the 30 days prior to starting naltrexone. Thus, we are reluctant to conclude that this is a meaningful difference between the groups.

In evaluating the respective "worth" of both treatments, cost-effectiveness should be considered. The therapeutic community is a relatively expensive form of treatment, requiring a residential facility. The naltrexone program on the other hand can be run on an outpatient basis and can be implemented by a pre-existing clinic which serves drug-abusing clients. This might suggest an economic advantage to naltrexone when the choice of treatment is unclear.

In summary, then, subjects participating in the therapeutic community who were matched to subjects participating in the naltrexone program showed comparable improvement in a variety of outcome variables. The between -groups comparison showed significant differences in only 2 of 18 items. We do not feel that these differences are clinically important and may, in fact, be artifacts. In short, we see no clear advantage of one treatment over the other. A prospective, randomized study would be desirable to further assess relative program benefits.

REFERENCES

Pale, R. N., Cabrera, L.S., Brown, J.D. Follow-up evaluation of drug abuse treatment. Am J Drug and Alc Abuse, 4(2):233-249, 1977.

Cohen, J. and Cohen, P. Applied Multiple Regression/Correlation Analysis for the Behavioral Sciences. New York: Laurence-Erlbaum Assoc.,1975.

Cronbach, L.J. and Furby, L. Psychol Bulletin, 74:68-80, 1970.

McLellan, A.T., Luborsky, L., O'Brien, C.P., and Woody, G.E. An improved diagnostic evaluation instrument for substance abuse patients: The Addiction Severity Index. J Nerv Ment Dis, 168(1):26-33, 1980.

Nunnally, J. Psychometric Theory. New York: McGraw-Hill, 1967.

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An Evaluation of Neonatal Abstinence Treatment Modalities[1]

Loretta P. Finnegan, Herman Michael, Betty Leifer, and Shobhana Desai

It has been well documented over the years that infants born to heroin- or methadone-dependent mothers have a high incidence of neonatal abstinence react ions. Recently, less potent opiates have been identified as also precipitating the neonatal opiate abstinence syndrome. These include propoxyphene hydrochloride (Darvon) (Tyson, 1974; Quillian and Dunn, 1976), codeine (Van Leeuwen et al., 1965), and pentazocine (Talwin) (Goetz and Bain, 1974; Preis et al., 1977). In addition, a number of non-opiate CNS depressants have been implicated. These have been reviewed by Ostrea et al. (1977) and include alcohol (Nichols, 1967), barbiturates (Bleyer and Marshall, 1972; Blumenthal and Lindsay, 1977). bromide (Rossiter and Rendel-Short, 1972). chlordiazepoxide (Athinarayanan et al., 1976), diazepam (Rementeria and Bhatt, 1977). ethchlorvynol (Rumack and Walravens, 1973), diphenhydramine (Parkin, 1974). and imipramine (Webster, 1973).

Neonatal opiate or CNS depressant abstinence syndrome is described as a generalized disorder characterized by signs and symptoms of central nervous system hyperirritability, gastrointestinal dysfunction, respiratory distress, and vague autonomic symptoms, which include yawning, sneezing, mottling, and fever. A high-pitched cry, increased muscle tone, tremors, and irritability develop. The infants tend to have increased deep tendon reflexes and an exaggerated Moro reflex. The rooting reflex is increased, and the infants are frequently seen sucking their fists or thumbs.

These infants' respiratory systems are also affected during the withdrawal stage, causing excessive nasal secretions, stuffy nose, and rapid respirations, sometimes accompanied by chest retractions, intermittent cyanosis, and apnea. Severe respiratory embarrassment occurs most often when the infant regurgitates, aspirates, and develops aspiration pneumonia. The high-pitched cry is similar to that of infants with central nervous system hyperirritability. Sucking reflexes are ineffectualand uncoordinated (Kron et al., 1976), causing extreme difficulties in feeding behavior. Sleep patterns are disturbed, and infants have excessive spontaneous generalized sweating.

The origin of the neonatal abstinence syndrome lies in the abnormal intrauterine environment. The fetus undergoes a biochemical adaptation to the presence of an abnormal agent in its tissues. Abrupt removal of the drug at delivery precipitates the onset of symptoms.

Withdrawal symptoms are found secondary to abstinence from opiate as well as nonopiate CNS depressants. Although infants born to barbiturate addicts may manifest symptoms similar to those of infants passively addicted to opiates, their symptoms tend to begin at a later age, and undernutrition at birth has not been a usual feature. Because the barbiturate withdrawal syndrome may not develop until an infant has been discharged from the nursery, it may not be treated unless suspicion has been aroused by the mother's symptoms or actions. Furthermore, there is a greater risk of seizure activity in infants withdrawing from barbiturates than in those withdrawing from opiates.

The time of onset of withdrawal signs ranges from shortly after birth to two weeks of age, but for the majority, signs appear within 72 hours. The type of drug used by the mother, her dosage, the timing of the last dose before delivery, the character of the labor, the type and amount of anesthesia and analgesia given during labor, and the maturity, nutrition, and presence of intrinsic disease in the infant may all play a role in determining the time of onset in the individual infant. Because of the variation in time of onset and in severity, a range of clinical courses may be delineated.

If mild symptoms appear, conservative measures such as swaddling, infrequent handling and demand feeding should be instituted. If the infants can no longer be managed by such conservative measures, drug therapy must be instituted. The objectives of drug therapy are to control symptomatology so that the infant sleeps or rests between feedings, takes in calories sufficient for weight gain and performs normally with regard to interaction with caretakers (Finnegan, 1979).

Many pharmacological agents have been used in the treatment of the neonatal abstinence syndrome and appear to be effective in relieving symptoms. The agents most commonly employed have been paregoric, phenobarbital and diazepam (Finnegan and MacNew, 1974).

Paregoric has been used for over 70 years with the rationale that narcotic abstinence symptoms are most specifically relieved by narcotic substitution. The major advantages of paregoric are its ability to be orally administered, its apparent lack of adverse effects, and its ability to provide a level of sedation that inhibits bowel motility (Finnegan and MacNew, 1974). Sucking has been found to be much closer to normal among paregoric-treated infants than among those treated with phenobarbital or diazepam (Finnegan, 1979). Disadvantages associated with paregoric are that large doses are often necessary to treat severe abstinence, and the duration of therapy can be longer with paregoric than with some other drugs.

Phenobarbital has been used extensively for neonatal abstinence as it supresses the major symptoms by a nonspecific central nervous system depression (Finnegan, 1979). Phenobarbital is especially effective in controlling irritability and insomnia. However, an occasional infant paradoxically becomes more irritated after treatment and although central nervous system symptoms are prevented by barbiturates, they do not prevent loose stools (Finnegan and MacNew, 1974). It has been shown that some infants are not fully controlled even at doses that produce plasma levels considered to be in the toxic range (Finnegan, 1979).

Diazepam therapy has also been advocated in the treatment of neonatal withdrawal. Nathenson and associates (1971) reported that the use of diazepam appears safe and effective and that a short course of diazepam therapy controls the symptomatology without serious side effects or rebound symptoms when it is discontinued. Kron et al. (1974) found that infants treated with diazepam became severely obtunded and their sucking reflex markedly diminished in comparison to infants treated with other drugs. In one study, seizures were seen more often in a group of infants treated with diazepam than in those treated with paregoric (Herzlinger et al., 1977).

In our previous studies of the treatment of neonatal abstinence (Finnegan et al., 1982), it was found that infants treated with paregoric required a second drug infrequently in comparison to those treated with phenobarbital or with diazepam. Of the infants treated with paregoric, 22% required an additional drug compared to 41% with phenobarbital loading, 42% with phenobarbital titration and 65% with diazepam. The purpose of this study was to further evaluate the efficacy of the three drugs for treatment of specific abstinence syndromes.

MATERIALS AND METHODS

In the nurseries at Thomas Jefferson University, paregoric, phenobarbital and diazepam were evaluated in the treatment of abstinence. Infants (n=139) were assessed and managed using the neonatal abstinence scoring system developed by Finnegan et al. (1975). Procedure dictated that the infants receive varying dosages of a specific drug dependent on abstinence severity. If the abstinence scores indicated a need for pharmacologic intervention, a randomized schedule was used in selecting the pharmacologic regimen unless clinically contraindicated.

The abstinence scores alsodictatedthe specific dose of the pharmacotherapeutic agents used to detoxify infants in two management regimens. The two include the loading dose and the titration regimens. With the score dose titration approach, the initial dose and all subsequent doses are determined by and titrated against the abstinence score. The infant is initially given a total dose which is divided into 3-6 equal doses at 4-8 hour intervals. If clinical observation and the results of the scoring system indicate the abstinence syndrome is not controlled, the dosage is increased.

When using the phenobarbital loading dose approach, an initial dose of 20 mg/kg is administered in an attempt to achieve an expected therapeutic serum level. If control is achieved, the initial dose is followed by daily maintenance doses of 5 mg/kg which can be adjusted in order to maintain the desired steady state phenobarbital level. If control is not achieved with the initial dose, the phenobarbital level is increased by administering phenobarbital at 10 mg/kg every 12 hours. This increase in serum level is continued every 12 hours until: 1) control is attained, 2) the serum level reaches 70 mg/ml, or 3) the infant demonstrates signs of phenobarbital toxicity.

For purposes of this study, the data obtained for the loading and titration methods of phenobarbital therapy were combined for an overall index of phenobarbital efficacy.

Abstinence, not controlled at the highest dosage of a specific drug, requires a second agent. The effectiveness of the drug therapies in this study was determined according to the necessity for administering a second agent. A history of drugs to which the infants were exposed in utero was obtained from medical records, social data forms, and routine urine toxicologies as required by law for methadone maintenance programs. Maternal drug abuse was divided into 2 categories as follows: 1. Narcotic use only which generally included methadone and/or heroin, and 2. Multi-drug abuse including a combination of narcotics and other rugs such as diazepam, alcohol, barbiturates, or amphetamines. Success or failure of the pharmacologic therapy was then analyzed according to maternal drug categories.

RESULTS

As Table 1 shows, treatment for infants exposed only to narcotic agents in utero was more often successful with paregoric than with either phenobarbital or diazepam. Paregoric was able to control withdrawal symptomatology in 93% of the infants while phenobarbital was only successful in 50% of the infants and diazepam was not at all successful in the treatment of any infant whose prenatal drug exposure was limited to narcotics ($p<.001$). When comparing only the paregoric and the phenobarbital treatments, the results remained significant ($p < .01$).

TABLE 1

Treatment Results for Infants Exposed Only to Narcotic Agents in Utero

Treatment Drug	Paregoric	Phenobarbital	Diazepam	Totals
Treatment Successes	13	13	--	26
Treatment Failures	1	13	5	19
TOTALS	14	26	5	45

Treatment for infants exposed prenatally to multiple drugs was more often successful with phenobarbital than with paregoric or diazepam (See Table 2). Phenobarbital treatment was successful in 89% of the infants while paregoric was successful in 61% and valium in 40% ($p<.001$). Again, the paregoric/phenobarbital comparison was significant ($p<.01$).

TABLE 2

Treatment Results for Infants Exposed to Multiple Drugs in Utero

Treatment Drug	Paregoric	Phenobarbital	Diazepam	Totals
Treatment Successes	11	54	6	71
Treatment Failures	7	7	9	23
TOTALS	18	61	15	94

In conclusion, these data suggest thatparegoric is the drug of choice for the control of abstinence symptomatology due exclusively to narcotic use and support the supposition that narcotic abstinence symptoms are most specifically relieved by narcotic substitution. Phenobarbital appears most efficacious when the infant has been exposed prenatally to multiple drugs. Diazepam appears the least efficacious of the three drugs. These results have definite implications for the management and treatment of specific neonatal abstinence syndromes.

REFERENCES

Athinarayanan. P., Pierog. S.H., Nigam, S.K., and Glass, L., 1976, Chlordiazepoxide withdrawal in the neonate. Am. J. Obstet. Gynecol., 124:212-213.

Bleyer. W.A., and Marshall, R.E., 1972, Barbiturate withdrawal syndrome in a passively addicted infant, J. Am. Med. Assoc., 221:185-186.

Blumenthal, I., and Lindsay, S., 1977, Neonatal barbiturate withdrawal, Postgrad. Med. J., 53:157-158.

Desmond, M.M., and Wilson, G.S., 1975, Neonatal abstinence syndrome: Recognition and diagnosis, Addic Dis., 2:113-121.

Finnegan, L.P. (Ed.), 1979, Drug dependence in pregnancy: Clinical management of mother and child, Services Research Monograph Series, National Institute on Drug Abuse.

Finnegan, L.P., Desai, S.. and Leifer, E.D., 1982, Detoxification of infants undergoing abstinence. Poster Session, Proceedings of the 44th Annual Scientific Meeting of the Committee on Problems of Drug Dependence, Inc., Toronto, Ontario, Canada, pp. 27-30.

Finnegan, L.P.. Kron, R.E., Connaughton, J.F., and Emich, J.P., 1975, A scoring system for evaluation and treatment of Neonatal Abstinence Syndrome: A new clinical and research tool. In Basic Therapeutic Aspects of Perinatal Pharmacology, P.O. Marselli, S. Grattini and F. Sereni, (Eds.), New York: Raven Press.

Finnegan, L.P. and MacNew, B.A., 1974, Care of the addicted infant, American Journal of Nursing, 74:685.

Goetz, R.L., and Bain, R.V., 1974, Neonatal withdrawal symptoms associated with maternal use of pentazocine. J. Pediatr., 84:887-888.

Herzlinger, R.A., Kandall, S.R., and Vaughn, H.G., Jr., 1977, Neonatal seizures associated with narcotic withdrawal, J. Pediatr., 91:638.

Kron, R.E., Litt, M.. and Finnegan, L.P., 1974. Behavior of infants born to narcotic addicted mothers, Proceedings of the 35th Annual Meeting of the Committee on Problems of Drug Dependence of National Research Council, Mexico City, 731-735.

Kron, R.E., Litt, M., Phoenix, M.D., and Finnegan, L.P., 1976, Neonatal abstinence : Effects of pharmacotherapeutic agents and maternal drug usage on nutritive sucking behavior, J. Pediatr., 88:637.

Nathenson, G., Golden, G.S.. and Litt. I.F., 1971, Diazepam in the management of Neonatal Narcotic Withdrawal Syndrome, Pediatrics. 48:523.

Nichols, M.M., 1967. Acute alcohol withdrawal syndrome in a newborn, Am. J. Dis. Child, 113:714-715.

Ostrea, E.M., Jr., Ting, B.C., and Cohen, S.N., 1977, Neonatal withdrawal from non-narcotic drugs. In Drug Abuse in Pregnancy and Neonatal Effects, J.L. Rementeria (Ed.), St. Louis: C.V. Mosby. pp. 165-178.

Parkin, D.E., 1974. Probable Benadryl withdrawal manifestations in a newborn infant, J. Pediatr., 85:580.

Preis, 0., Choi, S.J., and Rudolph, N., 1977, Pentazocine withdrawal syndrome in the newborn infant, Am. J. Obstet. Gynecol., 127:205-206.

Quillian, W.W., II, and Dunn, C.A., 1976, Neonatal drug withdrawal from propoxyphene. J. Am. Med. Assoc., 235:2128.

Rementeria. J.L., and Bhatt, K., 1977, Withdrawal symptoms in neonates from intrauterine exposure to diazepam, J. Pediatr., 90:123-126.

Rossiter. E.J.R., and Rendle-Short, T.J., 1972, Letter: Congenital effects of bromism, Lancet, 2:705.

Rumack, B.H., and Walravens, P.A., 1973, Neonatal withdrawal following maternal ingestion of ethchlorvynol (Placidyl), Pediatrics, 52:714-716.

Tyson, H.K., 1974, Neonatal withdrawal symptoms associated with maternal use of propoxyphene hydrochloride (Darvon), J. Pediatr., 85:684-685.

Van Leeuwen, G., Guthfie, R., and Stange, F., 1965, Narcotic withdrawal reaction in a newborn infant due to codeine, Pediatrics, 36:635-636.

Webster, P.A.C., 1973, Letter: Withdrawal symptoms in neonates associated with maternal antidepressant therapy, Lancet, 2:318-319.

FOOTNOTE

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Five-Year Follow-up of Opiate Addicts With Naltrexone and Behavior Therapy

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ABSTRACT

A group of 58 heroin addicts were treated with naltrexone and behavior therapy and followed for 5 years. At one-year post-treatment, almost half of the naltrexone-treated subjects were opiate free. Follow-up results at 5 years post-treatment indicate that over 90% of those patients treated with naltrexone became re-addicted for various periods of time. However, naltrexone-treated subjects did feel their treatment with naltrexone had provided them with the ability to remain opiate free for blocks of time. The results suggest that naltrexone is not a "cure" for opiate dependence, but is a medication which can be useful in protecting patients from re-addiction and is a modality patients should be encouraged to return to if they feel vulnerable to re-addiction.

INTRODUCTION

Narcotic antagonists are a class of drugs which received a great deal of attention in the 1970's for the treatment of opiate addiction.[1,2] Naltrexone in particular was touted as having tremendous potential for providing long-lasting, if not permanent, "cures" for opiate dependence. The possibility of developing a treatment for addiction which would allow addicts to develop drug-free lives rather than settle for opioid substitution provided by such treatments as methadone and LAAM was a tremendously appealing prospect.

Evaluations of naltrexone produced mixed conclusions. Naltrexone has been administered to over 1000 addicts and has been found to be well tolerated, physically, safe, and to produce a highly effective blockade against opiates.[1,2]

There is general agreement of those evaluating the clinical effectiveness of naltrexone that while an addict is receiving the antagonist, he rarely, if ever, challenges the blockade with heroin. Additional empirical support for the antagonist comes from subjective reports of addicts receiving naltrexone, which indicate that the antagonist rapidly reduces cravings and urges for opiates.

Clinical reports indicated the naltrexone may only be utilized by 5-10% of opiate addicts due to difficulties in inducting patients into treatment and problems involving a high rate of patient drop-out during the early stages of treatment.[1,2] However, for those patients who entered treatment with naltrexone, use of illicit opiates almost entirely stopped while on naltrexone, and one-year follow-up results indicated that approximately one-half remained opiate free.[4]

These results suggested that with some refinements of naltrexone induction procedures and the development of ancillary treatment, techniques, naltrexone could be conceptualized as a "cure" for some addicts' addiction problems.

Before accepting the idea that the best way to view naltrexone treatment is as a one-time intensive intervention resulting in a permanent elimination of an opiate dependence problem, a more longrange view is needed on the outcome of those patients who had been successfully treated with naltrexone. The purpose of this paper is to provide 5-year follow-up data on a group of subjects treated with naltrexone from 1974-1977.

METHOD

Subjects And Setting

Subjects were 58 male opiate addicts who received treatment from 1974-1977 at the Heroin Antagonist and Learning Therapy Project in Oxnard, California. Some demographic characteristics of the subjects are presented in Table One.

TABLE ONE

SUBJECT CHARACTERISTICS

N = 58

Mean Age at Treatment Initiation	26 Yrs.
Mean Age at First Use of Opiates	18.0 Yrs.
Mean Years Addicted to Opiates	7.9 Yrs.
Mean Years of Education	10.8 Yrs.
Number of Subjects with Prior Treatment Experience	16
Number of Anglo, Subjects	34
Number of Chicano Subjects	19
Number of Black Subjects	5

INTERVENTION PROCEDURES

Subjects were randomly assigned to one of three groups upon entering into treatment. The groups were:

1. Naltrexone Alone

   Subjects in this group receiving blocking doses of naltrexone on 3 clinic visits per week. For counseling or psychotherapy, they were referred to a local mental health facility.

2. Behavior Therapy Alone

   Subjects in this group received an intensive program combining behavior therapy techniques, such as role playing, contingency contracting, video feedback, relaxation training, systematic desensitization, social skills training, and structured employment training.

3. Naltrexone Plus Behavior Therapy

   Subjects in this group received both of the treatment programs described above.

To summarize the study results, subjects on naltrexone stayed in treatment approximately 6 months and stayed opiate free while on naltrexone. Behavior therapy group subjects stayed in treatment. Results of this study have been reported in a detailed report.[4]

FOLLOW-UP PROCEDURES

Last treatment contact for any subject was in August, 1977. Follow-up interviews were conducted in November-December, 1982. Subjects were contacted by telephone and in person and, asked to participate in an interview about their current situation, and asked to give a urine specimen to be screened for illicit drugs. Follow-up was greatly enhanced by the fact that Oxnard is a geographically isolated city with only one drug treatment program. Therefore, by having access to the treatment records of the one treatment facility along with the prior background information, such as family phone numbers collected during the patients' treatment, it was possible to collect follow-up data on 52 of 58 subjects. In all cases in which the subject was reported as being "opiate-free," this status was confirmed by a negative urinalysis. Subjects categorized as "opiate-positive" provided urine samples which assayed positive for opiates in all but six cases. In these six cases, subjects reported having used opiates within the prior 48 hours, but did not wish to give a sample to document this fact for fear of legal jeopardy.

RESULTS

This paper focuses on evaluating the long-term effect of treating addicts with naltrexone. Little attention is given to the follow-

p of the behavior therapy treatment since the conclusions reached at the end of the study's completion in 1978 was that the treatment had been almost totally ineffective. Therefore, the behavior therapy alone group can be viewed as a control population to compare the naltrexone-treated subjects against. Results from the two naltrexone treatments were not significantly different. Therefore, data from both naltrexone groups will be presented together.

Table Two presents a breakdown of the number of subjects contacted for follow-up.

TABLE TWO

NUMBER OF SUBJECTS CONTACTED FOR 5-YEAR FOLLOW-UP INTERVIEW

	Behavior Therapy Alone Group N = 15	Combined Naltrexone Group N = 43
Number of Subjects Contacted for Follow-Up	12 (80%)	35 (81%)
Number of Subjects Unable to Locate	1 (7%)	5 (12%)
Number of Subjects Dead*	2[a,b] (14%)	3[c,d,e] (7%)

*Causes of Death

a. Gunshot wound
b. Complication from hemophilia
c. Liverdisease
d. Stroke
e. Stomach cancer

One rather striking point illustrated by the data presented in Table Two is that 9% of the total sample are known to be dead. (This figure might be higher since two of the subjects we were unable to locate are rumored to be dead, but this was not confirmed). This figure clearly emphasizes the high mortality rate found in a group of addicts with a mean age of 32 years.

TABLE THREE

FOLLOW-UP STATUS AT 12-MONTH POST-TREATMENT INTERVIEW

Subject Status	Behavior Therapy Group N = 15	Combined Naltrexone Groups N = 43
Opiate-Free	4 (27%)	18 (42%)

Opiate-Positive	4 (27%)	7 (16%)
Incarcerated	6 (40%)	10 (23%)
In Other Treatment	0	2 (5%)
Out of Contact	0	6 (14%)
Dead	1 (7%)	0
Total	15	43

Table Three presents data on the follow-up status of subjects, one year post-treatment. These results were encouraging evidence which suggested that one treatment episode with naltrexone produced a one-year elimination of the opiate use in 18 of 37 (49%) subjects contacted for follow-up.

TABLE FOUR

FOLLOW-UP STATUS AT FIVE-YEAR POST-TREATMENT INTERVIEW

	Behavior Therapy Group N = 15	Combined Naltrexone Group N = 43
Opiate-Free	1 (7%)	7 (16%)
Opiate-Positive	4 (26%)	7 (16%)
Incarcerated	3 (20%)	8 (19%)
In Other Treatment	4 (26%)	13 (30%)
Out of Contact	1 (7%)	5 (12%)
Dead	2 (14%)	3 (7%)

Table Four presents a different picture on the five-year follow-up status of these same subjects. At the time of the five-year interviews, 1 of 12 (8%) of the subjects contacted for follow-up in the behavior therapy group were opiate free, while 7 of 35 (20%) of the naltrexone-treated subjects were opiate free. Of the 7 subjects opiate-free at 5 years, 4 reported having been re-addicted for various periods of time during the 5-year post-treatment interval. Therefore, only 3 of 35 subjects (9%) treated with naltrexone achieved a 5-year continuous period of opiate abstinence. A summary of the follow-up data, therefore, could be interpreted to indicate that about 50% of the patients treated with naltrexone were opiate free for one year, but that by 5 years post-treatment, fewer than 1 in 10 had sustained a treatment "cure."

This rather discouraging outcome does not accurately reflect the subjects' perception of the value of their treatment experience. Subjects in the behavior therapy group were fairly uniform in their opinion that their behavior therapy treatment attempt had been an interesting and novel experience. but had very little effect upon their subsequent pattern of drug usage. Subjects who had been in treatment with naltrexone reported, however, that although the treatment with naltrexone had not permanently eliminated their addiction problem, ait had provided them with a valuable experience. A majority of these subjects reported that the naltrexone treatment experience had provided them with a learning experience which indicated that it was possible to live for extensive periods of time on the street without the use of illicit opiates.

In order to illustrate this finding, subjects were asked to estimate the longest period during the post-treatment period that they had been opiate free while on the street. Table Five presents a breakdown of these intervals.

TABLE FIVE

LONGEST OPIATE-FREE PERIOD DURING
5 YEARS POST-TREATMENT

Longest Period Opiate Free	Behavior Therapy N = 15	Combined Naltrexone Group N = 43
One week or less	8 (53%)	9 (21%)
One week - six months	0	9 (21%)
Six months - two years	3 (20%)	8 (19%)
Two years - five years	1 (7%)	6 (14%)
Continuously since treatment termination	0	3 (7%)
Out of Contact	1 (7%)	5 (12%)
Dead	2 (14%)	3 (7%)

Of the 35 naltrexone-treated subjects contacted for 5-year follow-up, 26 (74%) reported they had sustained opiate-free intervals of more than one week. This is in comparison to only 4 of 12 (33%) of the behavior therapy group subjects. This comparison was statistically significant ($X^2 = 6.3$, $P < .02$). Even more impressive is the fact that 49% of the naltrexone-treated subjects were able to sustain opiate-free periods of 6 months or more during the post-treatment period. Therefore, while the naltrexone treatment did not produce a suppression of illicit opiate use for the entire

follow-up period, the naltrexone experience does appear to have produced a measureable effect. Naltrexone-treated subjects were able to sustain longer periods opiate free during the 5-year follow-up than behavior therapy controls.

DISCUSSION

The results of the study suggest that an extended treatment experience of naltrexone with or without ancillary therapy program is not effective in eliminating re-addiction liability beyond the treatment period. Naltrexone, in conjunction with the proper adjunct therapy program, will not permanently protect former addicts from re-addiction. However, a more realistic view of how naltrexone fits into the addiction treatment arsenal can be developed from the data collected.

Our 8-year experience with naltrexone suggests that naltrexone is a useful treatment tool which, for some addicts, can provide protection from re-addicton liability. Naltrexone does not cure opiate addiction, but gives addicts a mechanism for dealing with urges and cravings which can lead to re-addiction. Patients treated with naltrexone should not be given the hope that naltrexone will solve their opiate problem. Rather, they should be educated to the fact that naltrexone will eliminate their opiate problem for as long as they are in treatment. More importantly, they should be educated that following their naltrexone treatment they should return to treatment upon relapse, or if they feel vulnerable to relapse.

REFERENCES

1. Julius, D.A., and Renault, P.F., eds. Narcotic Antagonists: Naltrexone. National Institute on Drug Abuse Research Monograph 9. DHEW Pub. No. (ADM)76-387. Washington, D.C.: Superintendent of Documents, 1976.

2. Report of the National Research Council Committee on Clinical Evaluation of Narcotic Antagonists. Clinical evaluation of naltrexone in opiate dependent individuals. Archives of General Psychiatry 1978; 35: 335-340.

3. Callahan, E., Rawson;R., Glazer, M., McCleave, B., and Arias, B. Comparison of two naltrexone treatment programs; Naltrexone alone versus naltrexone plus behavior therapy. In: Julius, D.A., and Renault, P.F., eds. Narcotic Antagonists: Naltrexone, National Institute on Drug Abuse Research Mnograph 9. OHEW Pub. No. (ADM)76-387. Washington, O.C.: Superintendent of Documents, U.S. Government Printing Office, 1976. pp. 150-157.

4. Rawson, R.A., Glazer, M., Callahan, F., and Liberman, R.R. Naltrexone and behavior therapy for heroin addiction. In Krasnegor, N., ed. Behavioral Analysis and Treatment of Substance Abuse. National Institute on Drug Abuse Research Monograph 25. DHEW Pub. No. (ADM)79-839. Washington, D.C.: Supt. Docs., U.S. Govt. Print. Off., 1979. pp. 26-43.

Social Control as an Explanation of Sex Differences in Substance Use Among Adolescents

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A number of longitudinal studies of the use of alcohol, marijuana, cigarettes, and other substances by teenagers have indicated that males and females differ in their frequency of use of all substances except cigarettes (Kandel 1980; Bachman et al. 1981; Ensminger et al. 1982). Despite these sex differences, little attention has been paid to explaining why males are more frequent users. A major question is whether the processes that lead to substance use by females are the same as the processes that lead to substance use by males.

The antecedents of substance use may be distinct for males and females. First, the variables A, B, C, may lead to substance use for males while variables X, Y, Z, may be those that are important to female substance use. Second, the same variables may have different results for males and females--for example, school failure may increase substance use by males but reduce substance use for females. Third, the processes may operate similarly for males and females, but one sex may be much more likely to encounter certain risk factors than the other. For example, school failure may be related to substance use for both sexes, but males may fail more in school than females so that males' higher substance use would be partially explained by this variable.

In this paper we focus on social control theory as a potentially valuable explanatory framework for understanding substance use and compare male and female teenagers to see if social control processes relate to their substance use similarly. The research model we will be examining includes substance use, gender, and measures of social control.

Social Control

This theory as elaborated by Hirschi focuses attention on the strength of the individual's bonds with conventional institutions, norms, or persons (Hirschi 1969). The central thesis is that individuals are more likely to conform to societal standards when

their social bonds to conventional social order are strong. Through social bonds, individuals internalize the society's values and expectations regarding social behavior. The adolescent's tie to the school and the family thus become crucial variables from this perspective.

A potentially important aspect of social control theory is that it may account for the considerable variation in drug use and other kinds of antisocial behavior that occurs over the life course. Young people have higher rates of such activity than adults (Greenberg 1977). A social control perspective would suggest that this is because young people do not yet participate as fully as adults in important societal activities that strengthen social bonds.

Compared to boys, girls are generally considered as having more social bonds to teachers, parents, and conventional values (Jensen and Eve 1976; Turk 1969). Such differences, according to control theory, should relate to differences in the frequency of substance use.

Research Questions

In this paper we examine whether (1) social bonds relate to substance use for males and females and (2) whether the social bonds that relate to substance use operate in similar ways for males and females. The social bonds to be examined include teenage reports of bonds to home, school, and peers.

THE WOODLAWN STUDY

The data were gathered prospectively on a total population of first-grade children in Woodlawn, a poor, black, Chicago community. The children were assessed in first grade and were followed up ten years later at age 16 or 17. Data were also gathered in first grade and again at the time of the ten-year follow-up. These community epidemiological data were gathered on a total population of first-graders within a particular urban neighborhood.

In 1975-1976, we located and reinterviewed 939 (75%) of the mothers or mother surrogates of the 1,242 fami-lies from the 1966-1967 study. After the mother was interviewed and had given permission, the teenager was approached for reassessment. Of the 939 teenaged children of the reinterviewed mothers, 75% (n=705) participated in the reassessments, 14.5% refused to participate, and 10.4% had moved out of Chicago or were unavailable because they were in an institution or had unknown addresses. The study population for this paper consists of the 705 teenagers whom we reassessed.

In order to assess possible bias resulting from sample attrition, we compared the mothers and children whom we reinterviewed with those we did not, using the extensive early information we had on both. The mothers whom we could not reinterview were not distinctive in their 1966-1967 psychological well-being, early family income, welfare status, or the number or types of adults at home. They were more likely to have started child rearing in adolescence, had been more mobile before and during the child's first-grade year, and their children were more likely to have been. in parochial schools in first grade. We found little or no difference in the social adaptational status or psychological well-being between children reinterviewed and those not reinterviewed (Kellam et al. 1980).

METHODS AND MEASURES

Teenage Drug Use

The information on teenage drug use in this paper comes from responses to items in the What's Happening?, an instrument administered to the teenagers who participated in the follow-up sessions. The questions concerned the lifetime frequency of use of 12 categories of substances including alcohol and cigarettes. We focus on four categories in this paper: (1) beer or wine, (2) hard liquor, (3) cigarettes, and (4) marijuana or hashish.

The questionnaires were administered by two black college students (one male and one female) to adolescents in groups of five to eight. The college students emphasized confidentiality and trust issues. The assessment questions were presented visually on slides and orally on audiotape to control for reading ability differences and to standardize the pace and the general administration of the questions (Petersen and Kellam 1977).

Measures of Social Bonds

Social bonds were assessed through measures of the teenager's attachments and commitments to the important social fields of adolescence--family, school, and peers. These measures were obtained in the follow-up assessment. Cronbach alphas, a measure of reliability, for these constructs ranged from 0.65 to 0.68. The following items were used to measure bonds to these three areas--family, school, and peers:

Family Bonds

I enjoy being with members of my family.
Pow do your parents think you are doing at home?
How satisfied are you with your parents' opinion of how you're doing?

School Bonds

Doing well in school is important to me.
How far would you like to go in school if you had the chance?
How far do you think you will go in school?
How do your teachers think you are doing in school?
How satisfied are you with your teachers' opinion of how you are doing?

Peer Bonds

How many of your friends do you really feel close to?
How many close friends do you feel you can share secrets with about private feelings and problems?
How many days during the week do you usually spend time with your friends outside of school hours?
During the last month or so, about how many different friends did you spend time with?
How "in" do you feel with your social group?

RESULTS

Overall, both males and females reported frequent substance use. More than one-third of the males and about 16% of the females reported using marijuana and beer or wine 20 times or more. Fourteen percent of the males and five percent of the females used hard liquor frequently. As a comparison, rates of use of illicit drugs other than marijuana were low. Only one percent of these 16-17 year olds had tried heroin. About 8% reported using cocaine at least one time, while 12% reported using unprescribed codeine (Kellam et al. 1980).

Sex Differences in Substance Use

Males used significantly more beer or wine, hard liquor, and marijuana than did females, as shown by Pearson chi-square tests. There were no significant sex differences in the rates of cigarette use. See Figure 1 for the graphic representation of male and female use.

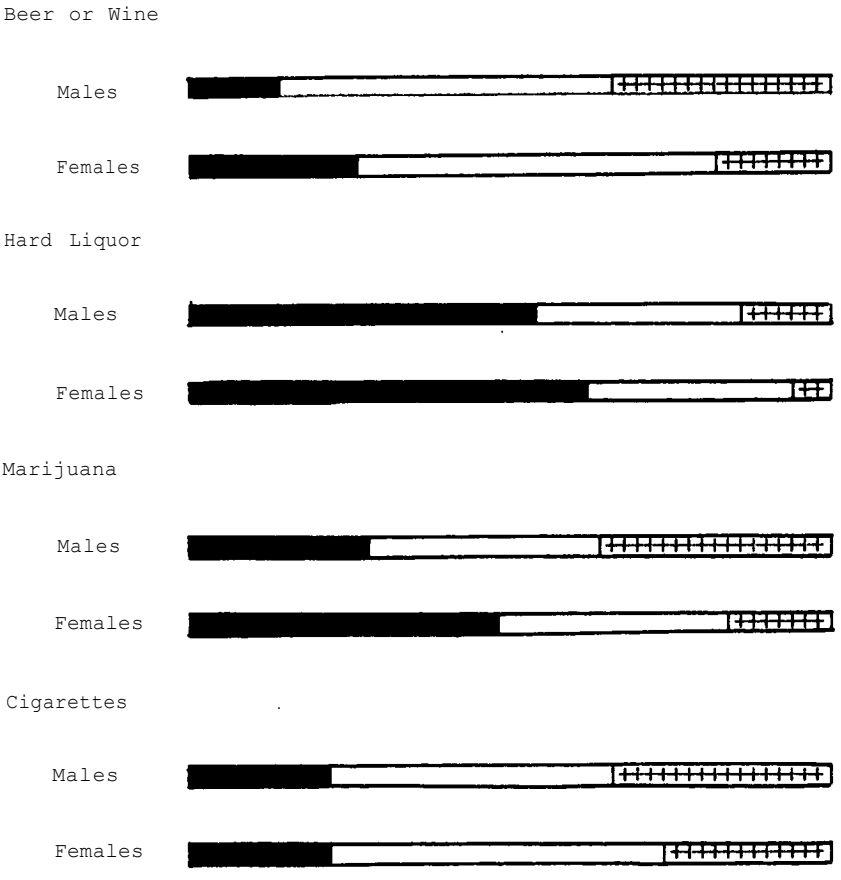
Teenage Social Bonds and Substance Use: Attachment to Family, School, and Peers

Social bonds are strongly related to substance use for both males and females. However, whether the social bonds are related similarly for males and females depends on the substance.

Table 1 shows the odds ratios of heavy substance use'to less use. The social bonds to family, school and peers are listed in rank order of their importance for each sex. For alcohol use the rank order of the odds ratio is reversed for females and males. For both beer or wine and hard liquor, family bonds rank the highest for females--family bonds are not related for males.

FIGURE 1

Teenagers' Reports of Beer or Wine,
Hard Liquor, Marijuana and Cigaretes
(Percentages)



SOCIAL BONDS TO FAMILY, SCHOOL, AND PEERS
RELATIVE ODDS OF HEAVY SUBSTANCE USE TO NONHEAVY USE

TABLE 1

Rank Order of Odds Ratios

	Females			Males		
Beer or Wine	1.	Family	4.7 *	1.	Peers	2.7 *
	2.	School	2.1	2.	School	2.0 *
	3.	Peers	1.5	3.	Family	1.9
Hard Liquor	1.	Family	4.3 *	1.	School	1.9 *
	2.	School	1.4	2.	Peers	1.9 *
	3.	Peers	1.2	3.	Family	1.3
Marijuana	1.	School	4.0 *	1.	School	3.9 *
	2.	Family	2.4 *	2.	Family	1.7
	3.	Peers	1.2	3.	Peers	1.6 *
Cigarettes	1.	School	1.8 *	1.	School	3.7 *
	2.	Peers	1.6	2.	Peers	1.4 *
	3.	Family	1.1	3.	Family	1.2

For family and school bonds the comparison is for low-bonded vs. high-bonded teens; for peer bonds the comparison is for high-bonded vs. low-bonded teens.

For males, peer bonds and bonds to school are most important, and neither are related to alcohol use for females. While social bonds are important for alcohol use for both males and females, they differ as to which social field influences their behavior.

We show here the ratio of the relative odds of heavy alcohol use to use for low-bonded compared to high-bonded teens. An odds ratio is a standard measure of association within four cells of a contingency table (see Fleiss 1973; Bishop et al. 1975) and in our case may be used to compare associations in tables for males and females. An odds ratio of one indicates no difference in the use of low-bonded compared to high-bonded teenagers. An odds ratio larger than one indicates that for family bonds and school bonds the low-bonded teenagers are higher alcohol users than are high-bonded teenagers, while for the peers bonds it indicates that high-bonded teenagers have higher alcohol use. The asterisk indicates that the likelihood ratio chi-square test of association was significant at the .05 level.

In contrast to the alcohol results, the rank order of social bonds is similar for males and females for marijuana and cigarette use. Attachment to school is ranked the highest in smoking marijuana or cigarettes for both females and males.

SUMMARY

For females, stronger family bonds are associated with less use for all substances except cigarettes. The odds of a female with low family bonds using alcohol (beer, wine, or hard liquor) are more than four times as large as the odds for a female with high family bonds. By comparison, for males the family bonds have no relation to substance use.

The strength of attachment to school is related to substance use for both males and females. The odds of a teenager--male or female--with weak bonds to school being a heavy marijuana user are four times greater than the odds for one with strong bonds to school. Similar results were found for males' cigarette use and alcohol use. For both sexes, but especially for males, low school bonds are thus associated with higher substance use.

Attachment to peers is very important for males' substance use but not females'; males who report strong attachments to their peers also report heavier use of all four substances. Although the direction of the relationships between peer bonds and substance use are the same for females as they are for males, the relationships for females are weaker; none are significant at the .05 level.

DISCUSSION

There are four important points worth considering:

First, certain subgroups differ in their likelihood of use of drugs. Males and females are an example. The level of attachment of individuals to institutions is another example. When these two are put together, we get even more information about the likelihood of using drugs, alcohol, and tobacco.

Second, when it comes to explaining why some people become heavy users we need to explore issues of attachment and bonds. An important question for future work concerns identifying the origins of social bonds for males and females.

Third, social bonds are an important part of understanding sex differences in substance use. For males, attachment to school and peer bonds were primary. For females, family bonds and bonds to school were important.

In regard to prevention efforts, the implications are that, at least for the, population studied here, the school context is an important arena for both males and females. The family is more important in the substance use of females and less important for the males. In systematic prevention research both sex differences and the two social fields of school and home will need to be part of the research design.

REFERENCES

Bachman, J.G., Johnston, L.D., and O'Malley, P.M. Smoking, drinking, and drug use among American high school students: Correlates and trends, 1975-1979. American Journal of Public Health, 71(1):59-69, 1981.

Bishop, Y.M., Fienberg, S.E., and Holland, P.W. with the collaboration of Light, R.J., and Mosteller, F. Discrete Multivariate Analysis: Theory and Practice. Cambridge, MA: MIT Press, 1975.

Ensminger, M.E., Brown, C.H. and Kellam, S.G. Sex differences in antecedents of substance use among adolescents. J Social Issues 38(2):25-42, 1982.

Fleiss, J.L. Statistical Methods for Rates and Proportions. New York: Wiley, 1973.

Greenberg, D.F. Delinquency and the age structure of society. Contemporary Crises, 1(2):189-223, 1977.

Hirschi, T. Causes of Delinquency. Berkeley: University of California Press, 1969.

Jensen, G.J., and Eve, R. Sex differences in delinquency: An examination of popular sociological explanations. Criminology, 13(4):427-448, 1976.

Kandel, D.B. Drug and drinking behavior among youth. Annual Review of Sociology, 6:235-285, 1980.

Kellam, S.G., Ensminger, M.E., and Simon, M.B. Mental Health in first grade and teenage drug, alcohol, and cigarette use. Drug and Alcohol Dependence, 5:273-304, 1980.

Petersen, A.C., and Kellam, S.G. Measurement of the psychological well-being of adolescents: The psycometric properties and assessment procedures of the How I Feel. Journal of Youth and Adolescence, 6(3):229-247, 1977.

Turk, A. Criminality and Legal Order. Chicago: Rand McNally, 1969.

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Criminality During the Life Course of Heroin Addiction

John C. Ball and David N. Nurco

STATEMENT OF THE RESEARCH PROBLEM

It has been established that opiate addiction in the contemporary United States is associated with exceedingly high crime rates (O'Donnell 1966; Chambers 1974; Ball et al. 1975; McGlothlin et al. 1978; Inciardi 1979; Gandossy et al. 1980; Barton 1980a, 198Ob; Clayton and Voss 1981). Indeed, recent studies have reported (Ball et al. 1982) that heroin addicts are frequently involved in criminal behavior on a daily basis and that, consequently, they comnithundreds and thousands of offenses per individual during their addiction careers. Furthermore, it is becoming apparent that the scope and magnitudeof the crime problem associated with opiate addiction is not only due to the frequency with which addicts commit "victimless" and lesser offenses, but that many of their offenses are serious and destructive (Chaiken and Chaiken 1982).

But further questions about the association of crime and addiction remain to be answered. One of the most crucial of these involves the continuity of crime among heroin addicts. What are the long-term consequences of this crime-drug relationship? Do active addicts become more, or less, enmeshed in criminal behavior over their adult years? Do the types of crimes they commit change? Or do they reach a high crime plateau which remains stable? What is the effect of successive abstinence periods upon criminality?

In order to investigate the life course of criminality among heroin addicts, three questions were addressed: (1) What specific types of offenses do addicts engage in over the years? (2) Do the types and frequencies of their criminality increase or decrease over the years? (3) How does criminality during successive periods of addiction compare with criminality during successive non-addiction periods?

SAMPLE AND RESEARCH PROCEDURE - Selection of the Baltimore Sample

In order to investigate the continuity of criminal behavior among opiate addicts over the years of their addiction, a probability-based sample of 354 male Baltimore addicts was selected for study. The Baltimore sample was selected because it was representative of the addict arrestee population of this city and, furthernmre, because unusually ccmprehensive life history data were available. With regard to representativeness, the 354 males were a stratified random sample selected from a population of 6,149 knmn opiate users arrested (or identified) by the Baltimore Police Department between 1952 and 1976. Of the 354 males, 159 were white and 195 were black.

Interview Procedure

Each of the 354 addicts was interviewed between July 1973 and January 1978 by specially trained interviewers who were familiar with the Baltimore addict subculture. Both the project staff's knowledge of the local addict street culture (i.e., its history; ecological, racial, and economic structure; major career pattems of criminality; current relationship with police; and availability of specific drugs) and the interviewers' interest in the daily problems and aspirations of the subjects were important requisites to obtaining comprehensive information in the interviews. The interview lasted some three hours, and the questions were focused upon six topics: drug use, criminal behavior, work, living arrangements, drug selling, and sources of income.

The Crime-Day Measures Employed

In order to investigate the extent of criminal behavior by these addicts accurately and comprehensively, an expanded set of crime-day measures was derived. These measures were developed from earlier research which employed a unidimensional crime-day conceptualization. The new measures include five types of crime-days:

Definition of terms:

Crime-Day Theft, (CD-1). A theft crime-day is defined as a 24-hour period during which a given individual engages in stealing property one or more times.

Crime-Day Violence, (CD-2). A violence crime-day is defined as a 24-hour period during which a given individual engages in one or more violent offenses.

Crime-Day Dealing, (CD-3). A dealing crime-day is defined as a 24-hour period during which a given individual engages in one or more drug sale offenses. (In this study, drug use and possession are not included as crimes.)

Crime-Day Con Games, (CD-4). A confidence crime-day is a 24-hour period during which a given individual engages in one or more confidence game offenses or forgery of checks or prescriptions.

Crime-Day Other, (CD-5). A crime-day other (or miscellaneous) is a 24-hour period during which an individual engages in one or more offenses which are not included in CD-1, CD-2, CD-3 or CD-4. These include illegal gambling, pimping, fencing and other offenses.

RESEARCH FINDINGS

The prevalence of the five types of criminality among the 354 Baltimore addicts during their nine year risk period is depicted in Table 1. The most frequent type of crime committed was theft of property which accounted for 37.9 percent of the total crime-days, or 293,308 of 774,777 crime-days. Next in frequency was drug sales, which accounted for 26.5 percent of the crime-days (or 205,692 crime-days). Third in frequency were other offenses, which accounted for 25.6 percent of the total crime-days (or 198,579 crime-days). These three types of crime-days (CD-1, CD-3 and CD-5) accounted for 90 percent of the overall crimes committed by the male addicts.

The remaining two types of crime-days, con games and violent offenses, accounted for, respectively, 7.9 percent and 2.1 percent of the crimes committed. The total number of confidence crime-days was 60,882, and the number of violence crime-days was 16,316.

The total number of crime-days committed by the 354 addicts during the nine years that they were on the street after the onset of their addiction was 774,777. This prevalence of crime meant that the average addict committed over two thousand offenses. The mean number of crime-days was 2,119.

TABLE 1. Total Crime-Days for Theft, Violence, Dealing, Confidence and Other Offenses for 354 Male Addicts

Type of Crime-Days	Number of Crime-Days	Mean Crime-Days Per Addict	Percent of Crime-Days of Each Type
1. Theft of Property	293,308	828.6	37.9%
2. Violent Offenses	16,316	46.1	2.1
3. Drug Sales	205,692	581.1	26.5
4. Confidence, Forg., etc.	60,882	172.0	7.9
5. other Offenses	198,579	561.0	25.6
TOTAL CRIME-DAYS	774,777	2,188.6	100.0

TABLE 2. Percent of Days in Each Addiction Period That Addicts Engaged in Crime, By Each of Five Types of Crime

Addiction Period	Mean Days	Number of Addicts	Percent of Each Period Engaged In: CD-1 'heft	CD-2 Violence	CD-3 Dealing	CD-4 Con Games	CD-5 Other	Percent of Days in Crime *
1.	815	354	34.2	2.3	23.1	7.4	27.6	69.8
2.	583	297	29.7	4.2	25.0	8.8	19.0	66.9
3.	470	226	35.1	0.4	29.8	8.5	21.6	70.9
4.	441	153	30.9	0.8	28.7	7.1	23.2	70.5
5.	453	100	49.9	0.3	17.7	7.4	14.4	70.5
6.	342	57	46.4	0.7	22.7	5.5	18.2	69.7
7.	393	38	63.2	0.2	32.9	2.4	15.9	92.2
8.	315	22	45.5	3.7	7.8	12.8	8.2	64.7
9.	360	13	48.8	3.8	7.1	3.7	34.5	69.8
10.	368	8	90.5	5.1	5.1	11.5	10.2	100.0
11.	385	6	37.9	5.2	7.8	67.5	42.9	88.3
12.	315	2	28.6	--	81.0	--	--	86.3
13.	720	2	--	--	27.1	11.6	--	27.1
14.	600	1	--	--	60.8	27.8	--	77.7

*This is the percent of total days in period which were compsite crime-days. Thus, in the first period of 815 days, 69.8 percent were days in which one or more types of crimes were committed; 30.2 percent of the days were non-crime days.

TABLE 3. Percent of Days in Each Non-Addiction Period That Addicts Engaged in Crime, By Each of Five Types of Crime

Off Period	Mean Days	Number of Addicts	Percent of Each Pet-id Engaged In: CD-1 Theft	CD-2 Violence	CD-3 Dealing	CD-4 Con Games	CD-5 Other	Percent of Days In Crime*
1.	887	319	9.2	0.1	6.3	0.5	8.2	22.4
2.	754	167	5.0	0.2	3.4	0.3	4.2	12.4
3.	625	78	2.0	0.0	4.4	0.7	6.3	11.9
4.	533	32	0.6	--	3.2	--	0.2	3.7
5.	639	14	15.7	0.0	1.3	--	--	15.8
6.	690	6	2.5	--	--	--	--	2.5
7.	750	2	--	--	--	--	--	0.0
8.	510	1	--	--	--	--	--	0.0

*Composite Crime-Days.

Note: In the above table, a dash indicates no crime-days in the period for the type of crime; 0.0 indicates less than 0.05 percent of crime.

Continuity of Crime Rates During Successive Addiction Periods

The continuity of crime during successive addiction periods is shown in Table 2. The percent of time in each of the five types of crime-days is tabulated for all 14 periods. The last column records the percent of days in each period that the addicts were engaged in crime of any type: this is the percent of each period that were composite crime-days (i.e., days during which one or more of the five types of crime were committed).

A major finding about the continuity of criminality during the addiction periods is that it is relatively stable in frequency. This stability is evident in the lack of variation of the five crime-day measures as well as in the composite crime-day findings. Thus, with regard to each of the five crime-day measures, the percent of time engaged in crime in successive periods usually does not differ from that of the first period by as much as ten percent. With regard to the overall amount of time in each addiction period that the male addicts were involved in crime, this too is quite stable. In only two of the first ten periods (7 and 10) does the amount of time involved in crime differ from the initial figure of 70 percent by more than five percent. There are, then, only minor variations in subsequent periods from the initial high rate of criminality in the first addiction period.

Crime Pates During Successive Off Periods

A major finding concerning criminality in the off periods is that it decreases in successive periods (Table 3). This is most evident in the sharp decrease in the overall percent of time that the addicts (or former addicts) engaged in crime from the first to the fourth off period - a decrease from 22.4 percent to 3.7 percent. This same trend is reflected in the five crime-daymeasureswhichdecreasein successive periods. Thus, during periods 2, 3 and 4, there was a decrease in crime-days in 13 of the 15 instances. Furthermore, there is evidence of a complete, or almost complete, cessation of crime after the fourth period.

INTERPRETATION AND CONCLUSION

In this follow-up study of a probability-based sample of Baltimore heroin addicts, it was found that the 354 male addicts maintained a high rate of criminality over their addiction careers. Thus, they committed offenses some 255 days a year while "on the street" and this high rate of criminality con-timed during their years at risk. Indeed, the continuity and stability of their frequent criminal behavior during their periods of addiction was remarkable.

Five crime-day measures were employed to analyze criminality within this sample over the risk years. It was found that theft was the most common offense as it accounted for 38 percent

of the total crime-days. Drug sales was second in overall frequency as it accounted for 27 percent of the crime-days. The "other crime" classification included 26 percent of the crime-days. The remainder of their crime involved violent offenses and con games; together these offenses accounted for ten percent of the total crime. This pattern, or configuration, of crime remained quite stable throughout their addiction periods.

While there is no support in the research findings for a maturation hypothesis with respect to the association between crime and opiate addiction, there was substantial support for the thesis that drug dependence is a major contributory factor leading to criminality among heroin addicts in the united States. In this regard, the difference between crime rates in the first addiction period and the first off period was striking (a mean of 255 crime-days per year vs. 82 crime-days per year). The comparable figure for the total number of crime-days during this first addiction and first off period was 273,049 and 68,999 for each of these two year periods.

The high crime-rates of the first addiction period continued in subsequent addiction periods. Thus, the 354 males committed well over 775,000 crimes during the nine-year risk period that they were free in the community and 88 percent of these were committed while they were addicted.

REFERENCES:

Ball, J.C., Levine, B.K., Demaree, R.G., and Neman, J.G. Pretreatment criminality of male and female drug abuse patients in the United States. Addictive Diseases, 1(4):481-489, 1975.

Ball, J.C., Rosen, L., Flueck, J.A., and Nurco, D.N. Lifetime criminality of heroin addicts in the United States. Journal of Drug Issues, Summer:225-239, 1982.

Barton, W.I. Drug Abuse History and Criminality of Inmates of Local Jails: Results of the 1978 LEAA Survey of Inmates of Local Jails in the U.S. Washington, D.C., DEA,.1980a, pp. 1-98. Drug histories and criminality: Survey of inmates of State correctional facilities, January 1974. The International Journal of Addictions, 15(2):233-258, 1980b.

Chaiken, J.M., and Chaiken, M.R. Varieties of Criminal Behavior. Santa Monica, The Rand Corporation, 1982.

Chambers, C.D. Narcotic addiction and crime: an empirical review. In: Inciardi, J.A. and Chambers, C.D., eds. Drugs and the Criminal Justice System. Beverly Hills: Sage, 1974. Chapter 5.

Clayton, R.R., and Voss, H.L. Young Men and Drugs in Manhattan: A Causal Analysis. National Institute on Drug Abuse Research Monograph 39. DHHS Rub. No. (ADM) 81-1167. Washington, D.C.: supt. of Docs., U.S. Govt. Print. Off., 1981.

Gandossy, R.P., Williams, J.R., Cohen J., and Harwood, H.J. Drugs and Crime. Washington: U.S. Government Printing Office, 1980.

Inciardi, J.A. Heroin use and street crime. Crime and Delinquency, July:335-346, 1979.

McGlothlin, W.H., Anglin, M.D. and Wilson, B.D. Narcotic addiction and crime. Criminology, 16(3):293-315, 1978.

O'Donnell, J.A. Narcotic addiction and crime. Social Problems, 13:374-385, 1966.

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Depressive Symptoms in Drug Abuse Treatment Clients: Correlates, Treatment, and Changes

Harold M. Ginzburg, Margaret Allison, and Robert L. Hubbard

INTRODUCTION

An association between drug use and depression has been observed in a number of studies (Robins 1974, Sutker 1971). These studies indicate that higher levels of drug use are associated with increases in depressive symptoms. In general, 30 to 50 percent of clients in drug abuse treatment studies are diagnosed as at least moderately depressed. These findings compare with estimates that 14-20 percent of the general population experience depressive symptoms at any given time (Midanik 1981), and 4-7 percent of the population may be diagnosable as having clinical depression (Weissman et al. 1975-1976). Thus, it is, clear that depression is a factor that must be considered in the treatment of drug abuse.

The effects of drug abuse treatment on depression, however, remain unclear. Woody and Blaine (1979) report that the high levels of depression at intake decrease over time. However, they also caution that suicide attempts are more common during withdrawal phases of treatment. In a long-term study of drug abusers, scores on depression scales decreased substantially regardless of the type of substance. abuse or the duration of treatment (Dorus and Senay 1980). It is apparent, then, that further research on the nature, prevalence, and course of depressive symptoms among clients in drug abuse treatment programs would be useful for a variety of purposes.

This paper describes the depressive symptomatology found in clients in drug abuse treatment programs in the Treatment Outcome Prospective Study (TOPS). In addition, correlations between the depressive symptoms and a number of demographic and outcome variables are presented and discussed. Finally, changes in symptom levels observed after three months in treatment are reported.

METHODOLOGY

Briefly, TOPS is a large scale, longitudinal, descriptive study of the effects of drug abuse treatment programs. The research tracks individuals who entered treatment during calendar years 1979, 1980, and 1981. Programs representing four major treatment modalities were included in the TOPS study, i.e., outpatient detoxification, methadone maintenance, residential, and outpatient drug free programs. Nearly 12,000 clients in 59 clinics in 10 cities located throughout the United States voluntarily participated in the study. Clients were interviewed at the time they entered treatment, at regular intervals while they remained in treatment, and periodically after they left treatment.

The interviews collected a wide range of information including demographic and background characteristics, drug and alcohol consumption, drug-and alcohol-related problems, prior treatment history for drug, alcohol or mental health problems, illegal activities, and family and social relationships. A set of three questions on depressive symptoms was also included in all of the TOPS interviews. These items sought self-reports of (1) having felt so depressed that one could not get out of bed in the morning, (2) having thought of suicide, or (3) having attempted suicide.

The TOPS three-item scale was validated against the Beck Depression Inventory, the CES-D (a depression scale developed by the National Institute of Mental Health, Center for Epidemiological Studies) and Koss and Butcher's Critical Items derived from the Minnesota Multiphasic Personality Inventory. Results of the validation study indicate that the scale is sufficiently valid to establish the internal validity of the depression data available in the overall TOPS database.

CORRELATES OF DEPRESSIVE SYMPTOMS

The TOPS data indicate that, at intake, more clients entering outpatient drug free (OPDF) or residential treatment programs reported experiencing depressive symptoms during the year prior to admission. Also, a substantially greater percentage of these clients report having attempted suicide than clients entering detoxification or methadone programs. Over 60 percent--roughly three times the rate in the general population--of all clients coming into TOPS drug abuse treatment programs state that they have experienced one or more of these symptoms.

Client Characteristics

In each modality women are more likely to report signs of depression than men (table 1). Depressive symptoms are most common among women entering residential, outpatient detoxification, and OPDF programs. About 66 percent of women entering methadone programs report depressive symptoms, compared to nearly 74 percent in the other modalities. Among male clients, there appears to be a

Table 1. Sex and Age by Depression Indicators During the Year Before Admission to Treatment

Sex/Age	n	Indicators of Depression: None	Felt Depressed	Suicidal Thoughts	Suicidal Attempts
Outpatient Detoxification					
Male					
< 25	70	40.0%	17.0%	37.1%	5.7%
26-30	137	46.0	21.9	30.7	1.5
> 30	192	51.6	20.8	24.5	3.1
Female					
< 25	50	20.0	24.0	34.0	22.0
26-30	37	29.7	37.8	21.6	10.8
> 30	32	28.1	25.0	37.5	9.4
Outpatient Methadone					
Male					
< 21	20	35.0%	25.0%	10.0%	10.0%
21-25	154	42.2	30.5	21.4	5.8
26-30	283	41.3	27.2	27.2	4.3
> 30	333	51.7	27.9	16.8	3.6
Female					
< 21	13	38.5	38.5	15.4	7.7
21-25	107	43.0	17.8	30.8	8.4
26-30	135	29.6	21.5	39.3	9.6
> 30	56	25.0	48.2	19.6	7.1
Residential					
Male					
< 21	141	36.9%	14.9%	24.8%	23.4%
21-25	210	40.0	17.1	31.4	11.4
26-30	176	44.3	15.9	34.1	5.7
> 30	159	47.2	20.8	24.5	7.5
Female					
< 21	42	14.3	14.3	31.0	40.5
21-25	73	28.8	12.3	35.6	23.3
26-30	39	17.9	17.9	30.8	33.3
> 30	23	21.7	21.7	43.5	13.0
Outpatient Drug Free					
Male					
< 21	162	46.9%	13.0%	30.2%	9.9%
21-25	166	36.7	21.1	31.3	10.8
26-30	126	42.1	15.9	34.1	7.9
> 30	109	44.0	11.9	31.2	12.8
Female					
< 21	81	24.7	7.4	46.9	21.0
21-25	97	26.8	14.4	37.1	21.6
26-30	71	26.8	22.5	28.2	22.5
> 30	74	31.1	18.9	35.1	14.9

Note. Rows add to 100%

negative relationship between age and depression indicators. Among female clients the relationship between age and depression indicators is not so straightforward. Older women in methadone programs are more likely to report depressive symptoms while, in other modalities, it appears that younger women may be more depressed. Overall, women under 21 are the most likely group to report some sign of depression. These findings are quite similar to those reported by Midanik (1981) based on a general population sample.

The weekly drug use pattern of clients entering drug abuse treatment programs was also correlated with the depression indicators. Seven patterns have been identified on the basis of the TOPS data. They are: (1) heroin and other narcotics; (2) heroin, not other narcotics; (3) narcotics, not heroin; (4) multiple nonnarcotics; (5) single nonnarcotic; (6) alcohol and/or marijuana; and (7) minimal drug use. It must be noted that membership in one of these pattern groups does not preclude the use of other drugs on a less than weekly basis.

It appears that heroin use, without the use of other narcotics, is not particularly associated with suicidal thoughts or attempts among clients in any of the treatment modalities. Similarly, clients in the alcohol/marijuana and minimal use pattern groups have relatively low rates of suicidal thoughts or attempts. Those at highest risk of suicidal behaviors and ideation are clients in the multiple nonnarcotic and other narcotics groups. Those in the single nonnarcotic group are at somewhat less risk among all but residential clients. These are especially noteworthy findings in view of the increasing numbers of polydrug abusers entering treatment (Bray et al. 1982) and have obvious implications for drug abuse treatment program planning.

In the TOPS interviews, clients were asked whether their drug abuse had caused them any problems of a medical, psychological, family, legal, educational, employment, or financial nature. As might be expected-, those who said they had more such problems were more likely to have experienced some sign of depression. Also, there were more frequent reports of suicide attempts from clients with more drug-related problems.

Not surprisingly, there is an unmistakable, positive relationship between prior mental health, treatment and depression indicators during the year before admission to drug abuse treatment, regardless of modality.

Ancillary Services

The data on which the following discussion is based were generated in interviews with clients after they had been in treatment for three months. Due to the short duration of detoxification treatment, few ancillary services are provided in programs of that modality. Therefore, they are not included in the discussion of services received in relation to depressive symptoms.

In methadone programs, there appears to be no relationship between depressive symptoms experienced during the first three months in treatment and the number of ancillary services received during the first three months of treatment. Clients who received psychological services were less likely than those who did not receive such services to say they had felt so depressed they couldn't get out of bed (8 percent versus 22 percent), but were more likely to say they had thought of or attempted suicide. The opposite was true for those receiving or not receiving medical services.

In residential treatment programs, clients who received no ancillary services were most likely to say they had no depressive symptoms during their first three months in treatment. Those receiving services for two or three types of problems (more than half the clients) were most likely to report some sign of depression. In general, there was a slight tendency for those who had received an ancillary service to be more likely to report some symptom of depression. None of the TOPS clients in residential treatment at three months reported suicide attempts during the first three months of treatment; 9 percent reported suicidal thoughts and 10 percent felt depressed.

Among OPDF clients there was little or no difference in the levels of depression indicators reported by those receiving or not receiving medical, family, or educational services. In contrast, those receiving psychological services were more likely to say they had been depressed or thought of suicide during their first three months in treatment. In nearly all cases, clients who received service for a given type of problem were more likely to say they had experienced some depressive symptoms during their first three months in treatment. Presumably, this indicates that those who are-more in need of treatment services are receiving them.

CHANGES IN DEPRESSIVE SYMPTOMS

Given the frequency of reports' of depression indicators and the potential effects of depression on treatment outcome and mental health in general, reducing levels of depression should be an important treatment goal. To examine the possible impact of treatment, responses in the TOPS interview forms at intake and at one month and three months in treatment were compared. Clients in detoxification programs were not included in this analysis because such programs are usually completed in less than one month.

Data from the TOPS intake and intreatment interviews indicate that there is a marked reduction in reports of depression indicators after one month in treatment. In general, the rate drops from about 60 percent at intake to an average of just over 30 percent at one month. Clients in residential programs show an even greater decrease to about 20 percent reporting depressive symptoms at one month. It is interesting to note that the rate increases somewhat at three months and then falls slightly at six months.

A finding of reductions this large so soon after admission into treatment may be partially explained by differences in the lengths of the time periods being compared, i.e., the year before admission, the first month of treatment, and the first or second three-month periods of treatment. On the other hand, it may suggest that much of the depression reported at intake could be of a transient situational type rather than a chronic mental health problem. Nevertheless, there is a substantial percentage of clients in all modalities who remain depressed. Early identification and treatment of the 20-30 percent of clients who may remain depressed for long periods could be important to the overall effectiveness of the drug abuse treatment program.

To assess how depression indicators change during the first three months in treatment as compared to the year prior to treatment for various subgroups of clients, a change scoring scheme was created. Based on the assumption of a hierarchy of severity (no symptoms < felt depressed < thought of suicide < attempted suicide), clients were categorized according to the most severe symptom reported at admission and again after three months in treatment. Clients who reported no depressive symptoms for either time period were classified as "no problem" (NP). Those who reported a less severe symptom at three months in treatment than they did at admission were placed in the "reduced depression" (RO) category. The "continued or increased depression" (CD) category comprised those clients who remained in or moved'to the "felt depressed" response group. Those who remained in or moved to either the "thought about suicide" or "attempted suicide" response groups were categorized in the "continued or increased suicidal tendencies" (CS) group. The percentages of clients in each of the categories are presented by modality in table 2.

Table 2. Changes in Depression Indicators Reported for the Year Before Admission and First Three Months in Treatment

	Outpatient Methadone	Residential	Outpatient Drug Free	Overall
NP	36.2%	38.9%	33.3%	36.3%
CD	13.5	5.0	3.8	9.2
CS	11.1	9.2	20.9	12.7
RD	39.2	46.9	42.0	41.8
	100.0 (n=658)	100.0 (n=338)	100.0 (n=264)	100.0 (n=1260)

NP=no problem;. CD=cont'd or increased depression; CS=cont'd or increased suicidal tendencies; RD=reduced depression

DISCUSSION

The findings reported in this paper have clear implications for program planning and clinical case management in drug abuse

treatment programs. Depressive symptoms are prevalent among clients in drug abuse treatment, especially young women and those who use two or more nonnarcotics on a weekly basis. Our findings also indicate that depressive symptoms fade rapidly for many clients but, at the same time, substantial proportions of clients continue to express some degree of symptomatology.

Further research aimed at the early detection of those whose symptoms are likely to persist would be of great benefit. Other research questions, such as whether depressive symptoms are a motivator for clients to seek treatment, how depression is related to specific drug use patterns, and whether or how depression might influence the effectiveness of drug abuse treatment are also in need of further investigation.

REFERENCES

Bray, R.M., Schlenger, W.E., Craddock, S.G., Hubbard, R.L., & Rachal, J.V. Approaches to the Assessment of Drug Use in the Treatment Outcome Prospective Study (RTI/1901/01-05S). Research Triangle Park, NC: Research Triangle Institute, 1982.

Dorus, W., & Senay, E.C. Depression, demographic dimensions, and drug abuse. Am J Psychiatry, 137(6):699-704, 1980.

Midanik, L. Alcohol use and depressive symptoms. In: Social Research Group, Draft Report on the 1979 National Survey. Berkeley, CA: School of Public Health, University of California, 1981.

Robins, P.R. Depression and drug addiction. Psychoanal Q, 48:375-386, 1974.

Sutker, P.B. Personality differences and sociopathy in heroin addicts and nonaddict prisoners. J Abnorm Psychol, 78(3): 247.-251, 1971.

Weissman, M.M., Myers, J.K., & Harding, P.S. Psychiatric disorders in a U.S. urban community: 1975-1976. Am J Psychiatry, 135(4):459-462, 1978.

Woody, G.E., & Blaine, J. Depression in narcotic addicts: Quite possibly more than a chance association. In: DuPont, R.L., Goldstein, A., & O'Donnell, J., eds., Handbook on Drug Abuse. Washington, DC: U.S. Government Printing Office, 1979.

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Striatal Dopamine Receptor Function in Morphine-Tolerant Dependent Rats: Influence of Hypothalamic Peptides

Hemendra N. Bhargava

INTRODUCTION

Chronic administration of opiates to rodents is associated with changes in the central dopaminergic systems. For instance, supersensitivity of brain dopamine receptors develops in rodents following chronic treatment with morphine (Lal 1975; Iwatsubo and Clouet 1975; Ritzmann et al. 1979; Bhargava 1980, 1981a) or human ß-endorphin (Bhargava 1981b). Some studies, on the other hand, have been unable to demonstrate the development of supersensitivity, and in some cases, have observed subsensitivity of dopamine receptors after chronic treatment with morphine (Kuschinsky 1975; Merali et al. 1975; Iversen and Joyce 1978). Dopamine receptor function has been studied by using ^{3}H-spiroperidol binding assays. In the rat, acute administration of morphine did not alter striatal ^{3}H-spiroperidol binding; implantation of two morphine pellets increased the dissociation constant (K_d value), and implantation of four morphine pellets reduced the number of binding sites (B_{max} value) and lowered the Kd value (Puri et al. 1978). Christie and Overstreet (1979) have found higher Kd values for ^{3}H-spiroperidol binding in the striatum of morphine-withdrawn rats.

Because of the existence of conflicting reports on changes in dopamine receptor system following chronic opiate administration, and also in an effort to seek a possible relationship between the opiate tolerance-dependence process and activity in the dopaminergic systems, the effect of chronic treatment with morphine to rats on the striatal ^{3}H-spiroperidol binding has been examined. In addition, the effect of two peptides, which have been shown to inhibit the development of tolerance to and dependence on opiates (Bhargava 1980, 1981a, c, d; Bhargava et al. 1980) on morphine-induced changes in ^{3}H-spiroperidol binding has been determined.

METHODS

Male Sprague-Dawley rats weighing about 250 g obtained from King Animal Laboratories, Oregon, WI. were housed in rooms with controlled temperature, humidity and light for 4 days before being used. The rats were given food and water ad libitum.

Cyclo(Leu-Gly) (CLG) was synthesized in these laboratories and its purity checked by thin layer chromatographic analyses (Bhargava 1981c). Melanotropin release inhibiting factor (MIF) was obtained from the Abbott Laboratories, N. Chicago, Illinois, through the courtesy of Dr. E.L. Woroch. The peptides were dissolved in distilled deionized water and injected subcutaneously. ^{3}H-Spiroperidol (specific activity 26.0 Ci/mmole) and d-butaclamol were obtained from New England Nuclear, Boston, MA.

The rats were made tolerant to and dependent on morphine by s.c. implantation of four morphine pellets (each containing 75 mg of morphine free base) during a 3-day period using a schedule described earlier (Bhargava 1977, 1978). Rats serving as controls were implanted with four placebo pellets. The schedule of vehicle or peptides, and morphine or placebo pellet treatment was similar to that described previously (Bhargava 1981a, c). The dose of each peptide was 2 mg/kg/day. All the pellets were removed from the rats, under light ether anesthesia. Twenty-four hours after the pellet removal, the rats were decapitated, their striata removed, and frozen at -70°C until assayed for ^{3}H-spiroperidol binding.

Implantation of four morphine pellets during a 3-day period produces a high degree of dependence (Bhargava 197.7) and a 6.5-fold tolerance to the analgesic effect of morphine (Bhargava 1978). Brain and plasma of morphine tolerant-dependent rats were shown to be devoid of morphine at the time the animals were sacrificed (Bhargava 1978).

The binding of ^{3}H-spiroperidol to rat striatal dopamine receptors was carried out as described previously (Creese and Snyder 1979). Specific binding of ^{3}H-spiroperidol was defined as the difference in binding in the absence and presence of 1 μM d-butaclamol. The concentration of protein in the striatal homogenates was determined according to the method of Lowry et al. (1951). The apparent dissociation constant (K_d) and the maximal binding capacity (B_{max}) were determined from the Scatchard plots generated by least square regression analyses. Four rats were used for each treatment group. The means of Bmax and K_d values in different treatment groups were analyzed by one- way ANOVA followed by the Schaffe's 'S' test.

RESULTS

Chronic administration of morphine to rats resulted in an enhanced affinity of ^{3}H-spiroperidol binding to striatal dopamine receptors but the number of binding sites did not change. Figure 1 shows the saturation curves and the Scatchard plots of ^{3}H-spiroperidol binding to striatal membranes of rats implanted with placebo or morphine pellets. The maximal number of binding sites (B_{max} values) in morphine and placebo pellet implanted rats were 141.5 ± 15.6 and 143.2 ± 9 fmoles of ^{3}H-spiroperidol bound per milligram of protein, respectively. Chronic treatment with morphine decreased the apparent dissociation constant (K_d value) to 49 pM from 111 pM found in placebo pellet-implanted rats.

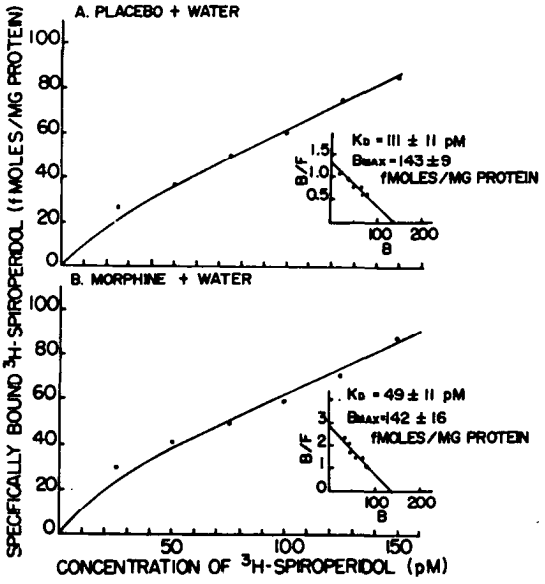

FIGURE 1

The saturation curves and Scatchard plots (insets) of ^{3}H-spiroperidol binding to striatal membranes of nontolerant (placebo pellets) and morphine tolerant-dependent (morphine pellets) rats.

Analysis of variance indicated a significant interaction $F(1,12) = 19.33$, $P < 0.001$. To seek a possible relationship between opiate tolerance-dependence and dopamine receptor function, the effect of two peptides, MIF and CLG, which have been shown to block the development of tolerance to and physical dependence on morphine, on striatal 3H-spiroperidol binding changes induced by chronic morphinization was investigated. Multiple injections of either MIF or CLG to rats implanted with placebo pellets had no effect on either the B_{max} or the K_d values of ^{3}H-spiroperidol nor did they alter the B_{max} values in rats implanted with morphine pellets (Table 1). Both peptides, however, reversed the changes in K_d values induced by chronic morphine administration, and these values were similar to those found in the placebo + water treated group (Table 1).

TABLE 1

Effects of Pro-Leu-Gly-NH_2 (MIF) and cyclo(Leu-Gly) (CLG) on ^{3}H-spiroperidol binding constants in the striata of rats treated chronically with morphine.

Treatment[a]	^{3}H-Spiroperidol binding parameters Mean± S.E.M. (N = 4)	
	Bmax (fmoles/mg protein)	K_d (pM)
Vehicle + Placebo	143.2 ± 9.0	111.3 ± 11.2
Vehicle + Morphine	141.5 ± 15.6	49.3 ± 10.7[b]
MIF + Placebo	138.9 ± 5.7	106.3 ± 10.7
MIF + Morphine	124.0 ± 14.4	88.2 ± 9.6[c]
CLG + Placebo	144.3 ± 7.4	104.4 ± 10.3
CLG + Morphine	118.5 ± 19.0	87.4 ± 8.8[C]

a Rats were injected with vehicle (water) or the appropriate peptide and then implanted subcutaneously with either 4 morphine or placebo pellets as described in the text. The pellets were removed 72 hr after the first implantation. Twenty-four hours after the pellet removal the animals were sacrificed, their striata removed and frozen at -70°C until receptor binding studies were undertaken.

b $p < 0.05$ vs vehicle + placebo group.

c $p < 0.05$ vs vehicle + morphine group.

DISCUSSION

As indicated in the introduction section, most studies indicate that the development of tolerance to and dependence on opiates is associated with the development of behavioral supersensitivity of brain dopamine receptors. However, considerable disparity exists in the literature for the biochemical evidence for dopamine supersensitivity using ^{3}H-spiroperidol binding assays. It is not clear whether this supersensitivity results from the proliferation of dopamine receptors or from the changes

in the affinity of the receptor ligands. It has been suggested that a high degree of dependence decreases, whereas a low degree of dependence increases, both the K_d and B_{max} values for ^{3}H-spiroperidol binding, respectively (Puri et al. 1978). Christie and Overstreet (1979) found no change in striatal ^{3}H-spiroperidol in morphine-tolerant rats, but observed an increase in K_d value in rats withdrawn from morphine. The present studies indicate that development of morphine tolerance-dependence process, which results in behavioral supersensitivity of brain dopamine receptors, results from enhanced affinity of the ligands to the receptors and not from the increased number of receptors. This observation is consistent with the report of Cross et al. (1978) who found a direct relationship between ^{3}H-spiroperidol affinity and behavioral supersensitivity to apomorphine.

MIF and CLG, which inhibit the development of tolerance to and dependence on morphine, were found to block the morphine-induced increased affinity of ^{3}H-spiroperidol to striatal dopamine receptors. It is thus possible that tolerance to and physical dependence on morphine involves changes in the affinity of ligands to dopamine receptors, and it may be one of the several mechanisms which may be involved in its genesis.

REFERENCES

Bhargava, H.N. Rapid induction and quantitation of morphine dependence in the rat by pellet implantation. Psychopharmacology, 52: 55-62, 1977.

Bhargava, H.N. Quantitation of morphine tolerance induced by pellet implantation in the rat. J Pharm Pharmacol, 30: 133-135, 1978.

Bhargava, H.N. Cyclo(leucylglycine) inhibits the development of morphine induced analgesic tolerance and dopamine receptor supersensitivity in rats. Life Sci, 27: 117-123, 1980.

Bhargava, H.N. The effect of melanotropin release inhibiting factor (MIF) and cyclo(Leu-Gly) on the tolerance to morphine-induced antinociception in the rat: a dose-response study. Br J Pharmacol, 72: 707-714, 1981a.

Bhargava, H.N. Enhanced response to apomorphine in rats treated with multiple injections of human beta endorphin and its blockade by Pro-Leu-Gly-NH_2 and cyclo(Leu-Gly). Life Sci 29: 1945-1949, 1981b.

Bhargava, H.N. The effects of peptides on tolerance to the cataleptic and hypothermic effects of morphine in the rat. Neuropharmacology, 20: 385-290, 1981c.

Bhargava, H.N. Inhibition of tolerance to the pharmacological effects of human beta endorphin in the rat by prolyl-leucyl glycinamide and cyclo(leucylglycine). J Pharmacol Exp Ther, 218: 404-408, 1981d.

Bhargava, H.N., Walter, R., and Ritzmann, R.F. Development of narcotic tolerance and physical dependence: effects of Pro-Leu-Gly-NH_2 and cyclo(Leu-Gly). Pharmacol Biochem Behav, 12: 73-77, 1980.

Christie, M.J., and Overstreet, D.H. Sensitivity of morphine-tolerant rats to muscarinic and dopaminergic agonists: relation to tolerance and withdrawal. Psychopharmacology, 65: 27-34, 1979.

Creese, I., and Snyder, S.H. Nigrostriatal lesions enhance striatal ^{3}H-apomorphine and ^{3}H-spiroperidol binding. Eur J Pharmacol, 56: 277-281, 1979.

Cross, A.J., Longden, A., Owen, F., Poulter, M., and Waddington, J.L. Interrelationship between behavioral and neurochemical indices of dopaminergic neurons. Br J Pharmacol, 63: 383, 1978.

Iversen, S.D., and Joyce, E.M. Effects in the rat of chronic morphine treatment on the behavioral responses to apomorphine. Br J Pharmacol, 62: 390, 1978.

Iwatsubo, K., and Clouet, D.H. Dopamine-sensitive adenylate cyclase of the caudate nucleus of rats treated with morphine or haloperidol. Biochem Pharmacol, 24: 1499-1505, 1975.

Kuschinsky, K. Does chronic morphine induce a supersensitivity of dopamine receptors in rat brain? Psychopharmacologia, 42: 225-229, 1975.

Lal, H. Narcotic dependence, narcotic action and dopamine receptors. Life Sci, 17: 483-496, 1975.

Lowry, O.H., Rosenbrough, N.J., Farr, A.L., and Randall, R.J. Protein measurement with Folin phenol reagent. J Biol Chem, 139: 265-275, 1951.

Merali, Z., Singhal, R.L., Hrdina, P.D., and Ling, G.M. Changes in brain cyclic AMP metabolism and acetylcholine and dopamine during narcotic dependence and withdrawal. Life Sci, 16: 1889-1894, 1975.

Puri, S.K., Spaulding, T.C., and Mantione, C.R. Dopamine antagonist binding: a significant decrease with morphine dependence in the rat striatum. Life Sci 23: 637-642, 1978.

Ritzmann, R.F., Walter, R., Bhargava, H.N., and Flexner, L.B. Blockage of narcotic-induced dopamine receptor supersensitivity by cyclo(Leu-Gly). Proc Natl Acad Sci, USA, 76: 5997-5998, 1979.

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Drug Preference and Mood in Humans: Mazindol and Phenylpropanolamine

L. D. Chait, E. H. Uhlenhuth, and C. E. Johanson

ABSTRACT

Normal human volunteers participated in two drug preference studies. In one study subjects (N=12) were allowed to self-administer either a dose of mazindol (MAZ) (0.5, 1.0 or 2.0 mg) or placebo. In the other study subjects (N=12) self-administered either a dose of phenylpropanolamine (PPA) (12.5, 25 or 50 mg) or placebo. In both studies subjects were free to leave after drug ingestion and filled out subjective report forms at the time of ingestion and 1, 3, and 6 hours later.

All three doses of MAZ were chosen less frequently than placebo (less than 1 out of 5 choice occasions). Ratings of drug liking also were less after MAZ than after placebo. The low dose of MAZ was most frequently perceived to be a tranquilizer, whereas the high dose was most often perceived to be a stimulant. Increases in ratings of Fatigue, Anxiety and Confusion, and' decreases in ratings of Elation and Positive Mood were obtained on the Profile of Mood States (POMS) after MAZ. Increases in ratings of "sedated" (visual analog scale - VAS) were also obtained after MAZ, as were increases in scores on the PCAG and LSD scales of the Addiction Research Center Inventor (ARCI). No significant changes were observed on ratings of "hungry" (VAS) after MAZ.

No dose of PPA was chosen with a frequency differing significantly from placebo (range 1.9 - 2.2 times out of 5 choice occasions). Ratings of drug liking after PPA did not differ from those after placebo. The low dose of PPA was most frequently perceived to be placebo, whereas the high dose was most often perceived to be a stimulant. Increases were obtained after PPA for ratings of Anxiety and Depression on the POMS and for ratings of "anxious," "down," "high," and "stimulated" on the VAS. No significant effects of PPA were obtained on the ARCI or on ratings of "hungry."

The results demonstrate that, unlike d-amphetamine and diethylpropion, MAZ and PPA are not preferred by subjects to placebo. In fact, MAZ actually proved to be aversive compared to placebo. Most of the subjective effects produced by the two drugs would generally be considered unpleasant, which corresponds to the failure by the subjects

to self-administer these two anorectics. These findings suggest that PPA and MAZ are of low dependence potential.

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The Use of Phenobarbital in Treating Abstinence in Newborns Exposed In Utero to Psychoactive Agents

Loretta P. Finnegan, Herman Michael, and Betty Leifer

Phenobarbital has been used extensively for neonatal abstinence as it suppresses the major symptoms by a nonspecific central nervous system depression. Two approaches to phenobarbital therapy are currently in use. With both approaches, the initial dose and all subsequent doses are determined by the severity of symptoms using an abstinence scoring system. The titration approach provides the infants with a total dose per body weight of 6-8-10 or 12 mg/kg per day which is divided into 3 equal doses at 8-hour intervals. The loading dose approach provides the infants with an initial dose of 20 mg/kg followed by maintenance doses sufficient to control the symptoms. The study infants were born to women using narcotics in combination with other illicit drugs during pregnancy. Of the 30 infants, 16 received the titration and 14 the loading dose regimen. All were treated successfully with phenobarbital. The relative efficacy of the two treatment modalities was determined by recording the number of hours necessary to achieve control of symptoms. Results revealed a statistically significant difference ($p < .01$) between the average time necessary to achieve control with the loading dose method (33 hours) and the average time necessary with the titration method (64 hours). These results suggest that although both methods successfully manage the symptoms of neonatal abstinence, the loading dose methodology provides more rapid and efficient control.

The Incidence of Violence in the Lives of Pregnant Drug-Dependent Women

Dianne O'Malley Regan, Betty Leifer, and Loretta P. Finnegan

Several factors have been shown to be associated with the occurrence of child abuse. They include: 1) parental drug and/or alcohol abuse, 2) extreme poverty, 3) chaotic life style, 4) abuse experienced by the parents when they were children, and 5) violence between the parents. Family Center (FC) is a multidisciplinary outpatient program providing methadone maintenance along with comprehensive medical and psychosocial services to pregnant drug-dependent women. Many of the women are poor and have experienced much chaos in their lives. They may, therefore, be considered to be at high risk for child abuse. To determine the levels of violence in the lives of these women compared to a control group of women, a questionnaire was developed and administered to: 1) a sample of 95 FC women, and 2) a matched sample of 69 non-drug-abusing women enrolled in our prenatal clinic. Analysis of the questionnaire responses showed that compared to control women, FC patients have experienced, and continue to experience, significantly more violence in their lives. Specifically, a greater proportion of the drug-dependent women reported having been beaten as children (27% vs. 14%, $p < .05$). Mothers were most often identified as the perpetrator of the beatings. Also, the experience of having been beaten as an adult (usually by the husband or partner) was reported by a significantly greater proportion of FC patients than control, women (73% vs. 20%, $p < .001$). Finally, there was a significantly difference between the proportion of patients in FC (29%) and the proportion of those in the control group (6%) reporting the experience of childhood rape or sexual molestation ($p < .001$). The high incidence of violent experience, both sexual and non-sexual, among FC women appears particularly critical considering that this group may already be at risk for child abuse according to other criteria. Evidence that abuse tends to be intergenerationally transmitted underscores the need for early intervention.

Cerebral Ventricular Changes in Newborns Exposed to Psychoactive Agents In Utero

Matthew Pasto, Leonard Graziani, Betty Leifer, Sandra Tunis, Theresa Matteucci, and Loretta P. Finnegan

In order to further evaluate the effects of psychoactive drugs taken during gestation on the developing nervous system, ultrasound studies of the brain were obtained in drug-exposed infants with neonatal abstinence and in a group of non-drug-exposed infants at 24 and 72 hours, and 1, 2, and 6 months following birth. The groups were comparable in birthweight, gestational age, Apgar scores, sex, race, and socioeconomic status. Mothers of the drug exposed infants had been maintained on methadone at an average daily dose of 41 mg, and many used unknown quantities of heroin, diazepam or amphetamines during gestation. Ultrasound examinations at 24 and 72 hours and 1 month revealed the presence of very small (slit-like) lateral ventricles in most of the drug-exposed infants but in few of the controls [26 vs. 9 at 24 hours, 30 vs. 5 at 72 hours, ($p < .001$), and 25 vs. 5 at 1 month ($p < .05$)]. The 2-and 6-month images failed to reveal signficant differences between the two groups of infants so that at 2 and 6 months, drug exposed infants were just as likely as controls to have normal lateral ventricles. The results suggest a relationship between slit-like ventricles and the period of neonatal abstinence which appears shortly after birth and is frequently manifested for as long as 6 months. However, the pathogenesis of the abstinence symptomatology was not defined by the current ultrasound studies. Slit-like ventricles may be due to a lack of visualization of fluid space within the ventricles, a diffuse compression of the ventricles bilaterally, or to decreased production or increased reabsorption of cerebrospinal fluid. Since increased intracranial pressure may in part be responsible, fontanel pressure measurements are being performed routinely on study and control infants. Furthermore, on a select number of subjects, we obtained computerized tomography (CT) examinations to determine whether the density of the periventricular white matter was consistent with edema. Results of the pressure measurements to date show that brain pressure is similar in drug-exposed and control infants. The CT examinations have not revealed any localized or generalized cerebral edema.

Depression, Self-Concept, and Violent Experience in Drug Abusing Women and Their Influence Upon Parenting Effectiveness

Dianne O. Regan, Betty Leifer, and Loretta P. Finnegan

Perinatal management of drug-dependent women is a challenge to health professionals due to the frequency with which the women experience psychiatric, psychological, and social difficulties. In an effort to provide comprehensive services and outpatient methadone maintenance for the high-risk population of pregnant drug-dependent women, Family Center was established. This investigation was aimed at describing levels of depression and self-esteem among women enrolled in Family Center, their experiences of violence, and the possible relationship of each to parenting effectiveness. To assess depression in a group of drug-free control women (n=26), the Beck Depression Inventory was administered to both groups. Results revealed that the drug-dependent women showed significantly more depression and that higher levels of depression were reported by drug-dependent women whose children had been referred to a child welfare agency. Additionally, levels of self-esteem in a group of drug-dependent women (n=28) were measured with the Beck Self Concept Test and compared with levels in a group of pregnant control women (n=29). Results revealed that the Family Center women had significantly lower self-esteem ($p < .01$); and for this group, self-concept was negatively related to parity ($p < .05$). Finally, a questionnaire on violence was developed and administered to drug-dependent women (n=95) and controls (n=69) to identify the number of past and present violent experiences. Significantly more of the drug dependent women had been beaten as children ($p < .05$) and as adults ($p < .001$). Also, significantly more Family Center women reported that they had been raped as adults ($p < .001$) and/or sexually molested as children ($p < .001$).

The results of this study demonstrate that, in addition to having problems directly associated with drug addiction, our sample of drug-dependent women have certain psychological and social difficulties compared to drug-free pregnant women. Because depression, low self-esteem, and the experience of violence have all been linked to problems in parenting, clinical intervention should address these issues to assure the best possible outcome for the drug-dependent woman and her children.

The Effects of RO15-1788, Alone and in Combination With Diazepam, on Ethanol-Induced Loss of Righting Reflex in Rats

B. A. Regan and D. E. Clody

ABSTRACT

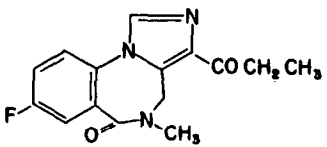
The depressant effects and motor impairments observed after treatment with either benzodiazepines or ethanol are synergistic. Although fatalities due to diazepam are rare, combining diazepam with alcohol can increase the potential for toxic effects.

RO15-1788, an imidazobenzodiazepine, is a novel and selective inhibitor of benzodiazepine binding. In vivo, RO15-1788 is relatively devoid of intrinsic pharmacological activity, yet it antagonizes many of the behavioral and pharmacological effects typically induced by the benzodiazepines. These studies were designed to determine the effects of RO15-1788, alone and in combination with diazepam, on ethanol-induced loss of righting reflex in rats (see Figure 1 for structures).

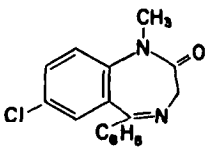
Rats were treated with diazepam alone (4 mg/kg p.o.) or with diazepam followed by RO15-1788 (20 mg/kg p.o.) 45 minutes later. Sixty minutes after diazepam, ethanol was administered, (3.2 g/kg i.p., the dose estimated to cause loss of righting reflex in at least 70% of the rats, see Figure 2), and the rats were then observed continuously for the incidence and duration of loss of righting (rat remained in supine position on a hard horizontal surface for at least 1 minute).

Diazepam alone caused a two-fold increase in the duration of ethanol-induced loss of righting in rats; this effect was reversed by the subsequent administration of RO15-1788. RO15-1788 alone did not exert significant effects on righting reflex. The results of this study, shown in Figure 3, suggest that RO15-1788 may be clinically useful in treating the depressant effects observed after acute overdosage with a combination of diazepam and ethanol.

FIGURE 1



Ro15-1788



DIAZEPAM

FIGURE 2

INCIDENCE OF ETHANOL INDUCED LOSS OF RIGHTING REFLEX (LRR) AND ACUTE LETHALITY IN RATS

Dose Ethanol g/kg i.p.	Exhibiting LRR[1] Treated	Dead Treated
6.4	8/8	7/8
4.8	7/8	6/8
3.2	24/32	4/32
1.6	0/8	0/8

ED_{50}[2] g/kg i.p. 2.8 (95% confidence limits 2.4-3.2)

LD_{50}[2] g/kg i.p. 4.4 (95% confidence limits 3.7-5.3)

[1]within 24hrs of treatment

[2]colculoted according to linear arc-sine transformation method of Finney

FIGURE 3

EFFECTS ON ETHANOL INDUCED LOSS OF RIGHTING REFLEX (LRR) IN RATS

Treatment (mg/kg p.o.)	Exhibiting LRR / Treated	$\bar{x}$ Duration ± SD (minutes)	Change from Control
Control	7/10	47.3 ± 23.9	–
Diazepam (4)[1]	8/10	98.6 ± 44.3[4]	+ 109 %
Ro15-1788 (20)[2]	7/10	32.3 ± 20.8	– 31 %
Diazepam (4) + Ro15-1788 (20)[3]	8/10	49.9 ± 36.9	+ 1%

[1] administered 60min prior to ethanol (3.2 g/kg i:p.)

[2] administered 15 min prior lo ethanol

[3] 45min between treatments

[4] statistically different from control ($p \leq 0.05$), two-tailed Student's t test

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Guanabenz Acetate: A New, Long-Acting Alpha-Two Adrenergic Agonist for Opioid Withdrawal

Forest S. Tennant, Jr., and Richard A. Rawson

ABSTRACT

Guanabenz Acetate (GA) is a new long-lasting alpha-two agonist. We found that it effectively suppressed opioid withdrawal in the majority of 47 opioid-dependent subjects. GA was usually given in twice per day dosages and did not appear to have as many side effects as clonidine. It may have greater acceptance among heroin addicts than clonidine.

INTRODUCTION

Guanabenz acetate (GA) is a new, long-acting alpha-two agonist with antihypertensive effects similar to clonidine hydrochloride. In addition, to its use in hypertension, clonidine has proved to be an effective agent for opioid withdrawal. Since clonidine is not an opioid and essentially has no abuse potential, its development for opioid withdrawal represents a significant clinical advance, and its use in opioid withdrawal has rapidly spread in the United States and abroad. When used in opioid withdrawal, however, clonidine may precipitate severe hypotension, and its common side-effects of lethargy and weakness have significantly limited its use, particularly in outpatient settings.[5-7] This study was undertaken to determine if GA can suppress opioid withdrawal while possibly avoiding some of clonidine's side effects.

METHODS

Forty-seven (47) subjects dependent upon the opioids, heroin (N=41) or methadone (N=6), were admitted to this pilot study and were administered GA. Heroin dependence was documented by a self-reported history of a minimum of three intravenous injections per day for at least six months; urine containing morphine; presence of fresh needle marks; and signs of opioid withdrawal. Methadone-dependent persons were on a standard methadone maintenance program; had a daily maintenance dose of 30 mg or less; and desired to withdraw to a drug-free state.

Subjects were informed they had to attend the clinic daily and would receive GA for a maximum of 14 days. The beginning dose of GA was 4 mg administered twice per day for a 24-hour total of 8 mg. Maximum dosage allowed was 4 mg administered four times per day for a 24-hour total of 16 mg.

On each day of attendance, subjects were assessed for withdrawal symptoms, side-effects, and blood pressure. A daily opioid withdrawal score was determined by assigning a numerical score of 0 for absent, +1- for mild, +2 for moderate, and +3 for severe to the following signs and symptoms: diaphoresis; rhinnorhea; lacrimation; yawning; piloerection; myalgia/arthralgia; chills; nausea/vomiting; insomnia; and anorexia. Each day, subjects were asked if they were experiencing any of 38 side effects including amnesia, blurred vision, constipation, delirium, dizziness, drowsiness, dry mouth, dysphoria, edema, hallucinations, headaches, lethargy, nocturia, numbness, photophobia, polyuria, sedation, tremor, tinnitus, and weakness. In addition, subjects were asked if they felt GA suppressed withdrawal; produced euphoria or a "high"; if it had a narcotic "feel," and if they had used heroin in the previous 24 hours. Urine samples were collected every other day and analyzed for the presence of morphine. Subjects were given the option to discontinue GA at any time, or enter a standard methadone or propoxyphene napsylate detoxification program.

RESULTS

Subjects were a very experienced group of opioid addicts as indicated by demographic and drug use characteristics listed in Table One. Forty (40) of 47 (85.1%) subjects returned for more than one clinic visit, and 33 of 47 (70.2%) reported that GA suppressed withdrawal (Table Two). Compared to a maximum of 30 possible points, withdrawal scores were very low. Mean scores were as follows: Day one, 5.3; Day two, 7.7; Day three, 5.6; Day five, 4.8. Only one (2.1%) subject reported that GA produced a "high" or "felt" like a narcotic. Five (5; 10.6%) chose to take GA for the maximum allowed time of 14 days. Mean retention was 5.0 days. Although 14 (29.8%) reported they ceased heroin use during GA administration, only five (5; 10.6%) demonstrated complete opibid withdrawal by producing a urine specimen devoid of morphine.

The beginning dose of 4 mg given twice per day was satisfactory for all but nine (9; 19.1%) subjects. Six (6; 12.8%) were given 4 mg three times per day for a total 24-hour dosage of 12 mg, and three (3; 6.4%) subjects requested a 24-hour dose of 16 mg.

One methadone maintenance patient elected to stop GA and return to methadone maintenance. The other five (5; 10.6%) dropped out of treatment within five days following admission. Seven (7; 14.9%) of the subjects elected to switch to standard methadone or propoxyphene napsylate detoxification during the study

period. No subject reduced either systolic or diastolic blood pressure by as much as 10 mm Hg during the study. Mean systolic blood pressures were as follows:

Day One - 115.0 mm Hg Day Three - 120.0 mm Hg
Day Five - 117.2 mm Hg Day Ten - 112.4 mm Hg

Mean Diastolic blood pressures were:

Day One - 74.5 mm Hg Day Three - 75.4 mm Hg
Day Five - 76.0 mm Hg Day Ten - 73.2 mm Hg

The major reported side-effects were dry mouth, lethargy, and weakness.

(Table Two)

DISCUSSION

Alpha-two agonists, including clonidine and lofexidine, apparently block opioid withdrawal by suppressing the major noradenergic nucleus, the locus coeruleus. The effects of electrical or pharmacological activation of this nucleus produce changes that resemble those of opiate withdrawal.[4,9] Clonidine has been shown in double-blind, placebo-controlled studies to suppress withdrawal, so it, therefore, has proved to be an effective opiate withdrawal agent.[3-5]

GA, a new, long-acting alpha-two agonist which has a longer half-life than clonidine, appeared to have some effectiveness in treating opioid withdrawal. In this pilot study, 40 of 47 (85.2%) stated that GA was effective in suppressing withdrawal symptoms. Withdrawal scores were low in those subjects who remained in the study. Several reports have shown that ineffective drug-dependence treatments invariably result in failure of patients to return to an outpatient setting, so the demonstration of return visits should be regarded as a measure of patient acceptance.[11,12] Subjects did not report that GA produces euphoria or feels like a narcotic, so GA's abuse potential is probably negligible.

GA appears to have some clinical advantages over clonidine. It has a half-life of 6 to 12 hours, and the majority of our subjects (38, 80.9%) required only a dose of 4 mg twice'per day (8 mg total in 24 hours) to adequately suppress withdrawal. In outpatients, .1 to .2 mg of clonidine must usually be administered four times per day to suppress opiate withdrawal, and this dose frequently causes hypotension and a degree of lethargy and weakness that causes many patients to shun treatment.[5-7,10] have recently reported on over 200 opioid-dependent outpatients we treated with clonidine.[6,7] Approximately 40% developed such severe lethargy that they refused to take clonidine at all, and we have not succeeded in getting more than 5% of patients to take clonidine for 14 days. In particular, we have not been

successful in promoting acceptance of clonidine in heroin addicts and have had to relegate its use almost entirely to patients who wish to withdraw from methadone maintenance.[6,7,10] The most common outpatient use we have found for clonidine is for transition from methadone to naltrexone maintenance.[10] Although the GA subjects reported here represent a relatively small number of subjects treated on a non-blind basis, there was lower incidence of lethargy and hypotension than that we have observed with clonidine. GA also was accepted by heroin addicts and many referred friends to the study. We have not observed clonidine to be a detoxification agent which heroin addicts prefer when methadone maintenance patients we attempted to withdraw either returned to methadone or dropped out of the study within five days. It is possible that clonidine and GA may have subtle pharmacologic differences that make the former more suitable for methadone and GA superior for heroin withdrawal. Superiority of one compound over another and the specific indications for each, however, must be determined by studies in other settings as well as double-blind comparisons.

GA appeared, in this pilot study, to be a useful withdrawal agent for some opioid-dependent persons. It appears equal or superior to clonidine in that it can usually be given in twice per day doses and has fewer side-effects. The theory that alpha-two agonists effectively treat opiate withdrawal by noradrenergic suppression is supported by data in this study.

ACKNOWLEDGEMENTS

Dr. Jerome Lackner suggested that this study be done and reviewed the manuscript when it was completed.

TABLE ONE
DEMOGRAPHIC AND DRUG USE CHARACTERISTICS
OF STUDY GROUP
N=47

Mean age (yrs)	29.5
Caucasian or Hispanic	47 (100%)
Females	12 (25.6%)
Total years heroin use (mean)	8.9
Number of previous treatment attempts (mean)	2.3
Heroin dependence on day of admission	41 (87.2%)
Methadone dependence on day of admission	6 (12.8%)

TABLE TWO
ASSESSMENTS AND OUTCOMES OF GUANABENZ (GA) SUBJECTS
N=47

Number returned for one or more clinic visits	40 (85.1%)
Number reported GA suppressed withdrawal	33 (70.2%)
Number reported GA produces euphoria high	1 (2.1%)
Number reported GA feels like a narcotic	1 (2.1%)
Number completed 14 days	5 (10.6%)
Mean number days in treatment	5
Number reported heroin cessation during treatment	14 (29.8%)
Number submitted morphine-negative urine after admission	5 (10.6%)
Number switched to methadone or propoxyphene detoxification	7 (14.9%)
Reported Side-Effects	13 (27.7%)
Dry Mouth	13 (27.7%)
Lethargy	9 (19.1%)
Weakness	8 (17.0%)
Drowsiness	6 (12.8%)
Headache	5 (10.6%)
Nocturia	5 (10.6%)
Dizziness	3 (6.4%)
Blurred vision	3 (6.4%)
Constipation	3 (6.4%)
Number had over 10 mm Hg drop in systolic blood pressure	0 (0%)

REFERENCES

1. Guanabenz: A New Antihypertensive Drug in The Medical Letter, The Medical Letter, Inc., New Rochelle, NY, 1983: 25: 11-12.

2. Gold, M.S., Redmond, D.E., Jr., Kleber, H.D. Clonidine in Opiate Withdrawal. Lancet 1978; 1: 929-930.

3. Washton, A.M., Resnick, R.B. Clonidine in Opiate Withdrawal: Review and Appraisal of Clinical Finding. Pharmacotherapy 1981; 1: 140-146.

4. Kleber, H.D., Gold, M.S., Riordan, C.E. The Use of Clonidine in Detoxification from Opiates. Bull Narcotics 1980; 32: 1-10.

5. Washton, A.M., Resnick, R.B. Clonidine for Opiate Detoxification: Outpatient Clinical Trials. Am J Psychiatry 1980; 137: 1121-1122.

6. Tennant, F.S., Jr., Rawson, R.A. Clonidine Hydrochloride for Detoxification of Narcotic Addicts: Annual Progress Report for 1981. Calfornia Research Advisory Panel, San Francisco, Calif. 1982.

7. Tennant, F.S., Jr., Rawson, R.A. Clonidine Hydrochloride for Detoxification of Narcotic Addicts: Annual Progress Report for 1982. California Research Advisory Panel, San Francisco, Calif. 1983.

8. Schneider, R.S., Linquist, P., Wong, E.T. et al. Homogeneous Enzyme Immunossay for Opiates in Urine. Clin Chem 1973; 19: 821-825.

9. Gold, M.S., Redmont, D.E., Jr., Kleber, H.D. Noradrenergic Hyperactivity in Opiate Withdrawal Supported by Clonidine Reversal of Opiate Withdrawal. Am J Psychiatry 1978; 136: 100-102.

10. Rawson, R.A., Washton, A.M., Resnick, R.B. et al. Clonidine Hydrochloride Detoxification from Methadone Treatment: The Value of Naltrexone Aftercare. In: Harris, L.S. ed. Problems of Drugs Dependence 1980. National Institute on Drug Abuse Research Monograph 34. Washington, D.C.: Supt. of Docs., U.S. Govt; Print. Off., 1981. pp. 101-108.

11. Anderson, W.H., O'Malley, J.E., Lazare, A. Failure of outpatients treatment of drug abuse, II: amphetamines, barbituates, hallucinogens. Am J Psychiatry 1972; 128: 1572-1576.

12. Tennant, F.S., Jr. Outpatient treatment and outcome of prescription drug abuse. Arch Intern Med 1979; 139: 154-156.

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Opioids Alter Tumor Cell Growth and Differentiation *In Vitro*

Ian S. Zagon and Patricia J. McLaughlin

INTRODUCTION

Heroin is an opioid that is best known for its analgesic and behavioral properties (Jaffe and Martin 1980). Several investigators have also demonstrated that perinatal exposure to heroin affects somatic, as well as neural, growth in both humans (Wilson 1975; Wilson et al. 1979, 1981) and laboratory-animals (Zagon and McLaughlin 1982). Heroin also appears to alter the growth of abnormal neural tissues (Zagon and McLaughlin 1981). Mice inoculated with neuroblastoma and receiving chronic treatment with heroin exhibit retarded tumor development and prolonged survival; these antitumor effects are not apparent when naloxone, a narcotic antagonist, is co-administered with heroin. These findings suggest that opioids, most likely through an interaction at the opiate receptor level, affect growth and development.

The present investigation was designed to explore the effect of heroin on cellular events under in vitro conditions, thus eliminating any confounding influences (e.g., drug metabolism, endorphin release, hormonal interaction) present in an intact biological system. Cultures of murine neuroblastoma cells were subjected to various dosages of heroin and cell proliferation (growth curves, mitotic coefficients) and morphological differentiation (cell! process lengths) were evaluated.

METHOD

Cell cultures. Murine tumor cells, S2OY neuroblastoma, cloned from the A/Jax mouse Cl300 neuroblastoma, were obtained from Dr. M. Nirenberg (NIH, Bethesda, MD); passage numbers 19-22 were utilized. Cells were grown in Falcon plastic flasks (75 cm^2) containing 15 ml Dulbecco's medium with 10% donor calf serum and 0.225% Na_2 CO_3 in a humidified atmosphere of 5% CO_2/95% air at 37°C. Cells were seeded at a density of $5x10^5$ cells per flask. Drugs were added 36 hr post-seeding; culture medium was changed every 24 hr thereafter. Cultures were observed daily by phase microscopy.

Drugs. Diacetylmorphine (heroin) was obtained from the National Institute on Drug Abuse, Bethesda, MD, and naloxone was a gift from Endo Laboratories, Garden City, NY. All drugs were prepared by dissolution in-sterile water. Control cultures received comparable volumes of sterile water.

Cell growth. Cells were harvested by rapping the culture flasks, and centrifuging the homogenate in culture medium. Harvested cells were counted with a hemacytometer at 400X magnification. Cell viability was determined by the trypan blue exclusion test. For each drug dosage at every time point monitored, 3 flasks were sampled. Cells from each drug dosage were counted at 12, 24, 48, and 72 hr after the addition of drug.

Mitotic index. Mitotic indices and assessment of differentiation were conducted on cells grown on 22 mm round coverglasses in 60 x 15 mm plastic tissue culture dishes (Falcon); cells were seeded at $2x10^5$ cells per dish. The coverglasses were fixed with 2 percent glutaraldehyde in 0.07 M Sorenson's phosphate buffer and 3 percent sucrose, stained with hematoxylin, inverted onto slides and mounted. Forty-eight hours after addition of heroin, at least 1000 cells were counted at 400X magnification from 4 or more coverglasses per drug treatment, and the percentage of mitotic cells calculated.

Cell differentiation. The formation of cell processes that were greater in length than the average cell diameter (40 µm) was considered indicative of "morphological differentiation" (Ishii et al 1978; Monard et al. 1973). Cells grown on coverglasses and fixed as described above were stained with cresyl violet, inverted onto slides, and mounted. At 48 hr post drug addition, the number of cells having processes greater than or equal to 40 µm was determined by light microscopy at 400X magnification; cells with multiple processes were only scored once. Cells were counted from fields selected in a random manner; at least 200 cells/coverglass from 3 or more coverglasses per drug treatment were examined.

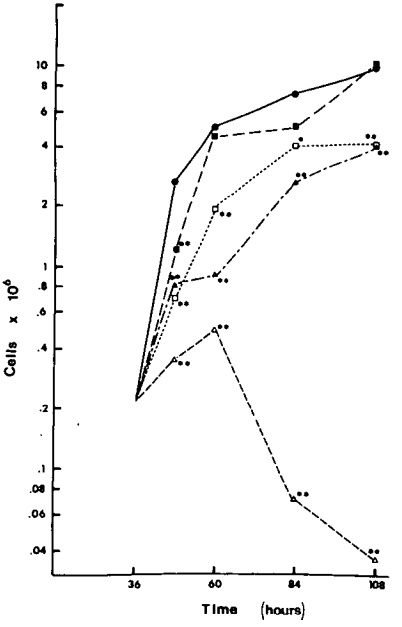
Statistics. The number of live cells attached to the culture flasks, as well as number of mitotic cells, were analyzed using analysis of variance, with subsequent comparisons performed with the Newman-Keuls test. Number of differentiated cells was analyzed using analysis of variance.

RESULTS

Cell growth and differentiation. Fig. 1 shows the effects on growth of neuroblastoma cells when heroin, at concentrations ranging from 10^{-2}M to 10^{-8}M, or sterile water, was added 36 hr after plating. The addition of heroin to cell cultures resulted in a decrease in the number of cells within 12 hr, with cultures receiving 10^{-2}M, 10^{-4}M, 10^{-6}M, and 10^{-8}M heroin having 87, 70, 73, and 48 percent, respectively, fewer cells than control cultures at this time point. Twenty-four hr after receiving drug, the number of cells in the 10-^{8}M heroin cultures was comparable to controls, but a dose-response reduction in cell number was observed for all other heroin-

treated cultures. At 72 hr post-drug administration when the control and 10^{-8}M heroin cultures were confluent, 10^{-6}M, 10^{-4}M, and 10^{-2}M heroin cultures had 54, 56, and 99 percent, respectively, fewer cells. Cytotoxicity, as evidenced by cell death, was only noted in cultures given 10^{-2}M heroin; fewer cells were observed in these cultures at 48 hr and 72 hr post-drug administration than recorded immediately prior to heroin treatment (i.e., at 36 hr).

FIGURE 1



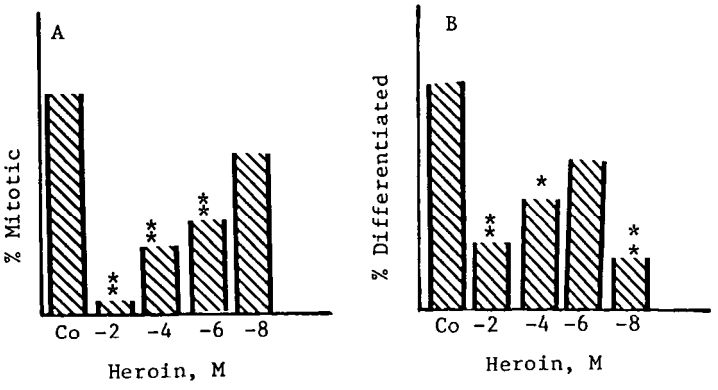
The number of viable S20Y neuroblastoma cells per culture flask plotted as a function of time. Heroin in concentrations of 10^{-8}M(■——■), 10^{-6}M (□····□), 10^{-4}M (▲.–.–▲), 10^{-2}M (△— —△), or sterile water (●_____●) was added to cultures 36 hr after seeding. Significantly different from controlsat $p<0.05$ (*) or $p<0.01$ (**)

The percentage of dividing cells in control and heroin-exposed cultures is presented in Fig. 2a. At 48 hr after drug treatment, a time of active cell proliferation in control cultures, cell cultures exposed to 10^{-2}M, 10^{-4}M, and 10^{-6}M heroin often had significant reductions in the number of mitotic cells (mitotic indices ranged from less than 1 to 14 percent). Cells treated with 10^{-8}M heroin were comparable to control cultures in mitotic coefficients at 48 hr post-drug administration.

In general, heroin was observed to inhibit differentiation (Fig. 2b), with a subnormal number of differentiated cells noted in the heroin-treated cultures. In contrast to the control cultures at

48 hr in which 25 percent of the cells were differentiated, only 3-6 percent of the cells in the 1^{0-2}M and 10^{-8}M heroin cultures were differentiated. Cultures receiving 10^{-4}M heroin had significantly fewer differentiated cells than controls, whereas 10^{-6}M heroin cultures had a comparable number of differentiated cells as control cultures.

FIGURE 2



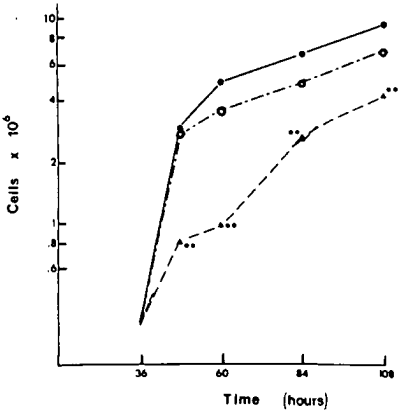
The precentage of mitotic (a) and differentiated (b) cells at 48 hr after addition of heroin in cultures of S2OY neuroblastoma cells. Heroin at concentrations of 10^{-8}M 10^{-6}M 10^{-4}M and 10^{-2}M or sterile water' were added 36 hr after seeding. Significantly different from controls at $p<0.01$ (*).

In order to determine if naloxone, an opiate antagonist, blocks the effects of heroin on cell growth, 36-hr cultures of neuroblastoma were treated with solutions of either 10^{-4}M heroin, 10^{-4}M heroin and 10^{-4}M naloxone, or sterile water. Cell cultures exposed to heroin alone had fewer cells than were present in control cultures at nearly all time points (Fig. 3). However, neuroblastoma cell cultures exposed to both heroin and naloxone were comparable to control cultures in growth.

DISCUSSION

The results of the present study confirm and extend previous reports (e.g., Willson et al. 1976; Simon 1971; Corssen and Skora 1964; North-Root et al. 1976) that opiate treatment of neural and non-neural cells and tissues maintained under in vitro conditions alters growth processes. As evidenced by growth curves and mitotic coefficients, heroin appears to exert an inhibitory effect on cell division of S2OY neuroblastoma cells that is dose-response in nature. At high drug dosages, cell death may even occur, since the number of cells recorded at 48 hr and 72 hr after addition of 10^{-2}M

FIGURE 3



The number of viable S20Y neuroblastoma cells per 75 cm culture flask plotted as a function of time. The cells were grown the presence of 10^{-4}M heroin(▲——▲), 10^{-4}M heroin and 10^{-4}M naloxone (o-.-.-o), or sterile water(●——●) Drugs were added 36 hr after seeding. Significantly different from controls at $p<.01$ (*).

heroin decreased below pre-drug levels.

Although an inhibition of cell proliferation is often accompanied by an increase in morphological differentiation (e.g., Prasad 1971; Bear and Schneider 1976), this did not appear to be the case with heroin. In the present investigation, heroin-exposed cultures that were growth retarded usually contained significantly fewer differentiated. cells relative to control cultures. Moreover, our results show that cell differentiation may be a more sensitive indicator of heroin's actions on cell function than growth inhibition, since cultures of neuroblastoma cells exposed to dosages of heroin that generally caused little growth retardation (i.e., 10^{-8}M) were noted to significantly alter activities related to morphological differentiation.

The present study demonstrates that heroin's action in perturbing cell division and differentiation may be related to certain specific features of opiates because concomitant administration of naloxone, a well-known opiate antagonist, blocked heroin's effects. Since the presumed locus of interaction between naloxone and narcotic drugs is the opiate receptor, and narcotic receptors are known to be present. in S20Y neuroblastoma cells (Klee and Nirenberg 1974), heroin's actions on cell activity appear to be mediated by opiate receptors. These receptors could directly regulate different physiological and biochemical processes related to cell function (e.g., changes in ion concentrations, enzyme activity, or membrane potential). Alternatively, the interaction of heroin with narcotic

receptors may trigger another sequence of events important to cellular integrity.

In an earlier study, we (Zagon and McLaughlin 1981) investigated the effects of heroin on mice inoculated with neuroblastoma. Daily SC injections of heroin, initiated 2 weeks prior to tumor cell inoculation of 10^6 S2OY cells, were observed to prolong median survival time by up to 50%, and to retard tumor growth. Furthermore a number of animals receiving heroin did not develop tumors within the post-inoculation period. Even when heroin treatment was initiated one week after tumor transplantation, a time when tumors were sizeable, at least one dosage (i.e., 6 mg/kg) of heroin did prove effective in retarding tumor growth and prolonging the lifespan of neuroblastoma-bearing animals. Extrapolation of in vitro data to in vivo studies suggests that heroin could be altering the course of neuroblastoma in mice by inhibiting cell proliferation.

Finally, heroin has been found to alter normal growth mechanisms. Children exposed to heroin in early life display a retardation in growth, including being smaller in stature, lighter in weight, and having smaller head circumferences. Laboratory studies in which heroin was chronically administered to pregnant rats also provided similar documentation of growth retardation (Zagon and McLaughlin 1982). The progeny of these heroin-treated mothers had lower birthweights than controls, indicating intrauterine problems in development. During the preweaning period (up to 21 days), these offspring continued to be subnormal in body weight. Thus, from these data as well as those mentioned earlier with our tumor experiments, heroin exerts an extremely potent effect on the growth of both normal and abnormal cells. Moreover, our data would suggest that mechanisms underlying this effect bear a distinct relationship to the opiate receptor.

ACKNOWLEDGMENT

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REFERENCES

Bear, G., and Schneider, F.J. The effect of medium pH on rate of growth, neurite formation and acetylcholinesterase activity in mouse neuroblastoma cells in culture. J Cell Physiol, 91: 63-68, 1976.
Corssen, G.; and Skora, I.A. "Addiction" reactions in cultured human cells. J Am Med Assn, 187:328-332, 1964.
Ishii, D.N., Fibach, E., Yamasaki, H., and Weinstein, I.B. Tumor promoters inhibit morphological differentiation in cultured mouse neuroblastoma cells. Science, 200:556-559, 1978.
Jaffe, E,H., and Martin, W.R. Narcotic Drugs. In: Goodman, L.S., and Gilman, A., eds. The Pharmacological Basis for Therapeutics. New York: MacMillan Publishing Co., 1980. pp. 494-535.
Klee, W.A., and Nirenberg, M. A neuroblastoma x glioma hybrid cell line with morphine receptors. Proc Natl Acad Sci USA, 71:3474-3477, 1974.

Monard, D., Solomon, F., Rentsch, M., and Gysin, R. Morphological differentiation of neuroblastoma cells in medium supplemented with delipidated serum. Proc Natl Acad Sci USA, 70:1894-1897, 1973.
North-Root, H., Martin, D.W., and Toliver, A.P. Binding of an opiate, levorphanol, to intact neuroblastoma cells in continuous culture. Physiol Chem Physics, 8:221-228, 1976.
Prasad, K.N. X-ray-induced morphological differentiation of mouse neuroblastoma cells in vitro. Nature 234:471-473, 1971.
Simon, E.J. Single cells. In Clouet, D.H., ed. Narcotic Drugs. New York: Plenum Press, 1971. pp. 310-341.
Willson, N.J., Schneider, J.F., Roizin, L., Fleiss, J.F., Rivers, W., and DeMartini, J.E. Effects of methadone hydrochloride on the growth of organotypic cerebellar cultures prepared from methadone-tolerant and control rats. J Pharmac exp Ther, 199:369-374, 1976.
Wilson, G.S. Somatic growth effects of perinatal addiction. Addict Dis, 2:333-345, 1976.
Wilson, G.S., McCreary, R., Kean, J., and Baxter, J.C. The development of preschool children of heroin-addicted mothers: a controlled study. Pediatrics 63:135-141, 1979.
Wilson, G.S., Desmond, M.M., and Wait, R.B. Follow-up of methadone-treated and untreated narcotic-dependent women and their infants: health, developmental and social implications. J Pediat, 98:716-722, 1981.
Zagon, I.S., and McLaughlin, P.J. Heroin prolongs survival time and retards tumor growth in mice with neuroblastoma. Brain Res Bull, 7:25-32, 1981.
Zagon, I.S., and McLaughlin, P.J. The effects of heroin and naloxone on the growth of neuroblastoma tumors in mice. In: Harris, L.S., ed. Problems of Drug Dependence, 1980. National Institute on Drug Abuse Research Monograph 34 DHHS Pub No. (ADM) 81-1058. Washington, D.C.: Supt of Docs., U.S. Govt. Print. Off., 1982. pp 394-398

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Annual Reports

A new format is being introduced this year to present the evaluation data from the testing facilities of the Medical College of Virginia (MCV) and the University of Michigan (UM). After an overall review by Dr. Jacobson and brief introductory presentations from each group, the data on individual compounds has been combined into a single report. NIH numbers only are used.

Certain data come only from one laboratory. Thus, the hotplate and Nilsen data come from Dr. Jacobson's laboratory at the NIH, while the rat infusion and other antinociceptive data come from MCV. The guinea pig ileum, rat vas deferens, and binding data come from UM. The monkey data is labeled separately as to its origin.

We hope this new format will make these annual reports easier to reference and more useful.

Biological Evaluation of Compounds for Their Dependence Liability. VII. Drug Testing Program of the Committee on Problems of Drug Dependence, Inc. (1983)

Arthur E. Jacobson

The Drug Testing Program of the Committee on Problems of Drug Dependence (CPDD) has continued uninterrupted over its ca. 35 years of existence, as an arm of the Committee directed towards the determination of the potency of analgesics and discernment of their dependence potential and abuse liability. The methodology which is utilized, and the actual researchers and affiliated groups, have changed somewhat over these three decades but the data which have been obtained from this Program, published annually in one form or other, stands as a basic framework of knowledge in the field. At the present time, two university groups, as well as my laboratory at the National Institutes of Health (NIH, NIADDK) are sponsored by the CPDD for this effort. Funding, in large measure, comes from the U.S. Government via the National Institute on Drug Abuse (ADAMHA), and from NIH for my laboratory. Details of the procedures for submission of samples from researchers, and of the in vivo and in vitro assays, were described in my 1980 report (Jacobson 1981). The work of the Drug Testing Program was expanded this year to include three other groups of researchers, in order to examine stimulants and depressants.

NEW TESTING PROGRAM - STIMULANTS AND DEPRESSANTS

In order to evaluate contemporary procedures which might be capable of determining the dependence potential and abuse liability of stimulants and depressants, three groups were chosen by the CPDD which had been involved in research in these areas for some time and had previously documented their procedures, These were groups under the direction of Drs. J. Brady and N. Ator in the Department of Psychiatry and Behavioral Sciences at the Johns Hopkins University School of Medicine, Drs. C. R. Schuster and C. Johanson in the Department of Psychiatry at the University of Chicago School of Medicine, and Dr. C. Gorodetzky at the Addiction Research Center of NIDA, in Lexington, Ky. Compounds were submitted to these groups in the same manner as those submitted to the groups which test analgesics, under a code number, with proof of purity (thin layer chromatograph), molecular weight, and some solubility data. Their preliminary reports were summarized at this Annual meeting by Dr. J. Woods, and will be printed in this Annual Report. The CPDD has

recommended that the work continue in 1984. Eventually, the submission of samples to these groups will be handled through the office of the Executive Secretary of the CPDD. The expansion of the program to include samples submitted by researchers interested in obtaining these data will be considered by the CPDD at the 1984 Annual Meeting, if the procedures prove to be useful for these purposes. A joint meeting of the Stimulant/Depressant Testing groups was held in April at the University of Chicago for a discussion of the results which had been obtained. Dr. H. Sorer, from NIDA, attended this meeting. Dr. Sorer's involvement in the Drug Testing Program has served to bring to the attention of NIDA management the relationship between the CPDD's program and NIDA's grants/contract programs in these biomedical areas.

PRECLINICAL DRUG TESTING PROGRAM ON ANALGESICS

The three involved groups, Drs. L. Harris, M. Aceto, and E. May in the Department of Pharmacology of the Medical College of Virginia (MCV), Drs. J. Woods, G. Winger, F. Medzihradsky, C. Smith, and J. Katz in the Department of Pharmacology of the University of Michigan (UM), School of Medicine, and myself at NIH, met with the NIDA representative, Dr. H. Sorer, prior to both the interim and annual meeting of the CPDD for general discussion of the program. A jointly sponsored paper, on the pharmacology of C-homobenzomorphans, is in the process of revision for the Japanese Journal of Pharmacology. Another paper, on the pharmacology of various peptides, is under consideration for publication by these groups in 1984. Two papers have previously appeared as the result of our joint collaborative effort (Woods et al. 1983, Jacobson et al. 1982).

NEW FORMAT FOR ANNUAL REPORT FROM UM/MCV

Individual Annual Reports from MCV and UM will continue to be distributed at the Annual Meeting of the CPDD. However, in order to better utilize the data which have been obtained from MCV and UM, it was decided to combine both reports into a single report for publication in the Proceedings. Thus, rather than seeking data on individual compounds in the separate reports, these data will be more readily observed from their combination. Effort will be made to identify the source of the data. All compounds will now be identified through a single number, the NIH number assigned to the compound, rather than through three separate numbering systems (i.e. - UM, MCV and NIH numbers). Indexing of this combined report in the Proceedings will be through the NIH number. The combined report will, as well as aid the individual perusing this literature, save considerable space in the printed volume, since antinociceptive data need only be stated once for the compound, and only one molecular structure will need to be listed rather than the duplication which was necessary for separate reports.

The combined MCV/UM report will list compounds in, more-or-less, random order. A more helpful ordering of them for medicinal chemists would be by classical molecular structural types (e.g.- morphinans, 4,5-epoxymorphinans, benzomorphans, pethidines, etc.).

Tables 2 through 7 present the data of the combined MCV/UM annual report in that classical framework. A highly abbreviated summary of the biological data which have been obtained is included for each of the compounds in the tables so that the reader can obtain an impression of the characteristics of these compounds compared with other members in that molecular class.

The Committee is indebted to the scientists at MCV and UM for the continued excellence of their work and for their major contribution to the field of analgesics represented by their Annual Report.

ORIGIN AND NUMBER OF SAMPLES SUBMITTED FOR EVALUATION -

The origin of the compounds examined by the Drug Testing Program has been classified into seven sources in table 1. It can be seen in that table that over 50% of the samples came from U.S. and foreign universities. About 65% of the university samples were from the U.S. The university samples combined with those from NIH constitute over 75% of the total number of submissions this year. Quite obviously, far fewer compounds are being sent by either U.S. or foreign pharmaceutical industries this year and last year than during. the previous three years. It is difficult to know whether this trend will be reversed in the future, since annual fluctuation in the percentage of submissions from each of the groups noted in table 1 has been the norm, and is to be expected. However, the major drop in the submission of samples from industrial sources seen over the past four years may be a cause of future concern to the program. The diminution of compounds sent to us by industry is only partially reflected in the number of compounds sent to MCV/UM in 1982-1983 and is just fortuitously mirrored by the reports to the CPDD from MCV/UM. The World Health Organization was responsible for our examination of one compound this year.

TABLE 1 - DRUG STATISTICS

	5/1/78-4/30/79	5/1/79-4/30/80	5/1/80-4/30/81	5/1/81-4/30/82	5/1/82-4/30/83	MEAN
COMPOUNDS RECEIVED AT NIH (FOR ALL PURPOSES)	124	121	131	137	174	137
NUMBER OF COMPOUNDS SENT TO MCV/UM[a]	28/26=54	51/50=101	67/81=148	43/59=102	54/37=91	49/51=99
NUMBER OF COMPOUNDS SUBMITTED FOR SDS TO MCV/UM[a]	22/16=38	26/23=49	28/25=53	19/18=37	28/25=53	25/21=56
REPORTS TO CPDD FROM MCV/UM[b]	32/48=80	41/43=84	51/52=103	77/51=128	47/28=75	48/44=93
SOURCE OF COMPOUNDS (%):						
U.S. INDUSTRY	25	41	28	8	12	23
FOREIGN INDUSTRY	6	22	18	0	8-10[c]	11
U.S. UNIVERSITIES	45	30	14	37	36	32
FOREIGN UNIVERSITIES	6	4	27	15	20	14
NATIONAL INSTITUTES OF HEALTH/ (CPDD)[d]	18	1	12	38	10 (10)[d]	16
DRUG ENFORCEMENT ADMINISTRATION	-	1	1	2	-	1
WORLD HEALTH ORGANIZATION	-	2	-	-	0-2[c]	1

a) Not necessarily reported in the Proceedings in the year denoted by the heading. The same compound may be sent to both MCV and UM, for different or duplicate assays.
b) Included in the Proceedings for the denoted year.
c) Sample sent directly from industry at request of W.H.O.
d) CPDD samples are reference compounds which serve to corroborate former work or add to data base.

MOLECULAR CLASSES OF EXAMINED COMPOUNDS

The epoxymorphinans were the most numerous of the various types of examined compounds. In table 2 and 3, the new compounds are shown to be amides at C-14 (NIH 9915-9917), quaternary amines of specific isomeric structure (NIH 9836-9837,9991-9995, C-6 substituted oximes and hydrazines (NIH 10001, 10003-10005, 10008) , and a C-5 methyl substituted endoethanooripavine (NIH 10064). The p-tolyl substituted N-ally1 group appeared to confer narcotic antagonist activity on NIH 9916. A p-chlorophenyl substituted N-allyl (NIH 9917) did not have that effect. Among the quaternary amines, only NIH 9994 appeared to have antinociceptive effect, as well as some narcotic antagonist activity. Its stereoisomer, NIH 9991, had no effect.

A limited nunber of morphinans, and only one homobenzomorphan, were examined this year. The new morphinans, as shown in table 4, had methoxy groups at C-4 (NIH 9959, 9997, and 10016), and an aromatic unsubstituted 14-hydroxymorphinan (NIH 10007) was also examined. The homobenzomorphan (NIH 9896) did not appear to act through opioid receptors.

The benzomorphans are shown in table 5. Enantiomeric compounds with disparate groups substituted on nitrogen were examined (NIH 10019, 10097-10098, 10167-10168). NIH 10168 was a fairly potent narcotic antagonist.

In table 6, the hydroxy-substituted phenylmorphans (NIH 10006 & 10154) showed considerable stereospecificity. The more potent isomer was morphine-like in SDS in monkeys.

The oxide-bridged decahydroisoquinoline in table 7 was one of the most interesting compounds examined this year. Although morphine-like in potency as an antinociceptive, it had little effect in GPI and VD, and that effect in the tissue preparations was not antagonizable. However, the compound binds to opioid receptors in RBH with high affinity. It did not appear to be morphine-like in SDS and was a potent narcotic antagonist.

ABBREVIATIONS USED IN TABLES 2 - 7

Antinociceptive assay (ED50, sc injection, mice) [Confidence limits are listed in MCV/UM report]: HP = hot plate; N = Nilsen; PPQ = phenylquinone; TF = tail flick; TFA = tail flick antagonism vs. morphine.

I = inactive, without a reasonable dose-response relationship, or insufficiently active for statistical analysis.

Receptor Binding Affinities:

RBH (EC50 by displacement of 0.5nM H-etorphine) = binding affinity, without sodium, to rat cerebrum membrane preparations, in nM (parenthesized nunber is ratio of +Na/-Na). The EC50 of morphine, for comparison = 14.0 (1.69). NE = no effect.

GPI = electrically stimulated guinea pig ilem EC50. E = x10 (parenthesized numbers are maximum percent inhibition at EC50); [bracketed, letters: A = antagonized by 10-7M naltrexone; NA q not antagonized by naltrexone; NE = no inhibition of twitch].

VD = electrically stimulated mouse vas deferens EC50 values. E = x10 (parenthesized numbers are maximun percent inhibition at EC50); [bracketed letters: A = antagonized by 10^{-8}M naltrexone; NA = not antagonized by naltrexone; NE = no inhibition of twitch; SA = slight antagonism by naltrexone]

Data From Monkey Colonies:

SDS = single dose suppression: NS = no suppression; CS = complete suppression; PS = partial suppression. (Parenthesized numbers = dose range studied, in mg/kg) . Potency comparison with morphine [M] may be stated, in brackets.

NW = studies in non-withdrawn monkeys: PW = precipitated withdrawal at dose levels, in mg/kg, indicated in parenthesis &/or comparison with naloxone [N], in brackets; NP = no precipitation; SP = slight precipitation

Other Studies:

RI = rat infusion: NS = no suppression; CS = complete suppression; PS = partial suppression.

PPD = primary physical dependence.

SA = self-administration : NE = no effect; High = codeine-like; IN = intermediate between saline and codeine; SE = slight effect.

Normal monkeys: M-like = morphine-like effect.

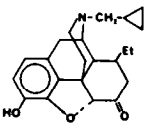
DD = drug discrimination.

The numbers used in the tables may be rounded. For precise values, and details of the procedures, see the MCV/UM report in these Proceedings.

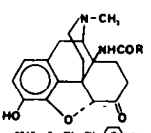
TABLE 2 - 4,5-EPOXYMORPHINANS[a]

7890: R=ALLYL (NALOXONE)

9930: R=CPM (NALTREXONE) (8503)



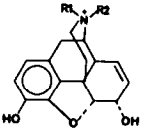
9508



9915: R= CH2-CH2-(Cl)

9916: R= CH=CH-(Me)

9917: R= CH=CH-(Cl)



9836: R1=Me, R2=ALLYL

9837: R1=ALLYL, R2=Me

9991: R1=Me, R2=CPM

9992: R1=Et, R2=Me

9993: R1=Me, R2=Et

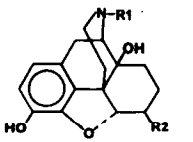
9994: R1=CPM, R2=Me

9995: R1=R2=Me

NIH#	MCV#	UM#	HP	N	PPQ	TF	TFA	RBH	GPI	VD	SDS	NM	OTHER
7890	4261	426	I	I	-	-	TIME COURSE						
9508	4162	1325	16.8	I	-	-	-						SA-SE PPD-LOW
9836	4277	1342	I	I	I	I	I				NS(5.6-17)	MP(5.6)	
9837	4278	1343	I	I	I	I	I				NS(5.6-10)	MP(5.6)	
9915	4255	-	0.3	-	0.2	0.3	I				CS(1)[2xM]		
9916	4256	1317	I	-	1.0	I	1.3	NOT SOLUBLE	NE[b]	6.4E-9(64)[A][b]	NS(10-40)		
9917	4257	-	0.95	-	-	0.5	I				CS(1,2)[6xM]		
(9930 8503	4002	1312 792)	I	-	-	-	TIME COURSE						
9991	4300	1348	I	-	I	I	I				NS(5.6,10)		
9992	4301	-	I	-	I	I	I						
9993	4302	1349	I	-	I	I	I				NS(5.6,10)		
9994	4303	-	I	-	4.3 11.9[c]	I	5.6 19.8[c]				NS(5.6,10)		
9995	4304	1350	I	-	I	I	I				NS(5.6,10)		

a) See text for explanation of abbreviations.
b) DMSO used as solvent. Result uncertain.

TABLE 3 - 4,5-EPOXYMORPHINANS (CONTINUED)[a]



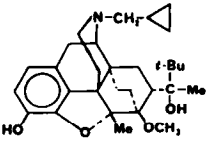
10001: R1=ALLYL, R2= 

10003: R1=CPM, R2= 

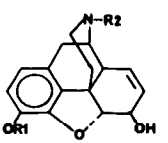
10004: R1=CPM, R2= =NOH

10005: R1=Me, R2= 

10008: R1=Me, R2= =NOH



10064



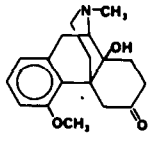
10122: R1=R2=Me (0002, CODEINE)

10124: R1=OH, R2=ALLYL (NALORPHINE)

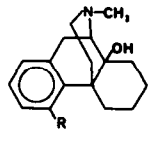
NIH#	MCV#	UM#	HP	N	PPQ	TF	TFA	RBH	GPI	VD	SDS	NM
10001	4308	-	I	I	-	-	TIME COURSE					
10003	4310	1372	I	I	I	I	TIME COURSE	2.3(0.33)	1E-7(95)[NA]	4.7E-6(94)[SA]		
10004	4311	1373	I	I	I	I	TIME COURSE	2.(0.46)	1.4E-5(97)[NA]	2.9E-5(76)[A]		
10005	4312	1374	0.56	0.45	0.1	2.2	I	13.5(1.45)	4.5E-9(79)[A]	4.6E-8(97)[A]		
10008	4314	1377	0.81	2.0	0.05	0.7	I	6.(1.16)	5.2E-9(74)[A]	6.3E-8(98)[A]		
10122 0002	4334 4125	1409	9.3	9.2	1.1	14.5	I	-	-	-	CS(4,8) CS(17)[0.3xM]	
10124 2105	4336	1411	13.8	27.0	I	I	0.5	-	-	-	NS(0.01-0.2) NS(1)	PW(0.1,0.4) PW(0.03-1) [0.1-0.12xN]
10064	4331	-	I	I	7.3	I	I					

a) See text for explanation of abbreviations.

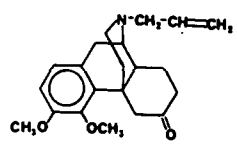
TABLE 4 - MORPHINANS AND HOMOBENZOMORPHANS[a]



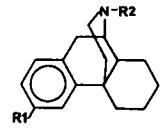
9959



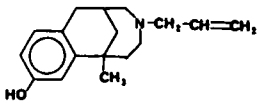
9997: R=OMe
10007: R=H



10016



9996: R1=COMe, R2=CPM
10123: R1=OH, R2=Me (LEVORPHANOL)

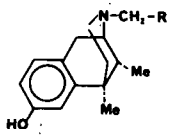


9896: (-)

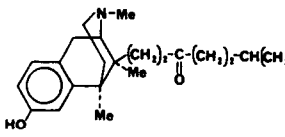
NIH#	MCV#	UM#	HP	N	PPQ	TF	TFA	RBH	GPI	VD	SDS	MW	OTHER
9896	4236	1291	I	I	4.	I	7.	NE	NE	NE	NS(0.13-2.)	MP(0.25-4)	
9959	4305	1340	0.16	-	0.04	0.6	I						
9996	4306	1367	1.3	0.13	0.5	1.3	I	22.4(1.2)	3E-7(45)[A]	5E-8(97)[NA]	PS		SA-IN
9997	-	1368	0.9	2.2	-	-	-	281(1.2)	2E-7(62)[NA]	9E-9(49)[NA]			
10007	4313	1376	4.2	4.2	1.0	4.7	I	661(0.76)	4E-7(63)[A]	5E-7(74)[A]			
10016	4318	1383	10.6	-	1.5	I	4.6	-	-	-	NS(1,3)	PW(1.) [0.01xN]	
10123 4590	4335	1410 510	0.21	0.25	0.05	0.1	I	-	-	-	CS(0.8,1.6)[3xM] CS(1.)[10xM]		

a) See text for explanation of abbreviations.

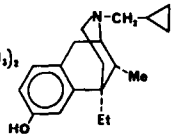
TABLE 5 - BENZOMORPHANS[a]



7958: R= CH=C(Me)$_2$
10019: (+)R= C≡CH
10097: (-)R= (CH$_2$)$_2$-CH(Me)$_2$
10098: (+)R= (CH$_2$)$_2$CH(Me)$_2$
10167: (+)R= CH=CH-Cl
10168: (-)R= CH=CH-Cl



9625

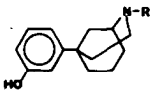


10165 (EXC)

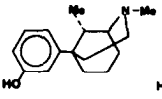
NIH#	MCV#	UM#	HP	N	PPQ	TF	TFA	RBH	GPI	VD	SDS	MW	OTHER
7958	4268	381	9.4	6.5								PS-ATYPICAL (1.25-10)	
9625	4176	1401	1.1										PPD-MILD
10019	4321	-	I	I	I	I	I				PS(1.25-10)		
10097	4332	-	3.1	-	0.9	20.6	I				CS(1.5,3)		
10098	4333	-	I	-	16.3	I	I				NS(3-12)	SP(6-18)	
10165 8848	4348	10165 975	0.09	-	0.04	0.4	I				NS-DEPRESSION (0.03-0.3) PS-CS(0.001-0.1)		NORMAL MONKEYS[b]
10167	4359	-	I	-	I	I	23.9				NS(0.25-0.4)	MP(1-12)	
10168	4360	-	I	-	1.7	I	0.004				NS(0.5-3)	PW(0.002-1) [3xN]	

a) See text for explanation of abbreviations.
b) Effect antagonized by 0.1 and 0.5mg naloxone.

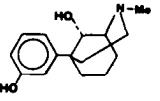
TABLE 6 - PHENYLMORPHANS[a]



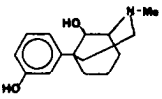
9892: R=Pr
9893: R=Bu



9955: (±)
9971: (-)
9972: (+)



10006

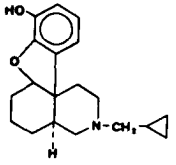


10154

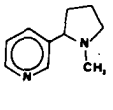
NIH#	MCV#	UM#	HP	N	PPQ	TF	TFA	RBH	GPI	VD	SDS	MM
9892	4262	1306	2.6	-	0.7	12.2	I				NS(1.7-3)	PM(3,5.6)[0.003mM]
9893	4263	1307	1.8	-	0.2	2.2	I				NS(1,3)	PM(0.3-1)[0.017mM]
9955	4275	-	9.3	I	I							
9971	4293	-	10.0	I	3.1	I	I					
9972	4294	-	I	I	I							
10006	4307	-	3.8	2.7	0.2	3.5	I				CS(0.25-16)[1mM]	
10154	4344	-	I	-	8.6	I	I				NS(6-18)	

a) See text for explanation of abbreviations.

TABLE 7 - MISCELLANEOUS[a]



9652



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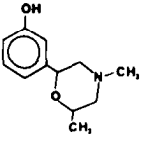

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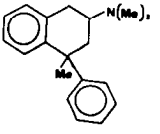
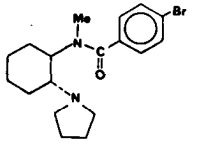
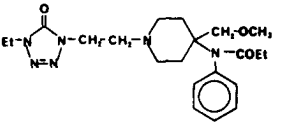


9940: (±)-TRANS



9962



10207 (ALFENTANIL)

NIH#	MCV#	UM#	HP	N	PPQ	TF	TFA	RBH	GPI	VD	SDS	MM	OTHER
9652	4179	1235	1.0	1.7	0.004	I	0.08	1.7(0.75)	2E-5(56)[NA]	2E-7(23)[NA]	NS(1.5-36)	PM[10mM]	
9733	4194	1230	2.2	19.6									PPD
9873	4229	1294	2.1	-	7.9	4.9	I	59(0.92)	3E-7(60)[A]	9E-8(57)[A]	CS(1.5,3)		
9940	4282	1326	I	-	4.8	I	I				NS(5.6,17)		
9962	4290	1345	5.7	-	6.8	3.7	I	2#35(1.96)	1E-7(85)[A]	4E-7(89)[A]	PS(3-12) NS(3-17)	SP(17) NORMALS,[b] MP(3-10) DD,SA	
10207	4374	-	0.04	-	0.1	1.6	I				CS(0.2)		

a) See text for explanation of abbreviations.
b) Morphine-like in normal monkeys (6-12mg/kg); EXC-like in DD; codeine-like to intermediate between codeine and saline in SA.

REFERENCES

Jacobson, A.E. Annual report: Biological evaluation of compounds for their dependence liability. IV. Drug testing program of the Committee on Problems of Drug Dependence, Inc. (1980). In: Harris, L.S. ed. Problems of Drug Dependence: 1980. National -Institute on Drug Abuse Research Monograph 34. Washington, D.C.: Supt. of Docs., U.S. Govt. Print. Off., 1981. pp. 287-296.

Jacobson, A.E., Schmidhammer, H., Hsu, F.-L., Rozwadowska, M.D., Atwell, L., Brossi, A., Aceto, M.D.,, Harris, L.S., Katz, J.L., Woods, J.H., and Medzihradsky, F. Structure-activity relationships of oxygenated morphinans. III. An exploration of the effect of the' aromatic oxygen and 6-keto group on antinociceptive activity, receptor affinity, and narcotic antagonism. In:. Harris, L.S. ed. Problems of Drug-Dependence: 1981. National Institute on Drug Abuse Research Monograph 41. Rockville, MD: The Institute, 1982. pp. 86-92.

Woods, J.H., Young, A.M., Medzihradsky, F., Smith, C.B., Aceto, M.D., Harris, L.S., and Jacobson, A.E. Zomepirac: Preclinical narcotic abuse liability evaluation. Arzheim.-Forsch/Drug Res. 33(1),2:218-222(1983).

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Evaluation of New Compounds for Opioid Activity (1983)

James H. Woods, Gail D. Winger, Fedor Medzihradsky, Charles B. Smith, and Jonathan L. Katz

The evaluation of new compounds by the programs at the University of Michigan and the Medical College of Virginia is coordinated by Dr. Arthur E. Jacobson, Medicinal Chemistry Section, NIADDK, National Institutes of Health, Bethesda, MD. The drugs, which come originally from pharmaceutical companies, universities, and government laboratories, are submitted to Dr. Jacobson, who performs the MOUSE ANALGESIA tests. Values obtained in these tests for some representative opioid drugs are given in Table I.

At the UM and MCV laboratories, drug samples arrive from Dr. Jacobson with only the following information: (1) an identifying NIH number, (2) molecular weight, (3) solubility information and (4) a recommended starting dose. Only after the evaluation is complete and the report submitted back to Dr. Jacobson are the chemical structure and the mouse-analgesia data released to the evaluating laboratory.

DEPENDENCE EVALUATION IN RHESUS MONKEYS

The single-dose suppression test (SDS) determines the ability of a drug to suppress the signs of withdrawal in monkeys which have been made dependent by the chronic administration of morphine (3 mg/kg every six hours). Compounds suspected of having morphine-antagonist properties are tested for their ability to precipitate the withdrawal syndrome in nonwithdrawn (NW) morphine-dependent monkeys. Nondependent monkeys (Normals) are used to determine whether the acute effects of the test drug are reversible by nalorphine or naloxone. In a primary dependence study (PDS), nondependent monkeys receive the test drug every six hours for 30 days to determine whether withdrawal signs will appear when the animals are challenged with an antagonist or when drug administration is discontinued.

Details of these techniques have been presented in the ANNUAL REPORT to the Committee in 1963 (Minutes of the 25th Meeting) by Deneau and Seevers (1963) and by Villarreal (1973).

SELF-ADMINISTRATION BY MONKEYS

Tests of self-administration determine the ability of the drug to maintain responding in monkeys trained to self-inject codeine. Each of at least three monkeys was studied with saline as a negative control and a number of doses of the test compound until a maximum rate of responding was obtained or until, in the absence of evidence of a reinforcing effect, directly observable changes in behavior were produced by the compound.

The schedule of intravenous drug delivery was a fixed-ratio 30; when a light above a lever was illuminated, the 30th response produced a five-second intravenous drug injection accompanied by another light that was illuminated during drug delivery. After each injection, a ten-minute timeout condition was in effect during which responses had no scheduled consequence and neither light was illuminated. Each of the two daily sessions consisted of 13 injections or 130 minutes, whichever occurred first. Other details of the procedure and initial findings with a variety of narcotics are given in previous reports (Woods, 1977; 1980).

Doses of the drugs are typically described in terms of moles/kg/injection (inj), to facilitate direct comparisons among drugs. Duplicate observations of codeine (7.5×10^{-7} mol/kg/inj; 0.32 mg/kg/inj) and of saline were obtained for each monkey. A saline substitution was conducted before and after the series of observations on a test drug; the control rates of codeine-reinforced responding were obtained by a random sampling of two sessions between the drug-substitution sessions. These data are represented in the following graphs with individual symbols for each of the monkeys; each symbol is the mean of duplicate observations for a given dose in each monkey. There are two additional types of averaged data presented. The closed circles indicate the averaged data for observations on the subset of monkeys used to indicate the codeine and saline rates of responding of 20 monkeys studied under the same conditions. The brackets indicate ± 3 standard errors of the codeine mean, and + 3 standard errors of the saline mean for the group of 20 monkeys. In all cases, the rates of responding given are those calculated during only the fixed-ratio portion of each session.

DISPLACEMENT OF STEREOSPECIFIC ^{3}H-ETORPHINE BINDING

Details of the binding assay were described in the 1978 ANNUAL REPORT (Swain et al., 1978). Briefly, aliquots of $_{3}$a membrane preparation from rat cerebrum were incubated with ^{3}H-etorphine in the absence and presence of 150 mM NaCl, and in the presence of different concentrations of the drug under investigation. Stereospecific, i.e., opiate receptor related, interaction of ^{3}H-etorphine was determined as the difference in binding obtained in the presence of an appropriate excess of dextrorphan

TABLE I

MOUSE ANALGESIA. Before submission to the The University of Michigan, all compounds are evaluated for analgesic activity by Or. Arthur E. Jacobson. Shown below are comparative data (ED 50 mg/kg) (95% Confidence Interval) from Hot Plate and Nilsen[d] assays. umol/kg

Compound	HOT PLATE (sc, mg/kg)	HOT PLATE (oral, mg/kg)	NILSEN (sc, mg/kg)	NILSEN (oral, mg/kg)
NIH #	(sc, umol/kg)	(oral, umol/kg)	(sc, umol/kg)	(oral, umol/kg)
Morphine sulfate NIH 0001, 9929	0.98 (0.83-1.1)	6.3 (4.7-8.3)	1.3 (1.0-1.7)	8.3 (6.0-11.4)
	2.9 (2.5-3.3)	18.9 (14.1-24.9)	3.9 (3.0-5.1)	24.9 (18.0-34.1)
Codeine phosphate NIH 0002	6.8 (4.5-10.2)	13.5 (9.7-18.7)	7.4 (4.9-11.0)	14.7 (9.2-23.3)
	17.1 (11.3-25.7)	34.0 (24.4-47.1)	18.6 (12.3-27.7)	37.0 (23.2-58.7)
Levorphanol tartrate NIH 4590	0.2 (0.1-0.3)	-	0.2 (0.16-0.3)	2.5 (1.7-3.7)
	0.5 (0.2-0.7)	-	0.5 (0.4-0.7)	6.2 (4.2-9.1)
Meperidine.HCl NIH 5221	5.3 (4.0-7.1)	-	-	-
	18.7 (14.1-25.0)	-	-	-
(-)-Metazocine.HBr NIH 7569	0.6 (0.5-0.9)	10.6 (8.0-14.1)	0.5 (0.3-0.7)	26.0 (21.0-33.0)
	1.9 (1.4-2.8)	34.1 (25.7-45.3)	1.6 (1.0-2.3)	83.6 (67.5-106.1)

TABLE II Continued

Dihydromorphine.HCl NIH 0123	0.19 (0.15-0.25) 0.6 (0.5-0.8)	0.9 (0.7-1.2) 2.8 (2.2-3.7)	0.2 (0.15-0.3) 0.6 (0.5-0.9)	1.8 (1.5-2.1) 5.6 (4.7-6.5)
Nalorphine.HCl NIH 2105	9.9 (5.7-17.1) 28.4 (16.4-49.1)	- -	23.0 (16.2-32.7) 66.1 (46.6-94.0)	- -
Cyclazocine NIH 7981	1.5 (1.1-2.1) 5.5 (4.1-7.7)	- -	0.1 (0.07-0.16) 0.4 (0.3-0.6)	- -
Pentazocine NIH 7958	9.3 (6.7-12.8) 32.6 (23.5-44.9)	- -	6.5 (4.4-8.8) 22.8 (15.4-30.9)	- -
Naltrexone.HCl NIH 8503	No dose response			
Naloxine.HCl NIH 7890	No dose response			
No antinociceptive activity in hot plate assay: Phenobarbital, amobarbital, diazepam, meprobamate, mescaline, oxazepam, flurazepam.				
Chloropromazine.HCl	1.1 (0.9-1.5) 3.2 (2.4-4.2)			

a) Eddy and Leimbach (1953); b) Jacobson and May (1965); c) Atwell and Jacobson (1978); d) Perrine, Atwell, Tice, Jacobson and May (1972).

and levorphanol, respectively. The potency of the drugs in inhibiting the stereospecific binding of ^{3}H-etorphine was determined from log-probit plots of the data. It Should be noted that since April 1982 the concentration of ^{3}H-etorphine in the binding assay was reduced from 3.0 nM to 0.5 nM, a concentration approaching the K_D of the radiolabeled opiate. This change was implemented in order to let the determined EC50 approximate the true K_i. of a given drug. However, due to the different concentration of the radiolabeled ligand, the EC50s determined since April 1982 are lower than those obtained previously. For the purpose of reference, Table II contains EC50 values of representative opiates determined in binding assays using 0.5 nM ^{3}H-etorphine.

INHIBITION OF TWITCH OF ISOLATED SMOOTH MUSCLE PREPARATIONS

In the past, submitted drugs have been evaluated on two smooth muscle preparations, the details of which were described in the 1978 ANNUAL REPORT (Swain et al., 1978). Shown in the following pages are the EC50's for the tested drug alone, for the drug in the presence of naltrexone (a pure opioid antagonist which is more effective against so-called "mu" agonists than against so-called "kappa" agonists), and for the drug in the presence of UM 979 (an antagonist which appears to be more effective against "kappa" than against "mu" drugs) (Smith, 1978). The maximum depression of the electrically induced twitch in each of the preparations is also indicated. The concentrations of both naltrexone and UM 979 used in tests of antagonism are always 10^{-7}M for the guinea-pig ileum and always 10^{-8}M for the mouse vas deferens. Recently, the drug evaluation procedure has been modified. The guinea-pig ileal preparation has not proven to be reliable and may give false positive results. Therefore, the preparation is only used as a supplementary assay. Drugs are still evaluated on the mouse vas deferens as described previously. There have been small additional modifications in procedure. First, naltrexone, 10^{-7}M, is the only antagonist used. Second, the ability of naltrexone, in an equimolar concentration, to reverse the inhibition of the twitch by active drugs is assessed. Finally, the ability of each drug conducted to reverse the inhibition of the twitch produced by a maximally effective concentration of morphine is measured in order to determine whether the unknown drug has antagonistic activity.

SUMMARY OF TESTS PERFORMED

The compounds which were evaluated at the University of Michigan during the past year and the individual tests which were performed are shown in Table III. Also shown are dates of Annual Reports in which results are reported of earlier tests on those compounds conducted at Michigan.

ACKNOWLEDGMENTS

This work was supported by Grant DA 00254-12 from the National Institute on Drug Abuse and by the Committee on Problems of Drug Dependence, Inc.

TABLE II

EC50 of representative opiates in displacing 0.5 nM ^{3}H-etorphine in a membrane preparation from rat cerebrum

	EC50 (M)		
Compound	-NaCl	+NaCl	+Na/-Na
UM 911	14.6	28.3	1.94
Morphine	14.0	23.6	1.69
Dextrorphan	6180	9820	1.59
UM 1071R	1.14	1.55	1.36
Ketazocine	10.7	14.1	1.32
Ethylketazocine	5.22	6.60	1.26
(-)SKF 10047	4.09	3.93	0.96
Etorphine	0.47	0.37	0.79
(-)Cyclazocine	0.85	0.53	0.63
Naltrexone	1.43	0.63	0.44

NOTE: Binding data for these and other compounds, determined in binding assays using 3.0 nM ^{3}H-etorphine, are included in the 1978 and 1981 ANNUAL Reports.

TABLE III

SUMMARY OF TESTS PERFORMED

NIH	UM	MCV	CHEMICAL CLASS AND/OR GENERAL NAME	SDS	NW	N	SA	GPI	MVD	BIND	PDS
9508	1325	4142	nordihydrocodeinone	-	-	-	1982	-	-	-	-
9625	1401	4176	benzomorphan	-	-	-	-	-	+	-	1982
9652	1319	4179	isoquinoline	-	-	-	-	+	+	-	-
9836	1342	4277	allylmorphinium	+	+	-	-	-	+	-	-
9837	1343	4278	methylnalorphinium	+	+	-	-	-	+	-	-
9873	1294	4229	morpholinyl phenol	-	-	-	-	+	+	+	-
9892	1306	4262	phenylmorphan	+	+	-	-	-	-	+	-
9893	1307	4263	phenylmorphan	+	+	-	-	+	+	+	-
9896	1291	4236	homobenzomorphan	-	-	-	-	+	+	+	-
9916	1317	4256	morphinone	-	-	-	-	+	+	-	-
9940	1326	4282	naphthylamine	+	-	-	-	-	+	+	-
9962	1345	4290	benzamide	+	-	+	+	+	+	+	-
9991	1348	4300	morphinium	+	-	-	-	-	+	-	-
9993	1349	4302	ethylmorphinium	+	-	-	-	-	+	-	-
9995	1350	4304	methylmorphinium	+	-	-	-	-	+	-	-
9996	1367	4306	morphinan	-	-	-	+	+	+	+	-
9997	1368		morphinan	-	-	-	-	+	+	+	-
10003	1372	4310	hydrazinaltrexone	-	-	-	-	+	+	+	-
10004	1373	4311	isonitrosonaltrexone	-	-	-	-	+	+	+	-
10005	1374	4312	hydrazioxymorphone	-	-	-	-	+	+	+	-
10007	1376	4313	morphinan	-	-	-	-	+	+	+	-
10008	1377	4314	isonitroso-oxymorphone	-	-	-	-	+	+	+	-
10016	1383	4318	morphinone	+	+	-	-	-	+	-	-
10122	1409	4334	codeine	+	-	-	-	-	+	-	-
10123	1410	4335	levorphanol	+	-	-	-	-	+	+	-
10124	1411	4336	nalorphine	+	+	-	-	-	+	+	-
10165	-	4348	ethylketocyclazocine	+	-	+	-	-	+	-	-
10207	-	4374	alfentanil	-	-	-	-	-	+	+	-

ACKNOWLEDGMENT: This work was supported by Grant DA 00254-12 from the National Institute on Drug Abuse and by the Committee on Problems of Drug Dependence, Inc.

AUTHORS: James H. Woods, Gail D. Winger, Fedor Medzihradsky, Charles B. Smith, and Jonathan L. Katz, from The Drug Abuse Basic Research Program, Department of Pharmacology, The University of Michigan, Ann Arbor, MI 48109

Dependence Studies of New Compounds in the Rhesus Monkey, Rat, and Mouse (1983)

M. D. Aceto, L. S. Harris, and E. L. May

Technical Assistants

F. Tom Grove, R. F. Jones, and S. M. Tucker

All the test drugs except vasopressin, pentazocine, and the dynorphin fragments were supplied by Dr. Arthur Jacobson, Medicinal Chemistry Section, NIADDK, under the auspices of the Committee on Problems of Drug Dependence, Inc. The chemical structures of the test compounds, excluding SKF 10,047 (-)-Nicotine, vasopressin and the dynorphin fragments, were unknown to us when they were originally submitted.

For the most part, the procedures described by Seevers and his colleagues (1936, 1963) and Deneau (1956) regarding the facilities and training of the monkeys were used and a brief description follows. The monkeys were injected with 3 mg/kg s.c. of morphine sulfate every six hrs for at least 90 days before being used. This dose schedule was reported by Seevers and Deneau (1963) to produce maximal physical dependence.

Modified procedures for the precipitated withdrawal (PPt-W) and single dose suppression (SDS) tests were reported by Aceto and co-workers (1977 and 1978). The PPt-W test was initiated by the injection of a test drug 2 1/2 hrs after an injection of morphine and the animals were observed for signs of withdrawal. The SDS test was started approximately 15 hrs after the last dose of morphine at which time the animals were showing withdrawal signs. The onset and duration of action of the test-drug were noted. In both tests, a vehicle control and an appropriate positive control (naloxone, 0.05 mg/kg or morphine sulfate, 3.0 mg/kg) along with three different treatments (doses) of a test compound were studied. Usually, three or four groups per compound were used. The observer was "blind" with regard to the treatment given. A minimum two-week washout and recuperation period between tests was allowed. In the primary physical dependence (PPD) test, the animals of a group received the drug every four to six hrs for 30-50 days. They were placed in abrupt withdrawal and challenged with naloxone periodically and were observed for signs of physical dependence. All potency estimates are rough approximations only.

The rat-infusion method was reported by Teiger (1974) and certain modifications are indicated as follows. Semi-restrained male, Sprague-Dawley rats were medicated by continuous infusion through indwelling, intraperitoneal cannula for six days with the drugs. Rats were anesthetized and each was fitted with a specially prepared cannula which was passed subcutaneously from the nape of the neck to the lateral side of the lower abdomen and then inserted in the peritoneal cavity. The cannula was anchored at both ends with silk sutures and attached to a flow-through; swivel mechanism which allowed the animal to move about in the cage and eat and drink normally. The swivel was connected to a syringe which was attached to a syringe pump. The animals received 7 to 10 ml of solution every 24 hrs.

In the substitution for morphine (SM) test, the animals first received morphine (50 mg/kg/24 hrs from days 3-6). Then, a test drug was substituted for two days. The morphine controls received an infusion of water. The animals were observed for changes in bodyweight and for behavioral withdrawal signs for one-half hr at 6,24,48,72 and/or 96 hrs after stopping the infusion of morphine.

In the primary physical dependence (PPD) study, the rats received test compound for six days and then were placed in abrupt withdrawal and observed as above.

Table 1

Comparative Data-ED_{50} mg/kg s.c. (95% C.L.) of Selected Standards in 3 Mouse Agonist-Antagonist Tests

Drug	Tail-Flick Test	Tail-Flick Antagonism Test	Phenylquinone Test
Pentazocine	15% at 10.0	18 (12.4-26)	1.65 (1.0-2.5)
Cyclazocine	17% at 1.0[a]	0.03 (0.02-.78)	0.011 (0.0046-0.03)
Nalorphine .HCL	None at 10.0	2.6 (0.69-9.75)	0.6 (0.25-1.44)
Naloxone .HCL	None at 10.0	0.035 (0.010-0.93)	No Activity
Naltrexone .HCL	None at 10.0	0.007 (0.002-0.02)	No Activity
Morphine Sulfate	5.8 (5.7-5.9)	- - -	0.23 (0.20-0.25)

[a]Mice were ataxic at 3.0 and 10.0 mg/kg but no further increase in reaction time was seen.

Three mouse tests were used in our laboratory at the Medical College of Virginia to provide a preliminary estimate of the potency and profile of activity of each *test* compound. The tests were the tail-flick agonist (TF) and the morphine antagonist (TF vs M) tests and the phenylquinone (PPQ) test (Dewey et al., 1970; Dewey and Harris, 1971). Reference-standard data for these tests are shown in table 1. In addition, Dr. Jacobson provided us with estimated starting doses. These doses were based on results obtained from the mouse hot plate (HP) (Eddy and Leimbach, 1953; Jacobson and May, 1965; Atwell and Jacobson, 1978) and Nilsen (N) (Parrine et al., 1972) tests from his laboratory. Reference data for these tests are shown in table 2.

Table 2

Comparative Data (ED_{50} mg/kg s.c.) [95% S.E.] from the Hot Plate and Nilsen Test

Compound	Hot Plate Test		Nilsen Test	
	Subcutaneous	Oral	Subcutaneous	Oral
Morphine Sulfate	0.98(0.83-1.1)	6.3(4.7-8.3)	1.3(1.0-1.7)	8.3(6.0-11.4)
Codeine Phosphate	6.8(4.5-10.2)	13.5(9.7-18.7)	7.4(4.9-11.0)	14.7(9.2-23.3)
Levorphanol Tartrate	0.2(0.1-0.3)	-	0.2(0.16-0.3)	2.5(1.7-3.7)
Meperidine .HCL	5.3(4.0-7.1)	-	-	-
(-)-Metazocine .HBr	0.6(0.5-0.9)	10.6(8.0-14.1)	0.5(0.3-0.7)	26.0(21.0-33.0)
Dihydromorphinone .HCL	0.19(0.15-0.25)	0.9(0.7-1.2)	0.2(0.15-0.3)	1.8(1.5-2.1)
Nalorphine .HCL	9.9(5.7-17.1)	-	23.0(16.2-32.7)	-
Cyclazocine	1.5(1.1-2.1)	-	0.1(0.07-0.16)	-
Pentazocine	9.3(6.7-12.8)	-	6.5(4.4-8.8)	-
Chlorpromazine .HCL	1.1(0.9-1.5)	-	-	

Naloxone .HCL and Naltrexone .HCL, no dose response.

Phenobarbital, Amobarbital, Valium, Oxazepam, Flurazepam, Mepromate and Mescaline are inactive on the hot plate test.

SUMMARY OF COMPOUNDS TESTED[a]

COMPOUND #			CHEMICAL CLASS	MOUSE	RAT	MONKEY
MCV	NIH	UM		TF, TFvsM, PPQ, HP, N	SM, PPD	SDS, PPt-W, PPD
4002	9930	1312	Naltrexone	+ (Time Course Study)		
4179	9652	1319	Isoquinoline	+ + + + +		+ +
4194	9733	1230	(-)-Nicotine			+
4255	9915		Morphinone	+ + + +		+
4256	9916	1317	Morphinone	+ + + +		+
4257	9917		Morphinone	+ + + +		+
4261	7890	426	Naloxone	+ (Time Course Study)		
4262	9892	1306	Phenylmorphan	+ + + +		
4263	9893	1307	Phenylmorphan	+ + + +		
4268	7958	381	Benzomorphan	+ +		+
4275	9955		Phenylmorphan	+ + + + +		
4277	9836	1342	Morphine Quaternary	+ + + + +		
4278	9837	1343	Nalorphine Quaternary	+ + + + +		

a) "+" indicates test results reported in this article

SUMMARY OF COMPOUNDS TESTED[a]

COMPOUND #			CHEMICAL CLASS	MOUSE					RAT	MONKEY
MCV	NIH	UM		TF,	TFvsM,	PPQ,	HP,	N	SM, PPD	SDS, PPt-W, PPD
4290	9962	1345	Benzamide				+			+
4290B	9962	1345	Benzamide							+ (Normal Monkeys)
4293	9971		Phenylmorphan			+	+	+		
4294	9972		Phenylmorphan			+				
4300	9991	1348	Morphine Quaternary	+	+	+	+			
4301	9992		Morphine Quaternary	+	+	+	+			
4302	9993	1349	Morphine Quaternary	+	+	+	+			
4303	9994		Morphine Quaternary	+	+	+	+			
4304	9995	1350	Morphine Quaternary	+	+	+	+			
4306	9996	1367	Morphinan	+	+	+	+	+		+
4307	10006		Phenylmorphan	+	+	+	+	+		+
4308	10001		Hydrazinonaloxone	+	+	+	+	+		
4308	10001		Hydrazinonaloxone		+ (Time Course Study)					

a) "+" indicates test results reported in this article

SUMMARY OF COMPOUNDS TESTED[a]

COMPOUND #			CHEMICAL CLASS	MOUSE	RAT	MONKEY
MCV	NIH	UM		TF, TFvsM, PPQ, HP, N	SM, PPD	SDS, PPt-W, PPD
4310	10003	1372	Hydrazinonaltrexone	+ (Time Course Study)		
4311	10004	1373	Isonitrosonaltrexone	+ (Time Course Study)		
4314	10008	1377	Isonitrosooxymorphone	+ + + + +		+
4321	10019		Benzomorphan	+ + + + +		+
4331	10064		5-Methylbuprenorphine	+ + + + +		
4332	10097		Benzomorphan	+ + + +		+
4333	10098		Benzomorphan	+ + + +		+ +
4334	10122	1409	Codeine	+ + + + +		+
4335	10123	1410	Levorphanol	+ + + + +		+
4336	10124	1411	Nalorphine	+ + + + +		+ +
4344	10159		Phenylmorphan	+ + + +		+
4348	10165		Ethylketocyclazocine	+ + + +		+
4348	8848		Ethylketocyclazocine			+ (Normal Monkeys)

a) "+" indicates test results reported in this article

SUMMARY OF COMPOUNDS TESTED[a]

COMPOUND #			CHEMICAL CLASS	MOUSE	RAT	MONKEY
MCV	NIH	UM		TF, TFvsM, PPQ, HP, N	SM, PPD	SDS, PPt-W, PPD
4353			Dynorphin-(1-13)			+ (i.v.)
4354			Dynorphin-(1-10)			+ (i.v.)
4356			Dynorphin-(1-6)			+ (i.v.)
4357			α-Neoendorphin			+ (i.v.)
4358			Vasopressin Tannate			+ +
4359	10167		Benzomorphan	+ + + +		+ +
4360	10168		Benzomorphan	+ + + +		+ +
4374	10207		Fentanyl	+ + + +		+

a) "+" indicates test results reported in this article

REFERENCES

Aceto, M.D., Flora, R.E., and Harris, L.S. The effects of naloxone and nalorphine during the development of morphine dependence in rhesus monkeys. Pharmacol, 15:1-9, 1977.

Aceto, M.D., Flora, R.E., and Harris, L.S. Caffeine elicited withdrawal signs in morphine-dependent rhesus monkeys. Eur J Pharmacol, 50:203-207, 1978.

Atwell, L., and Jacobson, A.E. The search fo r less harmful analgesics. Lab Animal, 7:42-47, 1978.

Oeneau, G.A. An analysis of factors influencing the development of physical dependence to narcotic analgesics in the rhesus monkey with methods for predicting physical dependence liability in man. Doctoral Dissertation, University of Michigan, 1956.

Dewey, W.L., Harris, L.S., Howes, J.F., and Nuite, J.A. The effects of various neurohumoral modulators on the activity of morphine and the narcotic antagonists in the tail-flick and phenylquinone tests. J Pharmacol Exp Ther, 175:435-442, 1970.

Dewey, W.L., and Harris, L.S. Antinociceptive activity of the narcotic antagonists analogues and antagonistic activity of narcotic analgesics in rodents. J Pharmacol Exp Ther, 179:652-659, 1971.

Dewey, W.L., and Patrick, G.A. Narcotic antagonists in the rat infusion technique. Proc. from the 37th annual meeting, Committee on Problems of Drug Dependence, NRS-NAS, U.S.A. 64-73, 1975.

Jacobson, A.E., and May, E.L. Structures related to morphine, XXI, 2'-Substituted benzomorphans. J Med Chem, 8:563-566, 1965.

Perrine, T.O., Atwell, L., Tice, I.B., Jacobson, A.E., and May, E.L. Analgesic activity as determined by the Nilsen method. J Pharm Sci, 61:86-88, 1972.

Seevers, M.H. Opiate addiction in the monkey. I. Methods of, study. J Pharmacol Exp Ther 56:147-156, 1936.

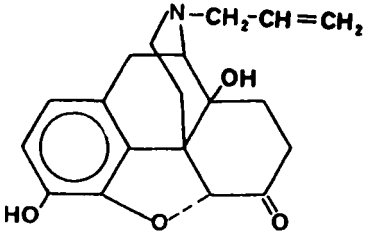
Seevers, M.H., and Oeneau, G.A. Physiological aspects of tolerance and physical dependence. In: Root, W.S. and Hofman, F.G., eds. Physiological Pharmacology, Vol. I. New York: Academic Press, 1963. pp. 565-670.

Teiger, O.G. Induction of physical dependence on morphine, codeine, and meperidine in the rat by continuous infusion, J Pharmacol Exp Ther, 190:408-415, 1974.

REPORT ON INDIVIDUAL COMPOUNDS

1983

NIH 7890. Naloxone hydrochloride.



MOUSE DATA-ED_{50} (95% C.L.) (mg/kg s.c.)

1) TF - Inactive at 10.0

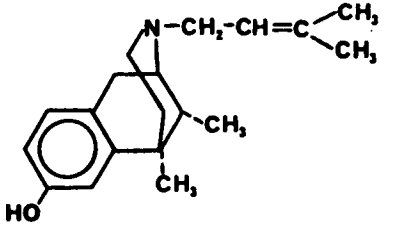
2) TF vs M - 0.035 (0.010 - 0.093

3) PPQ - No activity

4) HP - No dose-response

In this time course study, naloxone at 0.1 mg/kg produced 75% antagonism of the ED_{80} of morphine sulfate in the tail-flick test at 30 min, 60% antagonism at 60 min, and 15% antagonism at 120 min.

NIH 7958. 2'-Hydroxy-5,9-dimethyl-2-(3,3-dimethylallyl)-6,7-benzomorphan hydrochloride (Pentazocine).



NIH 7958 (Con'd)

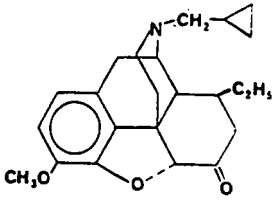
OBSERVATIONS IN THE MORPHINE DEPENDENT MONKEY (MCV)

MONKEY DATA (PPt-W)	# ANIMALS				
	Doses (mg/kg s.c.)	4	3	7	5
		7.5	7.5	5.0	2.5

3	9(Naloxone)	9(H_2O)
1.25	0.05	1.0 ml/kg

Pentazocine is an unusual antagonist. The drug rarely precipiated the withdrawal signs rigid abdominal muscle and vocalizes when abdomen palpated. At 5.0, 7.5 and 10.0 mg/kg it produced ataxia and slowing which are not considered to be withdrawal signs and at 10.0 it produced convulsions in 3/4 animals. Pentobarbital was used to control the convulsions in 2 of the animals. The withdrawal signs designated restless tremor, drowsiness, retching, vomiting, salivation, avoids contact and lying on side or abdomen were noted at the 2 higher doses. This drug could be classified as a partial antagonist. It is about 50 times less active than naloxone.

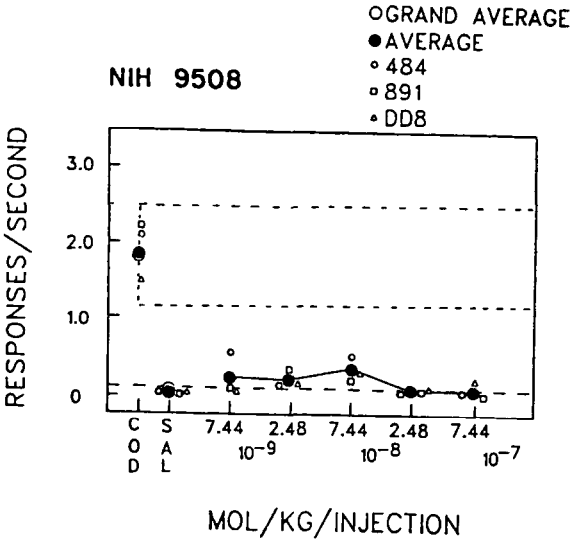
NIH 9508. N-Cyclopropylmethyl-8 beta-ethyl-N-nordihydrocodeinone hydrochloride.



MOUSE ANALGESIA, ED_{50} (mg/kg)

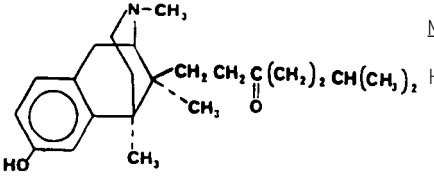
Hot Plate: 16.8 (10.5 - 26.9)
Nilsen: 50% at 100

DRUG SELF-ADMINISTRATION IN RHESUS MONKEYS (UM)



Rates of responding maintained by NIH 9508 were only slightly higher than those maintained by saline at doses of 7.44×10^{-9} and 7.44×10^{-8} mol/kg/inj). At doses of 2.48×10^{-8} and 7.44×10 mol/kg/injection, rates were no higher than those maintained by saline.

NIH 9625. 1-[(2-alpha,6-alpha,11S)-(±)-1-(1,2,3,4,5,6-Hexahydro-8-hydroxy-3,6,11-trimethyl-2,6-methano-3-benzazocin-11-yl]-6-methyl-3-heptanone methanesulfonate.



MOUSE ANALGESIA, ED_{50} (mg/kg)

Hot Plate: 1.1 (0.8 - 1.3)

INHIBITION OF TWITCH OF ELECTRICALLY DRIVEN MOUSE VAS DEFERENS

	EC50	Maximum Response
Drug alone	1.94×10^{-8} M	93.0
After naltrexone	2.60×10^{-7} M	92.7

NIH 9625, 3×10^{-5} M, did not antagonize an equimolar concentration of morphine. A maximally effective concentration of NIH 9625, 10^{-6} M, was reversed by an equimolar concentration of naltrexone.

PRIMARY DEPENDENCE STUDY IN RHESUS MONKEYS (UM)

In studies in withdrawn morphine-dependent monkeys conducted at The Medical College of Virginia, this drug neither suppressed nor exacerbated withdrawal. A primary dependence study was terminated prematurely as subjects were unconscious after injections (See CPDD Proceedings 1980, 81). The present study initially increased dose very slowly in an attempt to allow more tolerance to develop to the sedative effects of the drug.

Subjects. Three rhesus monkeys weighing from 4.4. to 5.5 kg were used. Each monkey lost about 1.0 kg over the 32 days of chronic drug administration.

Dosage Schedule. The drug was dissolved in water.

Day	Dose (mg/kg/6 hr, s.c.)
1	1.0
2	3.0
5	5.6
12	10.0
23	17.0
35	abrupt withdrawal

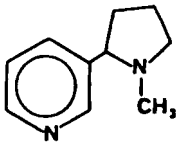
Subjects were tested for precipitation of withdrawal with nalorphine on days 15 and 29, and with naloxone on days 18 and 32.

Acute Effects. There were no obvious effects of 1.0 mg/kg. with the increase in dose to 3.0 mg/kg, there was some pupil dilation, scratching, and decreases in activity and eating.

Chronic Effects. Apparent tolerance developed to effects at 3.0 mg/kg by the third day so the dose was increased to 5.6 mg/kg with little increase in effects. Since the subjects were losing weight, that dose was maintained until the 12th day of the study when it was increased to 10.0 mg/kg. At this dose one monkey (FQ-35) was observed to assume peculiar postures with little muscle tone but could be aroused by observers. All three monkeys lost weight at this dose. Additionally, abscesses developed at injection sites. At 17.0 mg/kg a second monkey showed a loss of muscle tone and was lying on the cage floor. This subject also could be aroused by observers. On the fifth day at 17.0 mg/kg, monkey FQ-35 was found dead one hour after an observation indicating no obvious effects.

Dependence. Nalorphine. administered on days 15 and 30 produced very mild, if any, signs of withdrawal. Naloxone had greater effects than nalorphine. Subjects were observed to be lying on their sides, vocalizing, and restless after naloxone administration on day 33. With the abrupt discontinuation of drug administration, only very mild signs of withdrawal were observed in the two surviving monkeys.

NIH 9733. (-)-Nicotine di-l-tartrate.



MOUSE DATA-ED_{50} (95% C.L.) (mg/kg s.c.)

1) TF - 5.2 (2.7 - 10.0)

2) TF vs M - 1.6 (0.03 - 15.0)

3) PPQ - 1.3 (0.5 - 5.2)

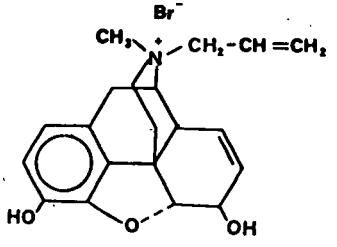
4) HP - 2.2 (1.6 - 3.0)

5) N - 19.6 (13.6 - 28.1)

PRIMARY DEPENDENCE STUDY IN RHESUS MONKEYS (MCV).

This primary physical dependence study in 5 non-tolerant rhesus monkeys was recently completed in which (-)nicotine-di-l-tartrate was administered chronically for 48 days. The treatment regimen included 6 injections per day on weekdays at 6:00 a.m., 9:00 a.m., noon, 3:00 p.m., 6:00 p.m. and midnight. The starting dose was 0.35 mg/kg SC (as base). This dose was gradually escalated with occasional reductions or adjustments, so that the animals received 4.9 mg/kg SC per injection by day 44. Effects were dose-related and when the dose of 3.5 mg/kg was reached, the animals exhibited salivation, lying on side abdomen, fighting, vocalizing, restlessness, drowsiness, wet-dog shakes, scratching, retching, coughing and vomiting. Convulsions were also seen. In spite of the severe effects produced by the chronic administration of nicotine, the animals continued to gain body weight throughout the study. A certain degree of tolerance developed which was quickly lost if the dose was reduced or when the animals were placed in abrupt withdrawal at 15 and 30 days; at the end of the study, few signs were observed. Naloxone (2.0 mg/kg s.c.) did not precipitate withdrawal signs. The animals appeared worse after nicotine administration than when the drug was withdrawn. In fact, the injection of nicotine elicited many signs designated as withdrawal signs. We realize that many mechanisms could account for these signs. Obviously, more studies are required to determine the significance of these results.

NIH 9836. N-Allylmorphinium bromide.



MOUSE DATA-ED_{50} (95% C.L.) (mg/kg s.c.)

1) TF - Inactive at 1.0 and 30.0

2) TF vs M - Inactive at 1.0 and 30.0

NIH 9836 (Cont'd)

3) PPQ - 14% at 1.0 and 30.0

4) HP - 0% at 20.0, 40% at 50.0 and 40% at 100.0

5) N - 0% at 20.0, 50.0, 100.0

INHIBITION OF TWITCH OF ELECTRICALLY DRIVEN MOUSE VAS DEFERENS

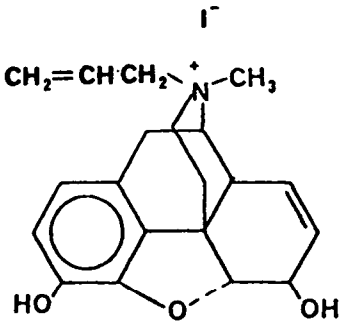
	EC50	Maximum Response
Drug alone	6.35×10^{-7} M	49.6
After naltrexone	8.24×10^{-8} M	37.3

Naltrexone, 3×10^{-5} M, caused a very slight reversal of the inhibition of the twitch caused by an equimolar concentration of morphine.

OBSERVATIONS IN THE MORPHINE-DEPENDENT RHESUS MONKEY (UM)

NIH 9836 failed to produce any effect in withdrawn monkeys (5.6 - 17 mg/kg) or in nonwithdrawn monkeys (5.6 mg/kg).

NIH 9837. N-Methylnalorphinium iodide.



MOUSE DATA-ED_{50} (95% C.L.) (mg/kg s.c.)

1) TF - Inactive at 1.0 and 30.0

2) TF vs M - Inactive at 1.0 and 30.0

3) PPQ - 30% at 1.0 and 38% at 30.0

4) HP - 10% at 20.0, 10% at 50.0 and 20% at 100.0

5) N - 0% at 20.0, 50.0 and 100.0

INHIBITION OF TWITCH OF ELECTRICALLY DRIVEN MOUSE VAS DEFERENS

	EC50	Maximum Response
Drug alone	7.10×10^{-9} M	30.5
After naltrexone	8.10×10^{-8} M	35.5

Naltrexone, in an equimolar concentration, reversed the inhibition produced by a maximally effective concentration of NIH 9837.

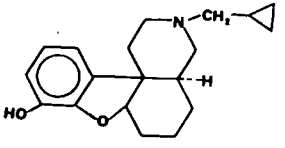
NIH 9837 (Cont'd)

NIH 9837, in an equimolar concentration, reversed the inhibition produced by a maximally effective concentration of morphine, 3 x 10^{-5} M.

OBSERVATIONS IN THE MORPHINE-DEPENDENT RHESUS MONKEY (UM)

NIH 9837 failed to affect either withdrawn (5.6 - 10 mg/kg) or nonwithdrawn monkeys (5.6 mg/kg). Drug supply depleted.

NIH 9652. 3-Cyclopropylmethyl-2,3,4,4a α,5,6,7,7a α-octahydro-1H-benzo[4,5]furyl[3,2-e] isoquinoline-9-01.



MOUSE DATA-ED_{50} (95% C.L.) (mg/kg s.c.)

TF - 26% at 10.0; 21% at 50.0

2) TF vs M - 0.08 (0.02 - 0.41)

3) PPQ - 0.004 (0.001 - 0.10)

4) HP - 1.0 (0.65 - 1.6)

5) N- 1.7 (1.2 - 2.5)

DISPLACEMENT OF STEREOSPECIFIC ^{3}H-ETORPHINE BINDING

NIH 9652, was not studied in the binding assay due to solubility limitations.

INHIBITION OF TWITCH OF ELECTRICALLY DRIVEN GUINEA PIG ILEUM

In concentrations which ranged from 10^{-9} M to 3 x 10^{-4} M, this drug caused only a slight inhibition of the twitch, which was completely antagonized by naltrexone and UM 979. In all preparations, this drug-increased the magnitude of the twitch at concentrations of 10^{-4} M and greater.

INHIBITION OF TWITCH OF ELECTRICALLY DRIVEN MOUSE VAS DEFERENS

	EC50	Maximum Response
	High Affinity	
Drug alone	3.47 x 10^{-7} M	53.6
After naltrexone	8.63 x 10^{-8} M	42.9
After UM 979	1.17 x 10^{-8} M	53.3

NIH 9652 (Cont'd)

	Low Affinity	
Drug alone	2.58×10^{-5} M	70.1
After naltrexone	4.48×10^{-4} M	63.4
After UM 979	5.20×10^{-5} M	73.3

NIH 9652 had a biphasic inhibitory action upon the vas deferens. Both naltrexone and UM 979 slightly antagonized the low affinity, but not the high affinity action of the drug.

COMMENT

Due to the limited solubility of NIH 9652, it was studied using DMSO as solvent for the smooth muscle preparations. Other compounds have not been studied under these conditions.

OBSERVATIONS IN THE MORPHINE-DEPENDENT MONKEY (MCV)

MONKEY DATA	# ANIMALS	1,	2,	1,	2,	3,
A. (SDS)	Doses (mg/kg s.c.)	36.0	18.0	12.0	9.0	6.0

1,	2,	2,	5(Vehicle),	4(Morphine)
4.5	3.0	1.5	1.0 ml/kg	3.0

In the dose range tested, the compound did not substitute for morphine.

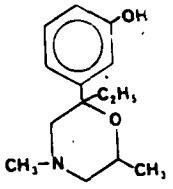
MONKEY DATA	# ANIMALS	1,	2,	2,	2,	1,
(PPt-W)	Doses (mg/kg s.c.)	36.0	18.0	9.0	4.5	2.25

1,	1,	1,	2,	3,	4,	3,	1,
1.125	0.56	0.28	0.14	0.035	0.00875	0.0022	0.0005

8(H_2O + Lactic Acid),	8(Naloxone)
1.0 ml/kg	0.05

Results: NIH 9652 precipitated withdrawal in the dose range 0.00875-36.0 mg/kg. At the 2 higher doses convulsions were seen which were terminated by giving morphine (2 x 2.0 mg/kg) and pentobarbital s.c. (20 mg). Some mild withdrawal signs were seen at 0.00219 mg/kg. The drug appears to be more potent than naloxone by one order of magnitude. Its duration of action is at least 2½ hrs.

NIH 9873. 3-(2-Ethyl-4,6-dimethyl-2-morpholinyl)phenol.



MOUSE ANALGESIA, ED50 (mg/kg)
Hot Plate 2.1 (1.6-2.8)

DISPLACEMENT OF STEREOSPECIFIC ^{3}H-ETORPHINE BINDING

EC50 of 59.1 nM in absence of 150 mM NaCl
EC50 of 54.6 nM in presence of 150 mM NaCl
Sodium response ratio = 0.92

INHIBITION OF TWITCH OF ELECTRICALLY DRIVEN GUINEA PIG ILEUM

	EC50	Maximum Response
Drug alone	2.94×10^{-7} M	59.8

Both naltrexone and U M 979 abolished all responses to NIH 9873. Concentrations of 10^{-4} M and higher caused increases in magnitude of twitch.

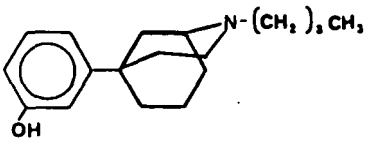
INHIBITION OF TWITCH OF ELECTRICALLY DRIVEN MOUSE VAS DEFERENS

	EC50	Maximum Response
Drug alone	9.49×10^{-8} M	57.3
After naltrexone	7.21×10^{-7} M	42.0
After UM 979	1.72×10^{-6} M	39.4

SUMMARY

Though somewhat less potent, NIH 9873 appears to be morphine-like upon both smooth-muscle preparations. The binding results also indicate the compound is less potent than morphine with a smaller sodium-response ratio.

NIH 9892. (+)-2-Butyl-5-(m-hydroxyphenyl)morphan hydrochloride.



MOUSE DATA ED_{50} (95% C.L.)
(mg/kg s.c.)

1) 12.2 (5.2 - 28.8

2) TF vs M - Inactive at 1.0 and 30.0

NIH 9892 (Cont'd)

3) PPQ - 0.7 (0.2 - 1.8)

4) HP - 2.6 (2.1 - 3.4)

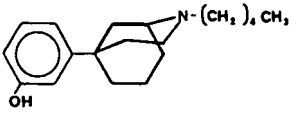
DISPLACEMENT OF STEREOSPECIFIC ^{3}H-ETORPHINE BINDING

EC50 of 221 nM in absence of 150 mM NaCl
EC50 of 224 nM in presence of 150 mM NaCl
Sodium response ratio = 1.01

OBSERVATIONS OF THE MORPHINE-DEPENDENT RHESUS MONKEY (UM)

NIH 9892 precipitated a withdrawal syndrome in the monkey with a potency 1/300 that of naloxone but with a comparable time course.

NIH 9893. (+)-5-(m-Hydroxyphenyl)-2-pentylmorphan hydrochloride.



MOUSE DATA-ED_{50} (95% C.L.) (mg/kg s.c.)

1) TF - 2.2 (0.8 - 6.5)

2) TF vs M - (0% at 1.0, 10.0 and 30.0

3) PPQ - 0.2 (0.1 - 0.7)

4) HP - 1.8 (1.6 - 2.7)

DISPLACEMENT OF STEREOSPECIFIC ^{3}H-ETORPHINE BINDING

EC50 of 51.6 nM in absence of 150 mM NaCl
EC50 of 41.7 nM in presence of 150 mM NaCl
Sodium response ratio = 0.87

INHIBITION OF TWITCH OF ELECTRICALLY DRIVEN GUINEA PIG ILEUM

	EC50	Maximum Response
Drug alone	6.56×10^{-7} M	86.9
After naltrexone	7.67×10^{-6} M	78.8
After UM 979	4.84×10^{-6} M	83.3

NIH 9893 (Cont'd)

INHIBITION OF TWITCH OF ELECTRICALLY DRIVEN MOUSE VAS DEFERENS

	EC50	Maximum Response
Drug alone	6.56×10^{-8} M	35.5
After naltrexone	5.22×10^{-8} M	26.5
After UM 979	2.96×10^{-8} M	18.3

At 10^{-4} M there was a contraction of the baseline.

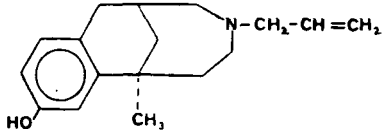
OBSERVATIONS IN THE MORPHINE-DEPENDENT RHESUS MONKEY (UM)

NIH 9893 precipitated a withdrawal syndrome with a potency of 0.016 that of naloxone with a comparable time course.

SUMMARY

NIH 9893 appears to have a morphine-like activity upon the guinea pig ileal preparation but not upon the vas deferens. Its actions in the monkey indicate significant antagonist activity.

NIH 9896. (-)-4-Allyl-1-methyl-10-hydroxy-2,3,4,5,6,7-hexahydro-1,6-methano-1H-4-benzazonine.



MOUSE ANALGESIA, ED50 (mg/kg)

Hot Plate: insufficient activity at 50 mg/kg

Nilsen: inactive at 5.0 mg/kg

DISPLACEMENT OF STEREOSPECIFIC ^{3}H-ETORPHINE BIINDING

NIH 9896 failed to displace significantly tritiated etorphine up to a concentration of 2×10^{-6} M.

INHIBITION OF TWITCH OF ELECTRICALLY DRIVEN GUINEA PIG ILEUM

At no concentration did this drug inhibit the twitch of this preparation. Concentrations of 10^{-6} M and greater caused increases in the magnitude of the twitch, and concentrations of 10^{-5} M and greater caused a baseline contraction of the preparation. Neither naltrexone nor UM 979 altered these responses.

INHIBITION OF TWTICH OF ELECTRICALLY DRIVEN MOUSE VAS DEFERENS

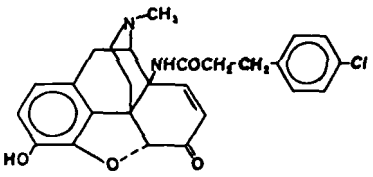
NIH 9896 did not inhibit the twitch of this ption at any concentration studied.

NIH 9896 (Cont'd)

SUMMARY

In the receptor binding assay and upon both the guinea pig ileum and the mouse vas deferens, NIH 9886 appears to be devoid of opiate-like activity.

NIH 9915. 14-ß-3-(4-Chlorphenyl)propionylaminomorphinone.



MOUSE DATA-ED_{50} (95% C.L.)

1) TF - 0.3 (0.1 - 0.6)

2) TF vs M - Inactive at 1.0 and 30.0

3) PPQ - 0.2 (0.1 - 0.3)

4) HP - 0.3 (0.2 - 0.4)

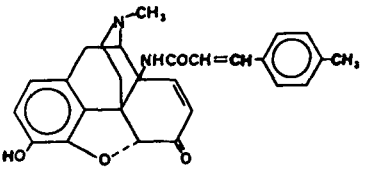
OBSERVATIONS IN THE MORPHINE-DEPENDENT MONKEY (MCV)

MONKEY DATA (SDS)	# ANIMALS Doses (mg/kg s.c.)	3, 0.25	3, 0.5	3, 1.0

3 (Vehicle dil HCl + H_2O or DMSO) 0.25 or 1.0 ml/kg	3(Morphine) 3.0

At the highest dose, the drug substituted completely for morphine. The onset of action was ½-1 hr and the duration was > 2½ hr. At the intermediate dose, the drug substituted partially for morphine and completely suppressed the withdrawal sign retching. Its potency is estimated as 2 times that of morphine.

NIH 9916. 14-ß-(4-Methylcinnamoyl)aminomorphi none.



MOUSE DATA-ED_{50} (95% C.L.) (mg/kg s.c.)

1) TF - 6% at 1.0, 10% at 10.0 and 35% at 30.0

2) TF vs M - 1.3 (0.2 - 6.9)

3) PPQ - 1.0 (0.3 - 3.0)

4) HP - 40% at 10.0 and 50.0, 20% at 20.0 and 70% at 100.0

NIH 9916 (Cont'd)

DISPLACEMENT OF STEREOSPECIFIC ^{3}H-ETORPHINE BINDING

NIH 9916 was not studied due to solubility problems.

INHIBITION OF TWITCH OF ELECTRICALLY DRIVEN GUINEA PIG ILEUM

At no concentration did NIH 9916 cause an inhibition of the twitch of this preparation.

INHIBITION OF TWITCH OF ELECTRICALLY DRIVEN MOUSE VAS DEFERENS

	EC50	Maximum Response
Drug alone	6.37×10^{-9} M	64.1
After naltrexone	3.62×10^{-8} M	61.4
After UM 979	3.15×10^{-8} M	42.9

For studies on the smooth-muscle preparations NIH 9916 was dissolved in dimethyl sulfoxide at a concentration of 10^{-3} M.

SUMMARY

NIH 9916 appears to have opiate activity upon the mouse vas deferens, but is devoid of activity upon the guinea pig ileal preparation. This pattern of response is extremely rare; and, in part may be due to conditions associated with the use of DMSO.

OBSERVATIONS IN THE MORPHINE-DEPENDENT MONKEY (MCV)

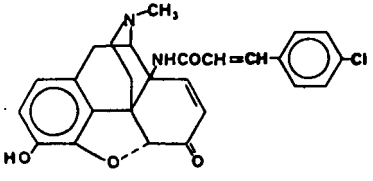
MONKEY DATA (SDS)	# ANIMALS	2	3	3
	Doses (mg/kg s.c.)	10.0	20.0	40.0

3 (DMSO), 1.0 ml/1g

3 (Morphine) 3.0

The compound did not substitute for morphine at any of the doses tested. However, it did reduce the incidence of retching and vomiting at all doses. The supply was exhausted.

NIH 9917. 14-ß-(4-Chlorocinnamoyl)aminomorphinone.



MOUSE DATA-ED_{50} (95% C.L.) (mg/kg s.c.)

1) TF - 0.5 (0.1 - 1.7)

2) TF vs M - Inactive at 1.0 and 30.0

NIH 9917 (Cont'd)

3) PPQ - Solvent (DMSO) active in this test

4) HP - 0.95 (0.7 - 1.3)

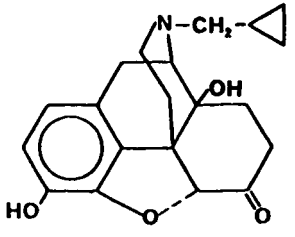
OBSERVATIONS IN THE MORPHINE-DEPENDENT MONKEY (MCV)

MONKEY DATA (SDS)	# ANIMALS Doses (mg/kg s.c.)	2, 0.25	2, 0.5	3, 1.0	1, 2.0

3 (DMSO) 1.0 ml/kg	3 (Morphine) 3.0

NIH 9917 substituted completely for morphine at the 2 higher doses. The onset was prompt and the duration of action was longer than that of morphine. At the highest dose, duration of action was approximately 5 hours. Its potency is about 6 times morphine.

NIH 9930. Naltrexone hydrochloride.



MOUSE DATA-ED_{50} (95% C.L.) (mg/kg.s.c.)

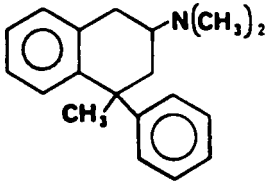
1) TF - None at 10.0

2) TF vs M - 0.007 (0.002 - 0.021)

3) PPQ - No activity

In this time-course study, naltrexone at 0.2 mg/kg produced 95% antagonism of the ED_{80} of morphine sulfate on the tail-flick test at 30 min., 81% antagonism at 60 min., and was inactive at 2 hr. In previous reports naltrexone is also numbered as NIH 8503.

NIH 9940. ±-trans-N,N-Dimethyl-1,2,3,4-tetrahydro-4-methyl-4-phenyl-2-naphthylamine hydrochloride.



MOUSE ANALGESIA, ED50 (mg/kg)

Hot Plate: 60% at 100

DISPLACEMENT OF STEREOSPECIFIC ^{3}H-ETORPHINE BINDING

NIH 9940, failed to displace tritiated etorphine significantly up to a concentration of 6×10^{-6} M.

NIH 9940 (Cont'd)

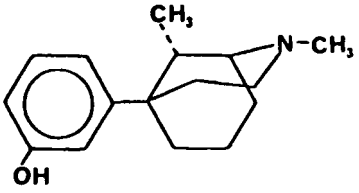
INHIBITION OF TWITCH OF ELECTRICALLY DRIVEN MOUSE VAS DEFERENS

At no concentration did NIH 9940 cause an inhibition of the twitch either in control preparations or in the presence of naltrexone. Concentrations of NIH 9940 of 3 x 10^{-6} M to 3 x 10^{-5} M caused large increases in the magnitude of the twitch.

OBSERVATIONS IN MORPHINE-DEPENDENT RHESUS MONKEYS (UM)

At 5.6 and 10 mg/kg, NIH 9940 had no apparent effects in withdrawn monkeys. At 17 mg/kg, the drug induced convulsions in one monkey and significant tremors in another.

NIH 9955. (±)-2,9α-Dimethyl-5-(m-hydroxyphenyl)morphan hydrochloride.



MOUSE DATA-ED_{50}(95% C.L.) (mg/kg s.c.)

1) TF-Inactive at 1.0 and 30.0

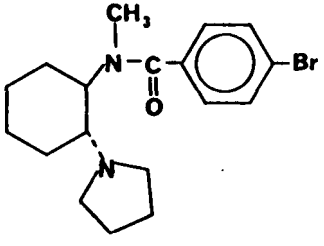
TF vs M - 5.0 (2.0 - 12.8)

3) PPQ - a. 19% at 1.0 and 28% at 30.0
b. 17% at 1.0, 20% at 10.0 and 17% at 30.0

4) HP - 10.0 (6.0 - 16.8)

5) N - 40% at 100

NIH 9962. trans-4-Bromo-N-methyl-N-[2(1-pyrrolidinyl)cyclohexyl]benzamide.



MOUSE DATA-ED_{50} (95% C.L.) (MG/KG s.c.)

1) TF - 3.7 (1.4 - 9.2)

2) TF vs M - Inactive at 1.0 and 30

3) PPQ - 6.8 (3.7 - 12.8)

4) HP - 5.7 (4.1 - 8.0)

NIH 9962 (Cont'd)

DISPLACEMENT OF STEREOSPECIFIC ^{3}H-ETORPHINE BINDING

EC50 of 2435 nM in absence of 150 mM NaCl
EC50 of 4767 nM in presence of 150 mM NaCl
Sodium response ratio = 1.96

INHIBITION OF TWITCH OF ELECTRICALLY DRIVEN GUINEA PIG ILEUM

	EC50	Maximum Response
Drug alone	1.27×10^{-7} M	84.7
After naltrexone	7.62×10^{-6} M	67.6
After UM 979	2.35×10^{-6} M	80.3

INHIBITION OF TWITCH OF ELECTRICALLY DRIVEN MOUSE VAS DEFERENS

	EC50	Maximum Response
Drug alone	4.2×10^{-7} M	89.0
After naltrexone	4.02×10^{-6} M	67.6
After UM 979	2.35×10^{-6} M	80.3

OBSERVATIONS IN MORPHINE-DEPENDENT AND NORMAL RHESUS MONKEYS (UM)

NIH 9962 failed to precipitate more severe withdrawal signs in the morphine-dependent monkey, and also failed to suppress the withdrawal syndrome. Rather, it produced signs of intoxication similar to those of kappa agonists.

OBSERVATIONS IN THE MORPHINE-DEPENDENT MONKEY (MCV)

MONKEY DATA (SDS)	# ANIMALS Doses (mg/kg s.c.)	2, 12.0	2, 9.0	4, 6.0	2, 3.0

4(dilHcl + H_2O) 1.0 ml/kg	4(Morphine) 3.0

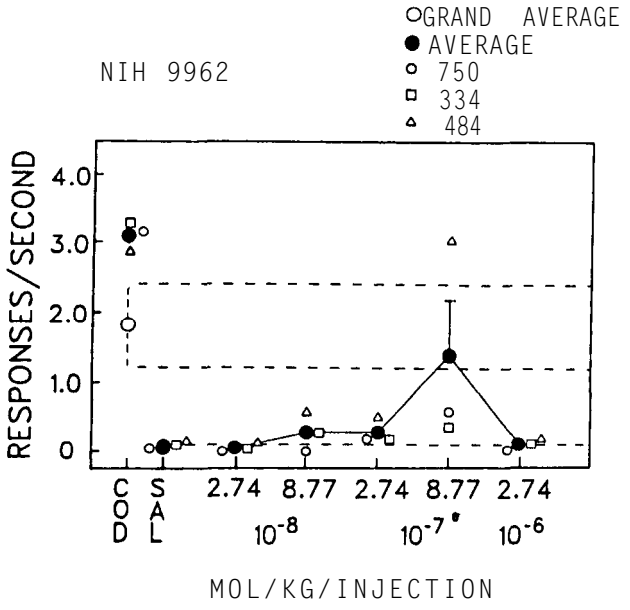
NIH 9962 suppressed some withdrawal signs at all doses. Thus, it substituted partially. Retching was the withdrawal sign that was suppressed the most. At 9.0 and 12.0 mg/kg the drug substituted completely for morphine in 2/3 of the animals. The action was slightly delayed and of brief duration, i.e., 1 hr. Tremors were observed in some monkeys at the three higher doses.

MONKEY DATA Normal Monkeys	# ANIMALS Doses (mg/kg s.c.)

NIH 9962 was given to normal non tolerant rhesus monkeys at doses of 12.0, 6.0 and 3.0 mg/kg s.c. respectively. Ataxia, lying on side, slowing, drowsiness, sagging (body) and scratch-

ing were the main effects noted. These effects developed promptly and began waning after 1 hr. Drowsiness persisted for 2½ hr. In another group of normal monkeys, 3 were given 12.0, 9.0 and 6.0 mg/kg s.c. respectively. One-half hr later they were challenged with 0.5 mg/kg s.c. of naloxone. The observed signs designated lying on side, ataxia, slowing, restlessness, drowsiness, sagging, and scratching quickly subsided. It appears that NIH 9962 produces many signs reminiscent of those seen after appropriate doses of morphine. However, the drug does not have a complete morphine-like profile.

DRUG SELF-ADMINISTRATION IN RHESUS MONKEYS (UM)



NIH 9962 maintained self-injection responding at high rates in one of three monkeys; in the two monkeys in which the drug maintained lower rates, the rates of responding were marginally above those of saline.

NIH 9962 produced drug-appropriate responding at 1.0 mg/kg in one monkey and 3.2 mg/kg in another; each trained to discriminate ethylketazocine from saline.

NIH 9962 (Cont'd)

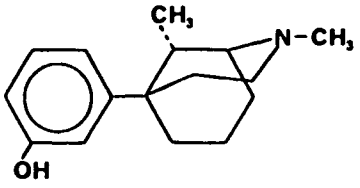
SUMMARY

NIH 9962 produced a novel spectrum of activity in the various preparations. Though somewhat less potent than morphine in the smooth-muscle preparations, its actions were reversed by both antagonists, a pattern of effects indicative of morphine-like compounds. Unlike morphine, it produced baseline contractures in both preparations at high concentrations.

The drug was markedly less potent than morphine in the binding assay. However, its in vivo activity is markedly different from morphine; in both the observational and drug discrimination studies NIH 9962 resembled ethylketazocine. The drug was considerably less potent than ethylketazocine in producing these actions. The actions of NIH 9962 were reversed by naloxone in the observational studies, suggesting that its actions are narcotic related.

In the drug self-injection studies, two of three monkeys maintained rates only marginally above saline; this is consistent with findings on other ethylketazocine'like agents. The third monkey, however, self-injected the drug at high rates. NIH 9962 should be studied in another set of monkeys to add evidence for the resolution of these findings. Thus, these data suggest that in the smooth muscle preparations the compound is morphine-like, but the behavioral data in the rhesus monkey suggest an ethylketazocine-like action. The drug should also be studied in monkeys trained to discriminate morphine-like agents from saline.

NIH 9971. (-)-2,9α-Dimethyl-5-(m-hydroxyphenyl)morphan hydrochloride



MOUSE DATA-ED_{50} (95% C.L.) (mg/kg s.c.)

1) TF - Inactive at 1.0 and 30.0

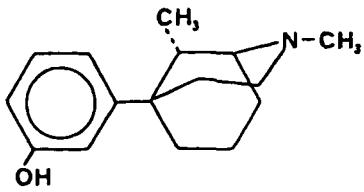
2) TF vs M - Inactive at 1.0 and 30.0

3) PPQ - 3.1 (1.1 - 9.3) 2.96 (0.91 - 9.61)

4) HP - 10.0 (7.2 - 13.9)

5) N - 25% at 50

NIH 9972. (+)-2,9α-Dimethyl-5-(m-hydroxyphenyl)morphan hydrochloride.



MOUSE DATA-ED_{50} (95% C.L.) (mg/kg s.c.)

1) TF - Inactive at 1.0 and 30.0

2) TF vs M - 4.5 (1.7 - 11.8)

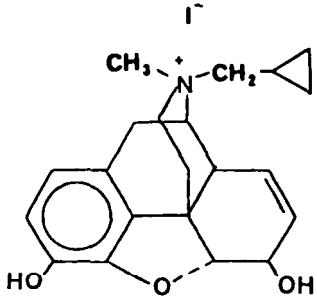
3) PPQ - a. 8% at 1.0 and 3% at 30.0

b. 0% at 1.0, 9% at 10.0 and 0% at 30.0

4) HP - Inactive

5) N- Inactive

NIH 9991. N-Cyclopropylmethylmorphinium iodide.



MOUSE DATA-ED_{50} (95% C.L.) (mg/kg s.c.)

1) TF - Inactive at 1.0 and 30.0

2) TF vs M - Inactive at 1.0 and 30.0

3) PPQ - 30% at 1.0, 4% at 30.0

4) HP - 20% at 20.0, 10% at 50.0 and 100.0

INHIBITION OF TWITCH OF ELECTRICALLY DRIVEN MOUSE VAS DEFERENS

	EC50	Maximum Response
Drug alone	7.79×10^{-8} M	53.2
After naltrexone	7.35×10^{-8} M	49.0
After UM 979		

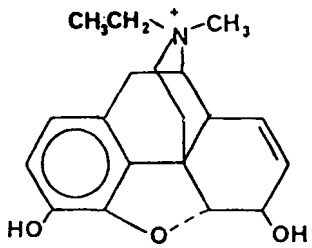
A very high concentration of naltrexone, 3×10^{-4} M, caused a very slight reversal of the inhibition produced by an equimolar concentration of NIH 9991. NIH 9991, 3×10^{-5} M, caused a very slight reversal of the effects of an equimolar concentration of morphine upon this preparation.

NIH 9991 (Cont'd)

OBSERVATIONS IN THE MORPHINE-DEPENDENT RHESUS MONKEY (UM)

At 5.6 and 10 mg/kg, NIH 9991 was without effect in withdrawn monkeys. Depletion of drug supply precluded full evaluation of in vivo activity.

NIH 9992. N-Methyl-N-ethylnormorphinium iodide.



MOUSE DATA-ED_{50} (95% C.L.) (mg/kg s.c.)

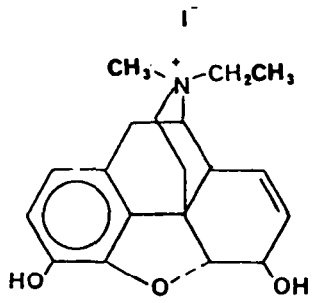
1) TF - 1% at 1.0 and 12% at 30.0

2) TF vs M - Inactive at 1.0 and 30.0

3) PPQ - 17% at 1.0, 40% at 10.0, 55% at 30.0 and 55% at 50.0

4) HP - 0% at 20.0 and 50.0

NIH 9993. N-Ethylmorphinium iodide.



MOUSE DATA-ED_{50} (95% C.L.) (mg/kg s.c.)

1) TF - Inactive at 1.0 and 30.0

2) TF vs M - Inactive at 1.0 and 30.0

3) PPQ - 0% at 1.0, 29% at 30.0

4) HP - 0% at 20.0 and 100.0

INHIBITION OF TWITCH OF ELECTRICALLY DRIVEN MOUSE VAS DEFERENS

	EC50	Maximum Response
Drug alone	4.89×10^{-8} M	56.0
After naltrexone	4.5×10^{-8} M	38.5

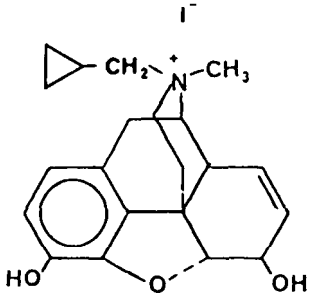
NIH 9993, in a concentration of 10^{-5} M, did not reverse the inhibition produced by an equimolar concentration of morphine. Naltrexone, 3×10^{-5} M, caused a very slight reversal of the inhibition produced by an equimolar concentration of NIH 9993.

NIH 9993 (Cont'd)

OBSERVATIONS IN THE MORPHINE-DEPENDENT RHESUS MONKEY (UM)

At 5.6 and 10 mg/kg, NIH 9993 was without activity in withdrawn monkey. Depletion of drug supply prevented full characterization of effects.

NIH 9994. N-Methyl-N-cyclopropylnormorphinium iodide.



MOUSE DATA-ED_{50} (95% C.L.) (mg/kg s.c.)

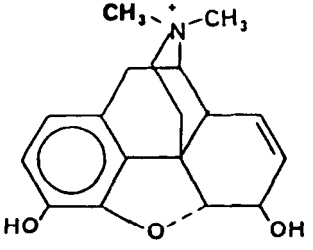
1) TF - 3% at 1.0 and 20% at 30.0

2) TF vs M - 26% at 5.0, 3% at 10.0, 40% at 30.0 and 82% at 60.0

3) PPQ - 11.9 (2.9 - 47.9)

4) HP - 30% at 50.0

NIH 9995. N-Methylmorphinium iodide



MOUSE DATA-ED_{50} (95% C.L.) (mg/kg s.c.)

1) TF - Inactive at 1.0 and 30.0

2) TF vs M - Inactive at 1.0 and 30.0

3) PPQ - 2% at 1.0, 15% at 30.0

4) HP - 0% at 20.0, 50.0 and 100.0

INHIBITION OF TWITCH OF ELECTRICALLY DRIVEN MOUSE VAS DEFERNS

	EC50	Maximum Response
Drug alone	2.77×10^{-7} M	55.1
After naltrexone	6.08×10^{-8} M	46.9

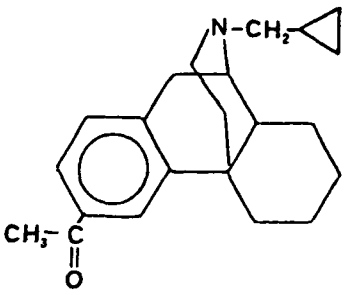
Naltrexone, 3×10^{-5} M, produced a very minute reversal of the inhibition produced by an equimolar concentration of NIH 9995. NIH 9995, 10^{-5} M, caused no reversal of the effects of an equimolar concentration of morphine upon this preparation.

NIH 9995 (Cont'd)

OBSERVATIONS IN MORPHINE DEPENDENT RHESUS MONKEYS (UM)

NIH 9995 was without significant effect in withdrawn monkeys at 5.6 and 10 mg/kg. Depletion of drug supply precluded full characterization of effects.

NIH 9996. (-)-1-[N-(Cyclopropylmethyl)morphinan-3-yl]ethanone D-tartrate.



MOUSE DATA-ED_{50} (95% C.L.) (mg/kg s.c.)

1) TF - 1.3 (0.6 - 2.6)

2) TF vs M - Inactive at 1.0, 10.0 and 30.0

3) PPQ - 0.6 - (0.3 - 1.0)

4) HP - 1.3 (1.0 - 1.8)

5) N- 0.13 (0.06 - 0.30)

DISPLACEMENT OF STEREOSPECIFIC ^{3}H-ETORPHINE BINDING

EC50 of 22.4 nM in absence of 150 mM NaCl
EC50 of 26.8 nM in presence of 150 mM NaCl
Sodium response ratio = 1.20

INHIBITION OF TWITCH OF ELECTRICALLY DRIVEN GUINEA PIG ILEUM

	EC50	Maximum Response
Drug alone	3.00×10^{-7} M	45.4
After naltrexone	3.70×10^{-6} M	53.6
After UM 979	completely blocked	

INHIBITION OF TWITCH OF ELECTRICALLY DRIVEN MOUSE VAS DEFERENS

	EC50	Maximum Response
Drug alone	4.66×10^{-8} M	96.9
After naltrexone	4.69×10^{-8} M	97.5
After UM 979	3.53×10^{-8} M	97.2

NIH 9996 (Cont'd)

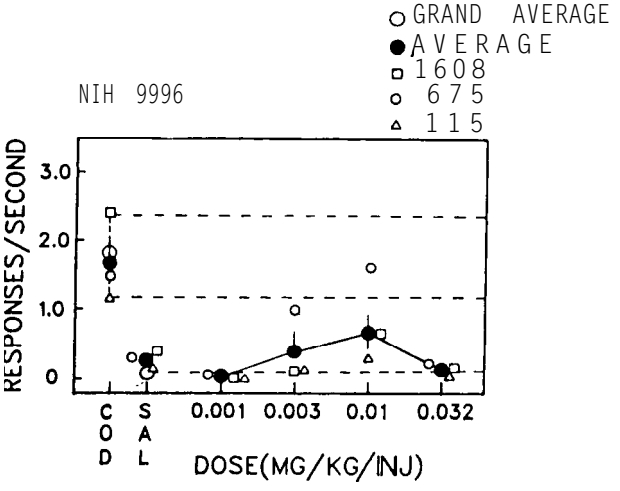
OBSERVATIONS IN THE MORPHINE-DEPENDENT MONKEY (MCV)

MONKEY DATA (SDS)	# ANIMALS	1	4	3	4	1
	Doses (mg/kg s.c.)	2.0	1.0	0.5	0.25	0.125

5 (Morphine) / 3.0, 5(H_2O) / 1.0 ml/kg

This compound abolished many withdrawal signs such as retching, vomiting, rigid abdominal muscles, vocalization when abdomen palpated, etc. However, tremor, slowing, drowsiness and body sag were seen at the higher doses. The drug substituted partially for morphine. Partial substitution does not necessarily indicate that the drug is morphine-like.

DRUG SELF-ADMINISTRATION IN RHESUS MONKEYS (UM)

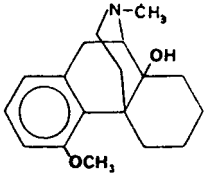


NIH 9996 maintained intermediate rates of drug self-injection, the highest rate at a dose of 0.01 mg/kg/inj.

SUMMARY

The findings on the smooth muscle preparations reported for NIH 9996 resemble a number of other compounds we have examined, including compounds such as baclofen. However, NIH 9996 has an affinity comparable to morphine, unlike baclofen, in the binding assay. Thus, NIH 9996 has a very interesting spectrum of action in these preparations. Unlike baclofen, NIH 9996 maintains drug self-injection responding. The potent antinociceptive activity and the ability of the drug to substitute partially for morphine in the dependent monkey also point to opioid-like activity for the drug.

NIH 9997. (-)-14-Hydroxy-4-methoxy-N-methylmorphinan.



MOUSE ANALGESIA, ED50 (mg/kg)

Hot Plate:	0.89 (0.65 - 1.24)
Nilsen:	2.2 (1.7 - 3.0)

DISPLACEMENT OF STEREOSPECIFIC ^{3}H-ETORPHINE BINDING

EC50 of 281 nM in absence of 150 mM NaCl
EC50 of 347 nM in presence of 150 mM NaCl
Sodium response ratio = 1.23

INHIBITION OF TWTICH OF ELECTRICALLY DRIVEN GUINEA PIG ILEUM

	EC50	Maximum Response
Drug alone	2.33×10^{-7} M	62.2
After naltrexone	2.12×10^{-7} M	45.2
After UM 979	blocked	-

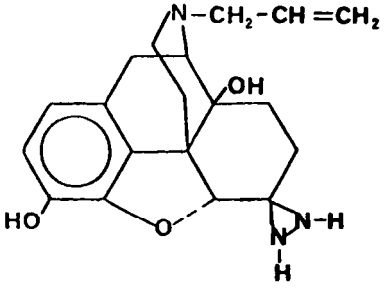
INHIBITION OF TWITCH OF ELECTRICALLY DRIVEN MOUSE VAS DEFERNS

	EC50	Maximum Response
Drug alone	8.68×10^{-9} M	48.9
After naltrexone	9.97×10^{-9} M	27.0
After UM 979	6.77×10^{-9} M	61.5

SUMMARY

NIH 9997 was less potent on each preparation than morphine. Its actions, however, on the smooth muscle preparations were not morphine-like.

NIH 10,001. 6-Desoxy-6,6-hydrazinaloxone.



MOUSE DATA-ED_{50} (95% C.L.)
(mg/kg s.c.)

1) TF - Inactive at 1.0 and 30.0

2) TF vs M - 0.06 (0.04 - 0.1)

3) PPQ - Inactive at 1.0 and 30.0

4) HP - 20% at 20.0, 0% at 50.0 and 100.0

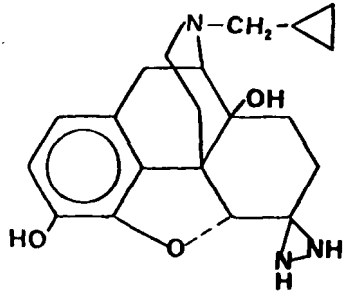
5) N - Inactive

NIH 10,001 (Cont'd)

TF vs M - Time Course Study
0.15 mg/kg AD_{80} oof NIH 10,001)

Time	% Antagonism
30 min	77%
1 hr	44%
2 hr	44%
4 hr	14%

NIH 10,003. 6-Desoxy-6,6-hydrazinaltrexone.



MOUSE DATA-ED_{50} (95% C.L.)
(mg/kg s.c.)

1) TF - Inactive at 1.0 and 30.0

2) TF vs M - 0.02 (0.01 - 0.03)

3) PPQ - Inactive at 10.0 and 30.0

4) HP - 20% at 50

5) N - Inactive at 100.0

TF vs M - Time Course Study
0.09 mg/kg (AD80 of NIH 10,003

TIME	% Antagonism
30 min	
1 hr	93%
2 hr	51%
4 hr	0%

DISPLACEMENT OF STEREOSPECIFIC ^{3}H-ETORPHINE BINDING

EC50 of 2.34 nM in absence of 150 mM NaCl
EC50 of 0.77 nM in presence of 150 mM NacL
Sodium response ratio = 0.33

INHIBITION OF TWITCH OF ELECTRICALLY DRIVEN GUINEA PIG ILEUM

	EC50	Maximum Response
Drug alone	1.09×10^{-7} M	94.6
After naltrexone	1.07×10^{-7} M	94.9
After UM 979	9.46×10^{-8} M	89.6

NIH 10,003 (Cont'd)

INHIBITON OF TWITCH OF ELECTRICALLY DRIVEN MOUSE VAS DEFERENS

	EC50	Maximum Response
Drug alone	4.74×10^{-6} M	94.3
After naltrexone	1.63×10^{-5} M	97.1
After UM 979	8.61×10^{-6} M	75.5

SUMMARY

NIH 10,003 is a very potent compound in the binding assay comparable to naltrexone with a lower sodium response ratio. Its inhibitory action upon the smooth muscle preparations was obtained at 10^{-7} M and higher suggesting that NIH 10,003 will be a potent naltrexone-like antagonist over a broad range of concentrations. At higher concentrations, the drug has a non-opiate component of inhibitory action in the guinea pig ileum and may have a narcotic component of inhibitory action in the mouse vas deferens.

This interestino compound should be studied more extensivelv in comparison to other naltrexone-like compounds [e.g., WIN 44,441 (NIH 9752), NIH 10,003].

NIH 10,004. 6-Desoxy-6-isonitrosonaltrexone.

MOUSE DATA-ED_{50} (95% C.L.)
(mg/kg s.c.)

1) TF - Inactive at 1.0 and 30.0

2) TF vs M - 0.08 (0.03- 0.2)

3) PPQ - Inactive at 1.0 and 30.0

4) HP - 20% at 20.0, 50.0 and 100.0

5) N - Inactive at 100.0

TF vs M - Time Course Study
0.3 mg/kg (AD_{80} of NIH 10,004)

Time	% Antagonism
30 min	91%
1 hr	70%
2 hr	68%
4 hr	11%

NIH 10,004 (Cont'd)

DISPLACEMENT OF STEREOSPECIFIC ^{3}H-ETORPHINE BINDING

EC50 of 1.96 nM in absence of 150 mM NaCl
EC50 of 0.90 nM in presence of 150 mM NaGl
Sodium response ratio = 0.46

INHIBITION OF TWTICH OF ELECTRICALLY DRIVEN GUINEA PIG ILEUM

	EC50	Maximum Response
Drug alone	1.40×10^{-5} M	97.4
After naltrexone	1.18×10^{-5} M	100
After UM 979	7.35×10^{-6} M	83.2

INHIBITION OF TWITCH OF ELECTRICALLY DRIVEN MOUSE VAS DEFERENS

	EC50	Maximum Response
Drug alone	2.88×10^{-5} M	75.9
After naltrexone	-	-
After UM 979	-	-

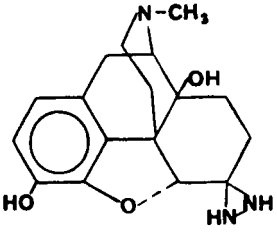
In all preparations, NIH 10,004 caused a marked contraction of the vas deferens at a concentration of 3×10^{-4}. Naltrexone markedly reduced and UM 970 completely abolished the response to the drug at 10^{-4} M.

SUMMARY

NIH 10,004 resembles naltrexone in the binding assay, yet its agonist actions on the smooth muscle preparations were not observed until concentrations on the order of 10^{-5} M were reached. This suggested that NIH 10,004 will be a potent antagonist in vivo as it was in the mouse. The inhibitory actions of NIH 10,004 upon the guinea pig ileum were more efficacious than morphine and were not reversed by either antagonist, suggesting that the drug has a non-narcotic component of action at high concentrations. Upon the mouse vas deferens, NIH 10,004 has a narcotic component of action since its inhibitory effects were reversed by both antagonists.

Obviously, this interesting compound should be studied in comparison to other naltrexone-like compounds [e.g. WIN 44,441 (NIH 9752) and NIH 10,003].

NIH 10,005. 6-Desoxy-6,6-hydrazioxymorphine.



MOUSE ANALGESIA, ED50 (mg/kg)

Hot Plate: 0.56 (0.40 - 0.77)
Nilsen: 0.45 (0.31 - 0.65)

DISPLACEMENT OF STEREOSPECIFIC ^{3}H-ETORPHINE BINDING

EC50 of 13.5 nM in absence of 150 mM NaCl
EC50 of 19.5 nM in presence of 150 mM NaCl
Sodium response ratio = 1.45

INHIBITION OF TWITCH OF ELECTRICALLY DRIVEN GUINEA PIG ILEUM

	EC50	Maximum Response
Drug alone	4.49×10^{-9} M	79.1
After naltrexone	3.53×10^{-6} M	55.6
After UM 979	3.81×10^{-7} M	39.5

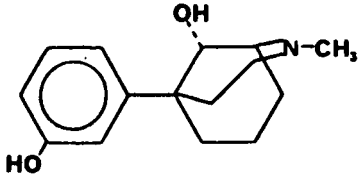
INHIBITION OF TWITCH OF ELECTRICALLY DRIVEN MOUSE VAS DEFERNS

	EC50	Maximum Response
Drug alone	4.58×10^{-8} M	96.6
After naltrexone	9.37×10^{-7} M	92.4
After UM 979	2.29×10^{-7} M	97.1

SUMMARY

NIH 10,005 has significant opiate-like activity upon each of the preparations. It was as potent as or slightly more potent than morphine in the various assays.

NIH 10,006. 9α-Hydroxy-5-(m-hydroxyphenyl)-2-methylmorphan hydrochloride.



MOUSE DATA-ED_{50} (95% C.L.) (mg/kg s.c.)

1) TF - 3.5 (2.5 - 5.0)

2) TF vs M - Inactive at 1.0 and 30.0

3) PPQ - 0.2 (0.07 - 0.4)

NIH 10,006 (Cont'd)

4) HP - 1.9 (1.5 - 2.6)

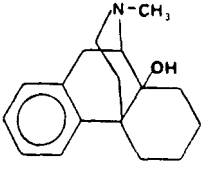
5) N - 2.7 (2.2 - 3.2)

OBSERVATIONS IN THE MORPHINE-DEPENDENT MONKEY (MCV)

MONKEY DATA (SDS)	# ANIMALS					
	Doses (mg/kg s.c.)	1, 16.0	3, 8.0	4, 4.0	3, 1.0	1, 0.25
		5(H_2O), 1.0 ml/kg			5(Morphine), 3.0	

This compound substituted completely for morphine in 2/3 at 1.0 mg/kg, 2/4 monkeys at 4.0 mg/kg and 2/3 monkeys at 8.0 mg/kg. The monkey receiving the highest dose did not require morphine at the noon injection. Drug supply was exhausted. Potency is about that of morphine.

NIH 10,007. (-)-14-Hydroxy-N-methylmorphinan.



MOUSE ANALGESIA, ED50 (mg/kg)

Hot Plate: 3.6 (2.7 - 4.8)
Nilsen: 4.2 (3.0 - 6.0)

DISPLACEMENT OF STEREOSPECIFIC ^{3}H-ETORPHINE BINDING

EC50 of 661 nM in absence of 150 mM NaCl
EC50 of 503 nM in presence of 150 mM NaCl
Sodium response ratio = 0.76

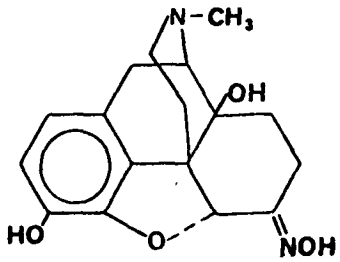
INHIBITION OF TWITCH OF ELECTRICALLY DRIVEN GUINEA PIG ILEUM

	EC50	Maximum Response
Drug alone	4.16×10^{-7} M	74.2
After naltrexone	7.28×10^{-5} M	70.6
After UM 979	1.35×10^{-6} M	39.3

SUMMARY

In the binding assay, NIH 10,007 was considerably less potent than morphine and the sodium response ratio was much lower. The inhibitory responses in the smooth muscle preparations produced by NIH 10,007 were antagonized by both antagonists, a pattern of results like that expected from a morphine-like drug. The contractures observed in both preparations at high concentrations are uncharacteristic of morphine.

NIH 10,008. 6-Desoxy-6-isonitroso-oxymorphone.



MOUSE DATA-ED_{50} (95% C.L.) (mg/kg s.c.)

1) TF - 0.7 (0.3 - 1.7)

2) TF vs M - Inactive at 1.0 and 30.0

3) PPQ - 0.05 (0.01 - 0.2)

4) HP - 0.8 (0.7 - 1.0)

5) N- 2.0 (1.4 - 2.9)

DISPLACEMENT OF STEREOSPECIFIC ^{3}H-ETORPHINE BINDING

EC50 of 5.95 nM in absence of 150 mM NaCl
EC50 of 6.91 nM in presence of 150 mM NaCl
Sodium response ratio = 1.16

INHIBITION OF TWITCH OF ELECTRICALLY DRIVEN GUINEA PIG ILEUM

	EC50	Maximum Response
Drug alone	5.22×10^{-9} M	73.8
After naltrexone	1.63×10^{-6} M	95.7
After UM 979	8.05×10^{-8} M	100

SUMMARY

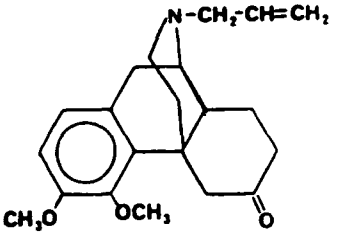
In both the binding assay and the guinea pig ileum NIH 10,008 was more potent than morphine; in addition the drug appears to be morphine-like in its actions upon the guinea pig ileum as demonstrated by its interactions with antagonists. The failure of UM 979 to antagonize NIH 10,008 upon the mouse vas deferens suggests that this is a very pure "mu" agonist upon this preparation.

OBSERVATION IN THE MORPHINE-DEPENDENT MONKEY (MCV)

MONKEY DATA (SDS) — # ANIMALS — Doses (mg/kg s.c.) — 3 / 2.0, 3 / 0.5, 2 / 0.125, 3(Morphine) / 3.0, 3(H_2O) / 1.0 ml/kg

At the highest dose, NIH 10,008 substituted completely for morphine. The drug acted promptly and had a long duration of action. Morphine was not required at the noon injection period. The drug is slightly more potent than morphine.

NIH 10,016. (-)-N-Allyl-3,4-dimethoxymorphinan-6-one.



MOUSE ANALGESIA, ED50 (mg/kg)

Hot Plate: 10.6 (8.0 - 14.1)

INHIBITION OF TWITCH OF ELECTRICALLY DRIVEN MOUSE VAS DEFERENS

	EC50	Maximum Response
Drug alone	1.23×10^{-7} M	36.0
After naltrexone	1.64×10^{-7} M	24.8

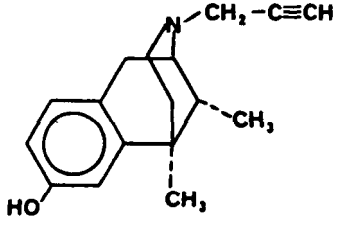
Naltrexone, in an equimolar concentration, reversed the inhibition produced by a maximally effective concentration of NIH 10,016. NIH 10,016, in an equimolar concentration reversed the inhibition produced by a maximally effective concentration of morphine sulfate.

NIH 10,016 is much less potent and efficacious than morphine. It appears to have both agonistic and antagonistic properties upon this preparation.

OBSERVATIONS IN THE MORPHINE-DEPENDENT RHESUS MONKEY (UM)

NIH 10,016 produced a prompt severe withdrawal syndrome at 1.0 mg/kg in nonwithdrawn monkeys. Depletion of drug supply precluded a determination of relative potency.

NIH 10,019. (+)-5, 9α-Dimethyl-2'-hydroxy-2-propynyl-6,7-benzomorphan hydrobromide.



MOUSE DATA-ED_{50} (95% C.L.) (mg/kg. s.c.)

1) TF - Inactive at 1.0 and 30.0

2) TF vs M - Inactive at 1.0 and 3.0

3) PPQ - Inactive at 1.0 and 30.0

4) HP - 10% at 20.0 and 50.0, 20% at 100.0

NIH 10,019 (Cont'd)

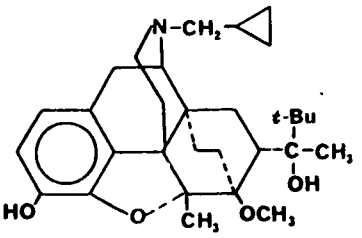
OBSERVATIONS IN THE MORPHINE-DEPENDENT MONKEY (MCV)

MONKEY DATA (SDS)	# ANIMALS	1	2	2	1
	Doses (mg/kg s.c.)	10.0	5.0	2.5	1.25

2(H2O)	2(Morphine)
1 ml/kg	3.0

At the 2 higher doses, severe ataxia was noted in one monkey and the other appeared catatonic. These effects lasted for about 30 minutes. The drug substituted partially and briefly for morphine. Partial substitution does not necessarily indicate that a compound is morphine-like. More work is recommended. Drug supply was exhausted.

NIH 10,064. 5-Methylbuprenorphine.



MOUSE DATA-ED_{50} (95% C.L.) (mg/kg s.c.)

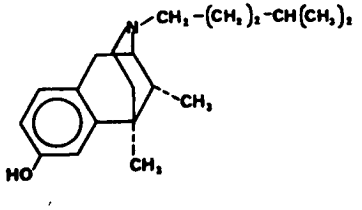
1) TF - Inactive at 1.0, 15.0 and 30.0

2) TF vs M- 0% at 1.0, 41% at 10.0 and 62% at 30.0

3) PPQ - 10% at 20, toxic at 50.0 (convulsions)

4) HP - Toxic at 20 (convulsions)

NIH 10,097. (-)-5,9α-Dimethyl-2'-hydroxy-2-(4-methylpentyl)-6, 7-benzomorphan hydrobromide.



MOUSE DATA-ED_{50} (95% C.L.) (mg/kg s.c.)

1) TF - 20.6 (9.9 - 42.4)

2) TF vs M - Inactive at 1.0, 10.0 and 30.0

3) PPQ - 0.9 (0.3 - 3.1)

4) HP - 3.1 (2.4 - 4.1)

NIH 10,097 (Cont'd)

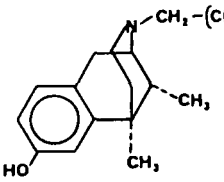
OBSERVATIONS IN THE MORPHINE-DEPENDENT MONKEY (MCV)

MONKEY DATA (SDS)	# ANIMALS	3,	3,	2,
	Doses (mg/kg s.c.	3.0	1.5	0.75

3(Morphine), 3.0 — 3(H_2O), 1.0 ml/kg

At the 2 higher doses, NIH 10,097 substituted completely for morphine in 3/3 monkeys at 3.0 mg/kg and 2/3 at 1.5 mg/kg. The onset was prompt and the duration short (approximately 90 min). The drug is approximately equipotent with morphine.

NIH 10,098. (+)-5,9α-Dimethyl-2'-hydroxy-2-(4-methylpentyl)-6, 7-benzomorphan hydrobromide.



MOUSE DATA-ED_{50} (95% C.L.) (mg/kg s.c.)

1) TF - Inactive at 1.0, 10.0 and 30.0

2) TF vs M - Inactive at 1.0, 10.0 and 30.0

3) PPQ - 16.3 (5.7 - 45.8)

4) HP - 70% at 100.0 convulsions

OBSERVATIONS IN THE MORPHINE-DEPENDENT MONKEY (MCV)

MONKEY DATA A. (SDS)	# ANIMALS	2	2	1
	Doses (mg/kg s.c.)	12.0	6.0	3.0

2(Alcohol, Tween 80 and H_2O), 1.0 ml/kg — 2(Morphine), 3.0

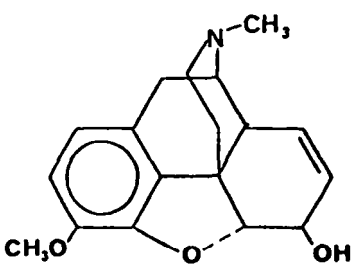
In the dose range of 3.0 - 12.0 mg/kg, NIH 10,098 did not substitute for morphine in dependent monkeys.

MONKEY DATA B. (PPt-W)	# ANIMALS	2	2	3
	Doses mg/kg s.c.)	18.0	12.0	6.0

3(Naloxone), 0.05 — 3(Alcohol + Tween 80 + H_2O), 1.0 ml/kg

The compound elicited some withdrawal signs but never produced a full withdrawal syndrome. At the 2 higher doses, 2 monkeys developed convulsions which were terminated with pentobarbital injections.

NIH 10,122.* Codeine phosphate.



MOUSE DATA-ED_{50} (95% C.L.) (mG/kg s.c.)

1) TF - 14.5 (8.1 - 2.0)

2) TF vs M - Inactive at 1.0, 10.0 and 30.0

3) PPQ - 1.1 (0.5- 2.5)

4) HP- 9.3 (6.7 - 12.8)

5) N - 9.2 96.4 - 13.0)

INHIBTION OF TWITCH OF ELECTRICALLY DRIVEN MOUSE VAS DEFERENS

NIH 10,122 produced a bfphasic dose-response relationship. The first phase of-inhibition occurred between concentrations of 10-10 M and 10-6 M. The second phase of inhibition occured in concentrations between 3 x 10^{-6} M and 3 x 10^{-4} M.

	First Phase of Inhibition	
	EC50	Maximum Response
Drug alone	2.05 x 10-9 M	43.1
After naltrexone	2.28 x 10-8,M	37.5

	Second Phase of Inhibition	
	EC50	Maximum Response
Drug alone	4.19 x 10-4 M	86.2
After naltrexone	6.12 x 10-5 M	49.2

OBSERVATIONS IN THE MORPHINE-DEPENDENT MONKEY (MCV)

MONKEY DATA (SDS)	# ANIMALS Doses (mg/kg s.c.)	3, 8.0	4, 4.0	2, 1.0	1, 0.25

$\frac{4(\text{Morphine})}{3.0}$, $\frac{4(H_2O)}{1.0\ ml/kg}$

NIH 10,122 substituted completely for morphine at the 2 higher doses. The onset of action was delayed for about 30 min and the duration was about 1½ hr at the highest dose and 1 hr at the next active dose. The drug is slightly less potent than morphine.

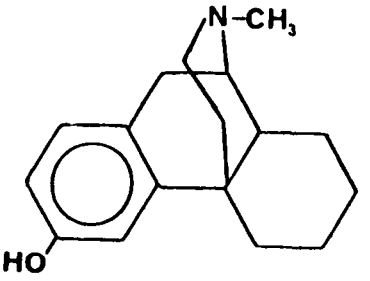
NIH 10,122 (Cont'd)

OBSERVATIONS IN THE MORPHINE-DEPENDENT RHESUS MONKEY (UM)

NIH 10,122 completely reversed the withdrawal syndrome at 18 mg/kg. This is in substantial agreement with historical controls.

*NIH 10,122 (Codeine) has previously been numbered NIH 00002.

NIH 10,123*. Levorphanol tartrate.



MOUSE DATA-ED_{50} (95% C.L.) (mg/kg s.c.)

1) TF - 0.1 (0.04 - 0.2)

2) TF vs M - Inactive at 1.0, 10.0 and 30.0

3) PPQ - 0.05 (0.1 - 0.2)

4) HP - 0.21 (0.16 - 0.28)

5) N - 0.25 (0.17 - 0.35)

DISPLACEMENT OF STEREOSPECIFIC ^{3}H-ETORPHINE BINDING

EC50 of 3.37 nM in absence of 150 mM NaCl
EC50 of 4.73 nM in presence of 150 mM NaCl
Sodium response ratio = 1.27

INHIBITION OF TWITCH OF ELECTRICALLY DRIVEN MOUSE VAS DEFERENS

	EC50	Maximum Response
Drug alone	1.48 x 10-7 M	80.2
After naltrexone	3.43 x 10-6 M	83.4
After UM 979		

MONKEY DATA (SDS)	# ANIMALS	1	3	3	3	1		
	Doses (mg/kg s.c.)	1.6	0.8	0.4	0.1	0.025		
							4(H_2O), 1.0 ml/kg	4(Morphine) 3.0

NIH 10,123 substituted completely for morphine at 1.6 and 0.8 mg/kg. The drug acted promptly and the duration of action was similar to morphine. The drug is about 3 x as potent as morphine.

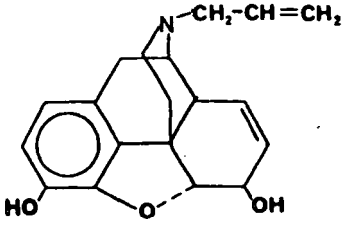
NIH 10,123* (Cont'd)

OBSERVATIONS IN THE MORPHINE-DEPENDENT RHESUS MONKEY

UM 1410 completely suppressed the withdrawal syndrome at 1.0 mg/kg. This is in substantial agreement with historical controls.

*NIH 10,123 (Levorphanol) has previously been numbered NIH 4590.

NIH 10,124.* Nalorphine hydrochloride.



MOUSE DATA-ED_{50} (95% C.L.) (mg/kg s.c.)

1) TF - 12% at 1.0, 19% at 10.0, 12% at 30.0

2) TF vs M - 0.5 (0.1 - 1.7)

3) PPQ - 3% at 1.0, 49% at 3.0, 43% at 10.0 and 60% at 30.0

4) HP - 13.8 (9.0 - 21.3)

5) N - 27.0 (18.5 - 39.5)

DISPLACEMENT OF STEREOSPECIFIC ^{3}H-ETORPHINE BINDING

EC50 of 8.10 nM in absence of 150 mM NaCl
EC50 of 5.43 nM in presence of 150 mM NaCl
Sodium response ratio = 0.67

INHIBITION OF TWITCH OF ELECTRICALLY DRIVEN MOUSE VAS DEFERENS

	EC50	Maximum Response
Drug alone	1.36×10^{-7} M	47.7
After naltrexone	8.88×10^{-7} M	54.7
After UM 979		

In the presence of morphine (3×10^{-5} M) a maximally effective concentration of morphine, an equimolar concentration of NIH 10,124 caused a partial reversal of the inhibition produced by morphine, which indicates that the drug has antagonistic activity. In summary, NIH 10,124 is a partial agonist. It is approximately 1/10 as potent as morphine and less efficacious.

NIH 10,124 (Cont'd)

OBSERVATIONS IN THE MORPHINE-DEPENDENT MONKEY (MCV)

MONKEY DATA A. (SDS)	# ANIMALS	2	2	1
	Doses (mg/kg s.c.)	0.2	0.05	0.0125

2(Morphine)	2(H_2O)
3.0	1.0 ml/kg

The compound did not substitute for morphine. It appeared to exacerbate withdrawal at the 2 higher doses. Vomiting was noted at the highest dose.

MONKEY DATA B. (PPt-W)	# ANIMALS	3	3	2
	Doses (mg/kg s.c.)	0.4	0.1	0.025

3(H2O)	3(Naloxone)
1.0 ml/kg	0.05

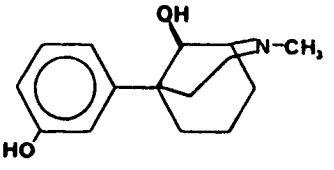
At the 2 higher doses, NIH 10,124 precipitated withdrawal in all the monkeys. The results were dose-related. Onset of action was prompt and the duration about 1½ to 2 hr. The drug is approximately 1/8 as potent as naloxone.

OBSERVATIONS IN THE MORPHINE-DEPENDENT RHESUS MONKEY (UM)

NIH 10,124 precipitated an intermediate grade of withdrawal at 0.1 mg/kg in nonwithdrawn monkeys. This is in substantial agreement with historical controls.

*NIH 10,124 (Nalorphine) has previously been numbered NIH 2105.

NIH 10,154. 9ß-Hydroxy-5-(m-hydroxyphenyl)-2-methylmorphan mandelate.



MOUSE DATA-ED_{50} (95% C.L.) (mg/kg s.c.)

1) TF - 1% at 1.0, 9% at 10.0, 31% at 30.0 and 46% at 60.0

2) TF vs M - Inactive at 1.0, 10.0 and 30.0

3) PPQ - 8.6 (4.2 - 17.6)

4) HP - 30% at 100.0

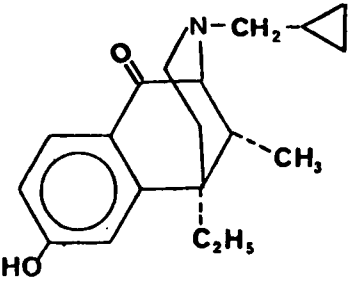
NIH 10,154 (Cont'd)

OBSERVATIONS IN MORPHINE-DEPENDENT MONKEYS - (MCV)

MONKEY DATA (SDS)	# ANIMALS Doses (mg/kg s.c.)	2 / 18.0	3 / 12.0	3 / 6.0	3(Morphine) / 3.0	3(H_2O) / 1.0 ml/kg

At-doses ranging from 3.0 to 18.0 mg/kg the drug did not substitute for morphine. The drug appeared to suppress retching and wet-dog shakes at all doses. Drug supply was exhausted.

NIH 10,165. Ethylketocyclazocine methanesulfonate.



MOUSE DATA-ED_{50} (95% C.L.) (mg/kg s.c.)

1) TF - 0.4 (0.1 - 1.0)

2) TF vs M - Inactive at 1.0, 10.0 and 30.0

3) PPQ - 0.04 (0.02 - 0.1)

4) HP - 0.09 (0.07 - 0.12)

AD50 for naloxone vs NIH 10,165 in TF - 0.1 (0.04 - 0.25)
AD50 for naloxone vs NIH 10,165 in PPQ - 0.02 (0.04 - 1.6)

INHIBITION OF TWITCH OF ELECTRICALLY DRIVEN MOUSE VAS DEFERENS

	EC50	Maximum Response
Drug alone	1.16×10^{-8} M	94.7
After naltrexone	1.51×10^{-7} M	94.7

NIH 10,165 in an equimolar concentration did not reverse the inhibition produced by a maximally effective concentration of morphine, 3×10^{-5} M; this concentration of NIH 10,165 added to the suppression of the twitch produced by morphine.

OBSERVATIONS IN THE MORPHINE-DEPENDENT MONKEY (MCV)

MONKEY DATA A. (SDS)	# ANIMALS Doses (mg/kg s.c.)	4 / 0.1	7 / 0.025	4 / 0.006	2 / 0.001	7(H_2O) / 1.0 ml/kg	7(Morphine) / 3.0

NIH 10,165 (Cont'd)

A. In a preliminary SDS study, one monkey received 0.25 mg/kg. Within 5 min., the animal showed severe ataxia, tremors, salivation, appeared depressed and became prostrate. Naloxone, (2 doses of 0.08 mg/kg) 5 min apart reversed all the signs except salivation. In the full SDS study, the drug substituted partially for morphine in all monkeys at the highest dose, and in reducing-the incidence of retching, vomiting, and vocalization when abdomen palpated. Relaxed abdominal muscles were also noted. In two monkeys receiving the 0.006 dose, the drug appeared to substitute completely for morphine for about 30 min. Interestingly, the drug also produced a number of dose-related signs, designated jaw and body sag, slowing, ptosis, ataxia, tremors, salivation, and prostration in one animal.

B. Special Study in Normal Non-Addicted Monkeys. Two monkeys were given 0.28 mg/kg of NIH 10,165. In one monkey, within 5 min., the animal was prostrate and stopped breathing. Naloxone (0.075) mg/kg ip and 0.075 mg/kg S.C. 5 and 15 min later revived the animal. The other monkey showed the signs ataxia, tremors, jaw and body sag, slowing, and was almost prostrate. Naloxone 0.5 mg/kg reversed the syndrome.

OBSERVATIONS IN THE MORPHINE-DEPENDENT RHESUS MONKEY (UM)

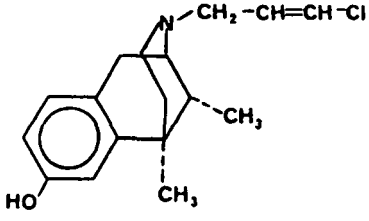
In partially withdrawn monkeys, NIH 10,165 (0.03 mg/kg) produced ataxia and sedation. At 0.1 mg/kg it increased the ataxia and produced pupil dilation. At higher doses, these effects were increased and ptosis and tremor were evident; however, signs of withdrawal were not altered otherwise.

Naloxone (0.2 mg/kg) reversed substantially the effects of 0.1 mg/kg NIH 10,165 in normal monkeys.

This is in substantial agreement with historical controls. Larger doses of naloxone were used earlier.

*NIH 10,165 (Ethylketocyclazocine) has previously been numbered NIH 8848.

NIH 10,167. (+)-2-(3-Chloro-2-propenyl)-5,9α-dimethyl-2'-hydroxy-6,7,-benzomorphan hydrobromide.



MOUSE DATA-ED_{50} (95% C.L.) (mg/kg s.c.)

1) TF - Inactive at 1.0, 10.0 and 30.0

2) TF vs M - 23.9 (17.6 - 32.6)

NIH 10,167 (Cont'd)

3) PPQ - 0% at 3.0, 0% at 10.0, and 55% at 30.0

4) HP - 30% at 50.0

OBSERVATIONS IN THE MORPHINE-DEPENDENT MONKEY (MCV)

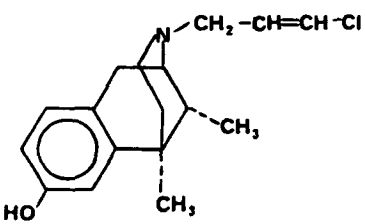
MONKEY DATA	# ANIMALS	3	3	1		
A. (SDS)	Doses (mg/kg s.c.)	4.0	1.0	0.25		
		3(Morphine) 3.0			3(H_2O) 1.0 ml/kg	

The drug did not substitute for morphine. At the highest dose, jaw sag, ataxia and slowing were observed. Some slowing was also observed at the intermediate dose.

MONKEY DOSE	# ANIMALS	1	2	2	1	
B. (PPt-W)	Doses (mg/kg s.c.)	12.0	8.0	4.0	1.0	
		2(Naloxone) 0.05			2(H2O) 1.0 ml/kg	

The drug did not precipitate withdrawal. It did produce a number of signs including slowing, ataxia, and tremors. One monkey receiving the 8.0 mg/kg dose showed all these signs and in addition fell off the perch and seemed unable to move. Two doses of 0.05 mg/kg of naloxone helped reverse the syndrome considerably. This type of activity resembles that produced by sigma agonists and PCP.

NIH 10,168. (-)-2-(3-Chloro-2-propenyl)-5,9α-dimethyl-2'-hydroxy-6,7-benzomorphan hydrobromide.



MOUSE DATA-ED_{50} (95% C.L.) (mg/kg s.c.)

1) TF - Inactive at 1.0, 10.0 and 30.0

2) TF vs M - 0.004 (0.002 - 0.008)

3) PPQ - 1.7 (0.5 - 5.9)

4) HP - 20% at 20 mg/kg

NIH 10,168 (Cont'd)

OBSERVATIONS IN THE MORPHINE-DEPENDENT MONKEY (MCV)

MONKEY DATA	# ANIMALS				
A. (SDS)	Doses (mg/kg s.c.)	2 0.5	2 0.125	2(Morphine) 3.0	2(H_2O) 1.0 ml/kg

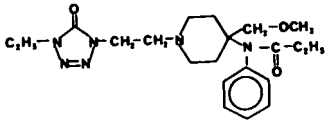
In the dose range tested, NIH 10,168 did not substitute for morphine. It seemed to exacerbate withdrawal.

MONKEY DATA	# ANIMALS					
B. (PPt-W)	Doses (mg/kg s.c.)	1 1.0	2 0.25	2 0.06	2 0.015	2 0.002

2(Naloxone) 0.05	2(H2O) 1.0 ml/kg

NIH 10,168 precipitated withdrawal at all the doses tested except the lowest dose. The drug acts promptly and its duration of action is similar to that of the reference compound naloxone. It is approximately 3 x more potent than naloxone.

NIH 10,207. Alfentanil hydrochloride.



MOUSE DATA-ED_{50} (95% C.L.) (mg/kg s.c.)

1) TF - 1.6 (1.0 - 2.5) 1.5

2) TF vs M - Inactive at 1.2, 10.0 and 30.0

3) PPQ - 0.1 (0.05 - 0.2) 2.8

4) HP - 0.04 (0.03 - 0.05)

NALOXONE AD50 vs NIH 10,207 in TF = 0.3 (0.1 - 0.6) 3.7
NALOXONE AD50 vs NIH 10,207 in PPQ 0 0.2 (0.1 - 0.5) 3.5

DISPLACEMENT OF STEREOSPECIFIC ^{3}H-ETORPHINE BINDING

EC_{50} of 133 nM in absence of 150 mM NaCl
EC_{50} of 290 nM in presence of 150 mM NaCl
Sodium response ratio = 2.18

NIH 10,207 (Cont'd)

INHIBITION OF TWITCH OF ELECTRICALLY-DRIVEN MOUSE VAS DEFERENS

	EC50	Maximum Response
Drug alone	9.49×10^{-7} M	75.7
After naltrexone	1.07×10^{-5} M	33.7
After UM 979		

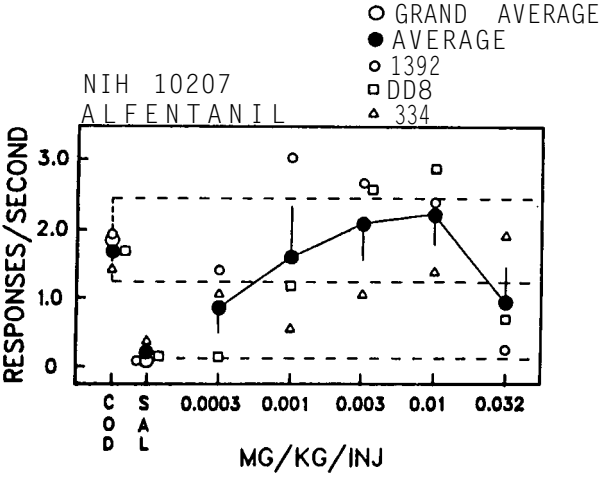
OBSERVATIONS IN THE MORPHINE-DEPENDENT MONEKY (MCV)

MONKEY DATA (SDS)	# ANIMALS	3,	1,	2,
	Doses (mg/kg s.c.)	0.2	0.05	0.0125

3(Morphine), 3.0 — 3(H_2O), 1.0 ml/kg

NIH 10,207 substituted completely for morphine in all the animals at the highest dose. The action was rapid and duration short (approx. 1 hr.). Scratching was also noted in these animals. At the peak effect the drug is 10-15 times more potent than morphine.

DRUG SELF-ADMINISTRATION IN RHESUS MONKEYS (UM)



COMMENT

Alfentanil maintained extraordinarily high rates of drug self-injection responding. One other narcotic test compound has maintained higher rates of responding in the rhesus monkey than codeine. (It, too, was a fentanyl analogue). Alfentanil also maintained rates of responding over a broad range of doses with

NIH 10,207 (Cont'd)

marked individual differences among monkeys in sensitivity to the compound. Alfentanil was 10 times more potent than morphine in terms of the maximal rate-maintaining dose (see attached figure).

Drug discrimination in rhesus monkeys. Alfentanil produced complete drug-appropriate responding in rhesus monkeys trained to discriminate codeine from saline. It was 30-100 times as potent as morphine in producing this effect. It has a rapid onset and brief duration of discriminative effect in the monkey.

This is a potent morphine-like compound in each of the assays described above. It may have been less active in the binding assay due to the use of etorphine as the tritiated ligand.The in vivo assays in primates suggest a potency range of 10-100 times that of morphine with a significantly shorter duration of action. This latter property in addition to its rapid onset may contribute to its capacity to maintain high rates of drug self-injection responding.

NIH 10,303. Dynorphin-(1-13): H-Tyr-Gly-Gly-Phe-Leu-Arg-Arg-iLE-aRG-Pro-Lys-Leu-Lys-OH.

OBSERVATIONS IN MORPHINE-DEPENDENT MONKEYS (MCV)

MONKEY DATA (SDS)	# ANIMALS				
	Bose (mg/kg i.v.)	3, 0.5	5, 0.25	5, 0.125	3, 0.062
		6(Saline) 2.0 ml			

Dynorphin-(1-13) at 0.5, 0.25 and 0.125 mg/kg i.v. suppressed withdrawal signs in a dose-related manner within 30 min. The effects were waning at 90 min.

NIH 10,304. Dynorphin (1-10) amide.

OBSERVATIONS IN MORPHINE-DEPENDENT MONKEYS (MCV)

MONKEY DATA (SDS)	# ANIMALS			
	Dose (mg/kg i.v.)	4, 0.5	4, 0.25	3(Saline), 2.0 ml
		3(Morphine) 3.0		

Dynorphin-(1-10) amide suppressed withdrawal signs at 0.5 mg/kg i.v.

NIH 10,306. Dynorphin-(1-6).

OBSERVATIONS IN MORPHINE-DEPENDENT MONKEYS (MCV)

MONKEY DATA (SDS)	# ANIMALS Dose (mg/kg i.v.)	4, 0.5	4, 0.25	3(Saline) 2.0 ml
				3(Morphine) 3.0

Dynorphin (1-6) did not suppress withdrawal signs at 0.25 or 0.5 mg/kg i.v.

NIH 10,307. α-Neo-Endorphin.

OBSERVATIONS IN MORPHINE-DEPENDENT MONKEY (MCV)

MONKEY DATA (SDS)	# ANIMALS Dose (mg/kg i.v.)	4, 0.5	4, 0.25	3(Saline), 2.0 ml
				3(Morphine) 3.0

α-Neo-Endorphin did not suppress withdrawal signs at either 0.25 or 0.5 mg/kg i.v.

NIH 10,308. Vasopressin tannate (Pitressin).

OBSERVATIONS IN MORPHINE-DEPENDENT MONKEYS (MCV)

MONKEY DATA A. (SDS)	# ANIMALS Dose (s.c.)	4, 20 units	4, 10 units	3(H_2O), 1.0 ml/kg
				3(Morphine) 3.0

Pitressin did not substitute for morphine. This substance may have produced more retching than the control animals at approximately 3 units/kg.

MONKEY DATA B. (PPt-W)	# ANIMALS Dose (s.c.)	2, 80 units	3, 40 units	4, 20 units
			2(H_2O), 1.0 ml/kg	2(Naloxone) 0.05

In doses up to approximately 25 units/kg pitressin did not precipitate withdrawal in these dependent monkeys.

ACKNOWLEDGMENT: This study was supported by National Institute on Drug Abuse contract #271-81-3830, Dr. Heinz Sorer, project officer.

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See 6-Desoxy-6-isonitrosooxymorphone
NIH 10,016 (MCV 4318, UM 1383)
See (-)-N-Allyl-3,4-dimethoxymorphinan-6-one
NIH 10,019 (MCV 4321)
See (+)-5,9α-Dimethyl-2'-hydroxy-2-propynyl-6,7-benzomorphan hydrobromide
NIH 10,064 (MCV 4331)
See 5-Methylbuprenorphine
NIH 10,097 (MCV 4332)
See (-)-5,9α-Dimethyl-Z'-hydroxy-2-(4-methylpentyl)-6,7-benzomorphan hydrobromide
NIH 10,098 (MCV 4333)
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NIH 10,154 (MCV 4344)
See 9ß-Hydroxy-5-(m-hydroxyphenyl)-2-methylmorphan fidelate
NIH 10,165 (MCV 4348)
See Ethylketocyclazocine methanesulfonate
NIH 10,167 (MCV 4359)
See (+)-2-(3-Chloro-2-propenyl)-5,9α-dimethyl-2'-hydroxy-6,7-benzomorphan hydrobromide
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NIH 10,207 (MCV 4374)
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See Dynorphin-(1-10) amide

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