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Opiate and Cocaine Addictions: Challenge for Pharmacotherapies

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KREEK, M. J. *Opiate and cocaine addictions: Challenge for pharmacotherapies.* PHARMACOL BIOCHEM BEHAV **57**(3) 551–569, 1997.—Neurobiological and behavioral studies, as well as basic and applied clinical research studies, may all contribute to the development of a pharmacotherapy for a specific addictive disease. This paper reviews recent findings from research work, primarily from one laboratory along with collaborative laboratories, that could have some relevance for the development of pharmacotherapy for cocaine dependency. The much earlier experiences of this laboratory in the development of a pharmacotherapy for opiate addiction will be addressed in the context of providing both some specific suggestions for addictive disease pharmacotherapy development and some warnings about the complexities of the introduction and implementation of a pharmacotherapy once developed. Finally, based on both the earlier perspectives and the more recent research findings, some very specific, though speculative, suggestions will be made about the development of novel pharmacotherapies for early opiate addiction, especially for cocaine abuse or addiction and prevention of relapse to cocaine use. The complex and diverse nature of the challenge for pharmacotherapy for the addictive diseases is presented, including specifically a mandate for broadening educational efforts concerning the basis of addictive diseases and the need for treatment, in parallel with the scientific efforts to develop increasingly sophisticated and targeted pharmacotherapies. © 1997 Elsevier Science Inc.

Cocaine Heroin Opiates Addiction Opioids Methadone Endogenous opioids Dynorphin Hypothalamus Nucleus accumbens Caudate putamen

THE development of a pharmacotherapy for cocaine addiction will entail several steps. Many of these are similar to the steps Professor Vincent P. Dole, the late Dr. Marie Nyswander, and I followed initially and in subsequent work (by the laboratory of Dr. Dole and, later, by my laboratory at the Rockefeller University, along with many other research groups) in the development of methadone maintenance treatment of opiate addiction. As part of our research work—first in developing a pharmacotherapy for opiate addiction and more recently aimed at the development of a pharmacotherapy for cocaine addiction, alcoholism, and other chemical dependencies—we have focused on both the neurobiological and the behavioral bases for addiction. We have also articulated specific goals and a rationale for the development of a specific pharmacotherapy.

This review is limited primarily to some clinical and basic research findings from my laboratory (the Laboratory of the Biology of Addictive Diseases at the Rockefeller University) over the last several years and from my related interinstitutional NIDA-supported Research Center. First, I will present recent findings from basic laboratory and clinical research of possible relevance for the development of a pharmacotherapy for cocaine addiction. Then, some of our experiences during the early and the more recent studies in the development of pharmacotherapy for opiate addiction will be reviewed briefly to highlight selected scientific, theoretical, and practical issues that may need to be addressed in developing a pharmacotherapy for cocaine dependency. Next, the relationship and possible commonality of the role of atypical responsivity to stressors in both cocaine addiction and opiate addiction, and the possible relevance for the development of pharmacotherapies, will be addressed, again with examples primarily from studies in my laboratory. Finally, I will offer some perspectives on the challenges to be faced in developing a pharmacotherapy for cocaine addiction.

VULNERABILITY FOR DEVELOPMENT OF ADDICTIONS

The intrinsic or acquired vulnerability for the development of addictions must be considered, and we have hypothesized that there are three different types of factors that contribute to vulnerability for the development of addictions, as well as the specific neurobiological or metabolic bases for each addictive disease. First, there may be genetic factors, most likely involving multiple genes. We have hypothesized that specific alleles of a group of specific genes, which could be called "candidate" genes, when present in specific combinations and

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when impacted upon either simultaneously or sequentially during or following acute or chronic exposure to a drug of abuse, may yield an intrinsic vulnerability for development of a specific addiction. It is unlikely that any single gene is central to the biological basis or the genetic basis of any addictive disease. However, many studies have begun to suggest that opiate addiction, and even cocaine dependency, like alcoholism, may be diseases in which genetic factors play a major or predominant role in a small to moderate percentage of afflicted persons. In each of these cases, clearly it must be the intersection of the genetic factors, as well as other specific host and environmental factors, acting in concert, that contribute to the development of an addiction. In each case, the drug of abuse must be introduced to the host, possibly on a subacute or chronic basis, before vulnerability is unmasked (46,60,62,64,65).

Early on, we had hypothesized—and (along with many other research groups) now have increasing evidence to document—that chronic exposure to a drug of abuse, such as opiates (which were studied initially), cocaine, and alcohol (more recently studied), may cause alterations in normal physiology that may persist long after illicit or excessive use of the drug or alcohol has ceased. Such changes may be persistent for a very long time or may even be permanent, following long cycles of addiction. We hypothesized many years ago (and then began to perform specific studies, which have provided the evidence) that these drug-induced abnormalities in physiology may involve alterations in neuroendocrine function, including changes in the function of the stress-responsive hypothalamic–pituitary–adrenal axis. Our group and others have subsequently shown that this is the case, based on basic clinical research studies in humans with addictive diseases (studied both during cycles of addiction and also in recent and long-term abstinent states) and in appropriate animal models.

We also hypothesized that atypical responsivity to stress or stressors, either on an inherent (possibly genetic) basis or due to drug-induced changes, may contribute to the acquisition and persistence of, and relapse to, illicit drug use. Other changes in normal physiology caused by chronic use of drugs of abuse may also contribute to craving and to drug-seeking behavior (45,46,61,62,65,108).

Finally, variable host responses, again of an intrinsic genetic or environmentally induced type, as well as set and setting, may contribute to the development of addiction. These contributing elements include environmental factors, the presence of other chronic diseases (including such diverse types as chronic liver disease, chronic pain, anxiety, or depression), or atypical responsivity to stressors, as well as the setting of acute and repeated use of drugs of abuse.

GOALS FOR A PHARMACOTHERAPY FOR AN ADDICTION

We have articulated goals for a pharmacotherapy for an addiction. These include prevention of withdrawal symptoms if any pertain. Withdrawal symptoms are profound and consistently observed, although with varying degrees of severity, following cessation of chronic opiate use. Although different and dramatic symptoms have also been described in an outpatient clinic setting following cessation of long-term "binge" pattern cocaine abuse, when studies are carried out in a controlled inpatient setting, very few signs and symptoms of cocaine withdrawal are observed (6,25,32,61,66,124).

Of the greatest importance, any pharmacotherapy should also reduce "drug hunger" or drug craving, because these factors contribute enormously to the desire to (and the actual event of) relapse during short- or long-term periods of abstinence (25,58,62,64,65).

The third goal of treatment, and one articulated by the late 1960s by our research team, is to normalize any physiological functions that may be disrupted by chronic drug use. We have hypothesized that normalization may be critical for the reduction and elimination of "drug hunger" or drug craving (45,46, 61,62,73,74).

We have proposed that a rationale of any pharmacotherapy for addiction should be to normalize both physiological and behavioral function and to lead to the cessation of drug abuse. This can be accomplished through targeting a treatment agent at a specific site of action, such as a receptor or transporter, or alternatively, targeting the medication to some physiological system that has been significantly deranged by chronic drug abuse (25,62,64,65).

INVOLVEMENT OF THE ENDOGENOUS OPIOID SYSTEM IN SPECIFIC ADDICTIVE DISEASES

We hypothesized many years ago that disruption of the endogenous opioid system, including the opioid peptides and their specific opioid receptors, may be involved in each of three specific addictive diseases: heroin addiction, cocaine dependency, and alcoholism. In my laboratory, we are continuing to address questions of the role and the precise mechanisms of this disruption (62,70).

Since the mid-1980s, using techniques of molecular biology, we have been addressing quantitative questions about the localization of specific components of the endogenous opioid system in appropriate animal models (including rats and, more recently, guinea pigs and transgenic mice) and, to the extent possible, in human tissue (3,86,118,145,146,147). With a goal of developing increasingly sensitive and specific techniques for quantitation of the levels of gene expression, as part of my NIDA Research Center, Dr. C. Inturrisi and colleagues in collaboration with Drs. A. Branch and H. Robertson have developed a modified solution hybridization RNase protection assay for the quantitation of levels of messenger RNA (3,152). In this modified technique, only long riboprobes are used, to increase sensitivity and specificity of the method. Standard calibration curves are constructed for each gene of interest and for an internal standard gene by use of both sense strand and radioactive-labelled antisense riboprobes made from subcloned cDNA. In most of our studies, 18S ribosomal RNA has been used as the internal standard because it has been shown not to be altered by such factors as aging or chronic drug exposure (3,118,152). In addition, to allow studies of multiple brain regions in individual small animals such as rats and mice, before and after perturbations of specific types, a step of trichloroacetic acid precipitation of the hybridized species followed by harvesting and radioactivity counting is used, with independent confirmation of the presence of a single predominant hybridized species for each type of riboprobe.

Using this sensitive and specific new solution hybridization RNase protection assay, we have gone back and remapped the levels of mRNAs of genes of interest in specific brain regions in individual rats (3,118–121,132). Special attention has been given to those regions involved both in the reinforcing effects of drugs of abuse (i.e., the mesolimbic–mesocortical dopaminergic system) and, in part, in their locomotor effects (i.e., the nigrostriatal dopaminergic system and the hypothalamus, which is the site of the tuberoinfundibular dopaminergic system and the site of origin of other peptides of the stress-responsive hypothalamic–pituitary axis). We have found that

each of these areas has abundant amounts of both enkephalin mRNA and dynorphin mRNA, with especially high levels found in: a) the nucleus accumbens, the brain region that has been found by many groups to be of singular importance with respect to the reinforcing effects of drugs of abuse; b) the caudate putamen, which has been found to have many implications for the locomotor effects and possibly part of the sensitization effects of drugs of abuse; and finally, c) the hypothalamus, the site of origin of many neuropeptides involved in controlling neuroendocrine functions (3,118,119,121).

The initial successful cloning of one type of opioid receptor, the delta opioid receptor, using a rodent hybrid cell line, was a feat accomplished simultaneously, but independently, in late 1992 by Dr. C. Evans working at the University of California–Los Angeles and Dr. B. Kieffer working in Strasbourg, France (26,40). Next, genes for the other two opioid receptor types, which were defined much earlier using increasingly selective chemicals, the mu and kappa opioid receptors, were cloned by many groups, first in rats, mice, or guinea pigs, as well as in other species (7,8,109,127,139,143). Then, the human gene for each of these opioid receptor genes was cloned (91,116,117,140).

An orphan opioid receptor has also been identified that does not bind any of the opioid ligands with high affinity (4,9,27,93,141,142). However, at least one endogenous ligand currently named "nociceptin" or "orphanin" has recently been identified and defined structurally to be very similar to dynorphin A_{1-17}, with similar processing in vitro; the gene for this peptide was cloned recently by two groups working independently (92,110,144).

Using probes for the kappa and mu opioid receptors, we have gone back to remap the rat brain to determine the actual levels of mRNAs (121–123,132,134,135). We have found abundant levels of both mu and kappa mRNAs in the nucleus accumbens, the amygdala, and other regions of the mesolimbic–mesocortical dopaminergic system, as well as in the caudate putamen and substantia nigra of the nigrostriatal system and in the hypothalamus—that is, in the same brain regions where opioid peptide mRNAs are abundant and the sites of known actions of drugs of abuse (120–122,132,134,135).

USE OF THE "BINGE" PATTERN COCAINE ADMINISTRATION MODEL FOR NEUROBIOLOGICAL STUDIES OF COCAINE EFFECTS

For many years my group, and others, have identified that "binge" pattern cocaine abuse is one of the most common patterns of illicit use of this drug in humans. Consequently, in 1988–1989, I developed an experimental model for use by my laboratory and my NIDA Research Center in which cocaine is administered in a "binge" pattern to rodents maintained in a stress-minimized controlled environment. In this model, cocaine is administered by the intraperitoneal route in three doses, each 1 h apart, in the hours just before the predominant sleep period of the rodent; this schedule is thus related to the pattern of early evening use of cocaine in the "binge" pattern by humans. Dose responses have been determined using cocaine doses ranging from 2.5 mg/kg/dose up to 20 mg/kg/dose. Subsequently, the majority of our studies have been conducted in rats using 10 or 15 mg/kg/dose. Many physiological assessments have been made and several behavioral studies have been conducted in which animals are studied using computerized techniques for constant monitoring, with animals maintained in their home cage throughout the study. In addition, many different neurochemical and molecular biological studies have been conducted.

Although there are many other animal models that have contributed enormous amounts of important information, the studies reported herein will be focused primarily on the "binge" pattern of cocaine administration, with all administration of cocaine performed by the investigators, which is the model we have used in recent years for many of our molecular biological, neurochemical, and behavioral studies. Different kinds of information may be forthcoming when cocaine (or any drug) is administered in a single dose as opposed to being administered on a subacute or chronic basis. Similarly, different findings may be obtained when multiple doses are administrated in a regular pattern (a strategy commonly used in pharmacological studies), as contrasted with the "binge" pattern; however, whether or not such differences pertain to cocaine administration has not been fully studied. Also, it has already been shown that very different findings may be forthcoming when multiple doses of any drug are administered regularly (or irregularly, as cocaine is in the "binge" pattern), as contrasted with infusion of a similar amount of the drug by pump over a 24-h period in a steady-state fashion.

Of great importance, many studies using self-administration models for exposure to any drug of abuse may provide similar or very different information from what is obtained in studies using investigator administration of the drug. In this context, significantly, many of the molecular biological and neurochemical findings we have made using the "binge" pattern of cocaine administration are essentially identical to findings made by other investigators using self-administration models. The value of studies conducted using "yoked" animals has been documented by many investigators; completely divergent findings may be made when the effects of a drug in a "yoked" animal are compared with the effects in an animal self-administering the drug of abuse. However, the similarities in findings between our investigator-administered "binge" pattern model and those made in the self-administration model support the validity of the "binge" pattern model of cocaine administration, at least for the types of studies reviewed herein.

Some Behavior Effects of "Binge" Pattern Cocaine Administration

In behavioral studies, we have found, as many have before us, that following a single-dose administration of cocaine (but in this case, following each of the three initial doses in the "binge" pattern), there is an abrupt increase in locomotor activity, beginning on day 1 (133). In a study conducted by Drs. E. Unterwald and A. Ho, we found that the enhanced activity reappeared on a daily basis after administration of each dose of cocaine, with some exaggerated responses beginning to appear (though without significance) by day 4 or 5. On days 6 through 8 of "binge" pattern cocaine administration, the responses seemed to be similar to those on the first day of treatment. However, by day 10 or 11, a significantly exaggerated increase in activity was noted after each dose; by day 13, significantly greater activity was observed following each dose of cocaine in animals receiving the chronic "binge" pattern of administration as compared with those same animals on day 1 (133) (see Figs. 1, 2).

Dopaminergic System Effects of "Binge" Pattern Cocaine Administration

Studies by Dr. R. Spangler and Mr. C. Maggos were conducted to determine the impact of "binge" pattern cocaine administration on dopamine transporter levels, primarily in the

substantia nigra as well as in the ventral tegmental area, the major regions of cell bodies of dopaminergic neurons. In these studies, they found that mRNA levels for the dopamine transporter were not altered by 14 days of "binge" pattern cocaine administration (87,88). Similar findings have been made by other groups with respect to the peptide levels (i.e., the actual amounts of transporter present) during chronic cocaine administration by different paradigms, including at least one other multiple-administrations paradigm. It has also been shown by several other groups that mRNA levels (measured primarily by Northern blot techniques or by in situ hybridization techniques) are not altered during chronic cocaine administration.

We have gone on to ask the question of whether or not chronic "binge" pattern cocaine administration alters the density of the dopamine receptors, as measured by quantitative autoradiography. Drs. E. Unterwald and A. Ho and colleagues have shown that D_2 receptors are significantly increased in density after 7 days of "binge" pattern cocaine administration, specifically in the rostral caudate putamen, the rostral nucleus accumbens, and the olfactory tubercle. However, after 14 days of cocaine administration, these alterations were no longer seen, suggesting that the normal status had been restored with respect to density of D_2 receptors. In this in vitro quantitative autoradiography technique, endogenous ligand is removed by washing the slices, and dynamic regulation of receptor binding is not possible (133). However, in their studies, Unterwald et al. found that the D_1 receptor densities, which were only modestly altered following 7 days of cocaine administration, were significantly increased in specific brain regions after 14 days of "binge" pattern cocaine administration (133). The increases in D_1 receptor densities as determined by quantitative autoradiography were found throughout the nucleus accumbens, the olfactory tubercle, as well as in the ventral pallidum (133). Also, D_1 receptors were modestly increased in density in the substantia nigra on days 7 and 14 (133). Because in these studies the concentrations of the D_1-directed ligand (the selective D_1 antagonist SCH 23390) used should have been adequate to label most (over 98%) of the D_1 binding sites, the data were interpreted as indicating an increase in βmax for D_1 receptors (133). A significant positive correlation within animals was found between D_1 receptor density increases in the nucleus accumbens and in the olfactory tubercle following 14 days of "binge" pattern cocaine administration when compared with the enhanced or sensitized locomotor activity 1–3 h following cocaine administration on day 13 in the same animals.

Provocatively, very different findings with respect to binding may be made using different kinds of techniques, including positron emission tomography. In studies ongoing in my laboratory in collaboration with that of Dr. H. Tsukada in Hamamatsu, Japan, using a rodent model, in which both D_1- and D_2-type receptors have been studied by PET using selective ligands, findings have been made that suggest there may be reductions in total dopamine receptor binding availability (128). Similar results have been obtained in humans by the group of Drs. N. Volkow and J. Fowler at the Brookhaven National Laboratory, in studies in which D_2- but not D_1-type

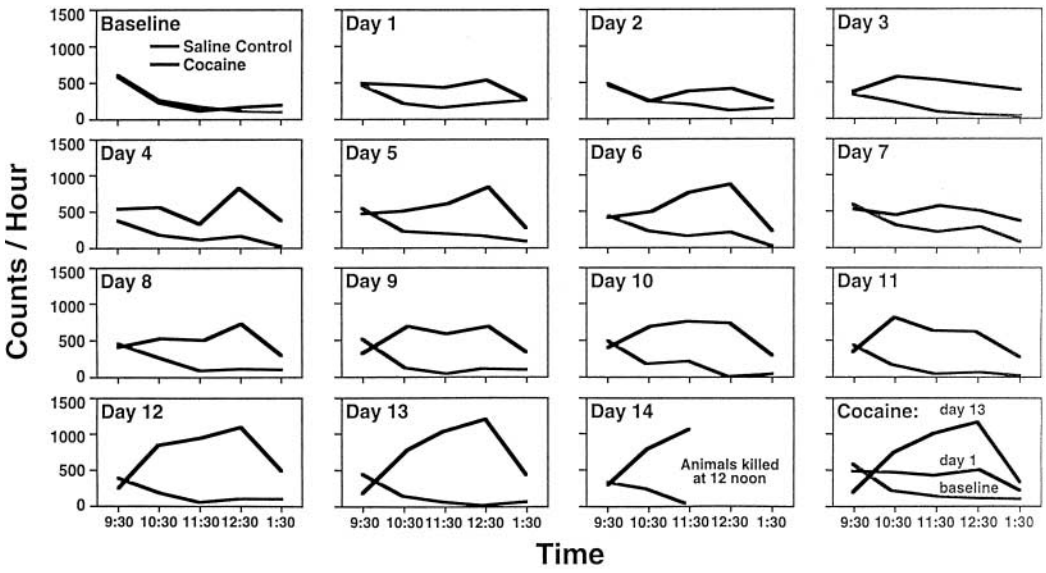

FIG. 1. Effect of daily "binge" cocaine administration on activity. Cumulative photocell interruptions (mean ± SEM; $n = 6$ per treatment group) during the hour ending at the time indicated are given for saline- and cocaine-injected animals on the day before the start of the study (baseline) and on each of days 1 through 14 of treatment. Animals were injected with saline or cocaine at 0930, 1030, and 1130 h daily. Photocell counts were higher for the cocaine-injected animals on each day of drug administration. Behavioral sensitization was evidenced by a greater increase in activity on day 13 of cocaine treatment than on the first day of treatment (bottom right). Comparing cocaine-treated with saline-injected controls, sensitization was seen by examination of the difference counts for each group between day 1 and day 13 [two-way ANOVA, group $F(1, 10) = 6.5$, $p < 0.05$]. Looking only at the cocaine-treated animals, sensitization was demonstrated by a greater number of total photocell counts over the 3 h after cocaine injections on day 13 of cocaine administration as compared with the first day of cocaine administration (paired $t = 2.08$, $df = 5$, $p < 0.05$, one-tailed). [Originally published in Unterwald et al. (133), *J. Pharmacol. Exp. Ther.* 270:1387–1397, 1994.]

dopamine receptors ligands were used (137,138). In our work, further findings suggest that this is due to cocaine-induced alterations in ligand affinity (128).

Microdialysis Studies of Extracellular Fluid Dopamine Levels During "Binge" Pattern Cocaine Administration

All of these changes with respect to the dopaminergic system confirm and extend work by many other groups. After cocaine binds to the dopamine reuptake transporter, a brisk rise in dopamine levels in extracellular fluid (ECF) occurs, then levels return essentially to baseline within 3–5 h after the last cocaine administration, as shown by many groups, including my laboratory (89). In further findings from the microdialysis studies of Dr. I. Maisonneuve, we have learned that, following chronic "binge" pattern cocaine administration (duration 14 days), the baseline levels of dopamine are significantly lowered both in the caudate putamen region and in the nucleus

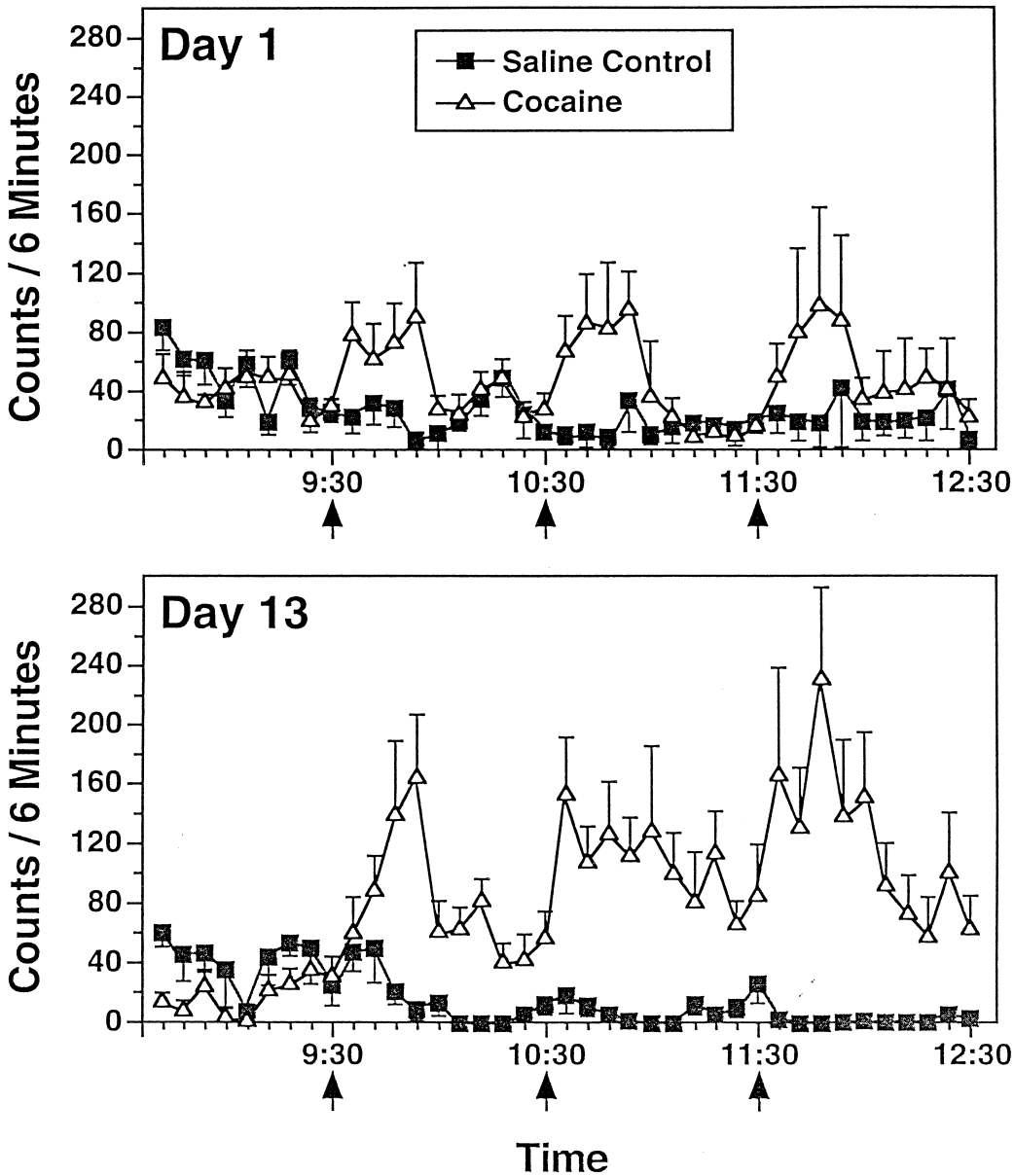

FIG. 2. Effect of binge cocaine administration on activity on day 1 and day 13. Photocell counts (mean ± SEM; $n = 6$ per treatment group) for each 6-min period are shown for the hour before and the 3 h after the three saline and cocaine injections. Animals were injected with saline or cocaine at 0930, 1030, and 1130 h daily (arrows). On day 1, the activity of the cocaine-injected animals was significantly higher than that of the saline-injected animals [treatment × min postinjection, $F(9, 90) = 3.77$; $p < 0.001$]. Behavioral sensitization was evidenced by a greater increase in activity on day 13 of cocaine administration than on the first day of treatment. In addition, the duration of the cocaine-induced hyperactivity was greater on day 13 than on day 1. [Originally published in Unterwald et al. (133), *J. Pharmacol. Exp. Ther.* 270:1387–1397, 1994.]

accumbens (90). Again, these findings are modest yet highly significant and may support a much earlier concept of "inadequate dopamine reserve" or "depletion" following chronic cocaine administration. However, the ability of ECF dopamine levels to surge following each dose of cocaine is similar in animals pretreated by "binge" pattern cocaine and in those pretreated with "binge" pattern saline alone (90). It should be noted, however, that the actual levels of dopamine measured by microdialysis in the ECF are significantly lowered at all time points in the animals receiving chronic cocaine treatment.

Thus, in the microdialysis studies, we have found significant changes in the dopamine system: a lowering in basal ECF levels of dopamine and lowered absolute levels in response to chronic cocaine administration, although with similar amplitude of rise. We have found, using this "binge" pattern model, that "sensitization" develops with respect to the locomotor activity effects. In this paradigm, unlike the earlier paradigms, there is no protracted period of abstinence following chronic cocaine administration, but rather, regular daily use, though with irregular time intervals between cocaine doses due to the "binge" pattern of administration. Also, changes in D_1 receptors seem to predominate over D_2 (based on quantitative autoradiography), and these changes in D_1 receptor density are positively correlated with the enhanced locomotor activity effects of cocaine. These findings have suggested to us for some time that, in addition to an appropriate search for a pharmacotherapeutic agent for cocaine dependency targeted to a cocaine-selective region of the dopamine transporter, a D_1 dopamine-type receptor-selective agonist or partial agonist might be effective in management of cocaine craving, and thus possibly in preventing relapse to cocaine abuse.

Numerous studies of the treatment of opiate dependencies have found that an agonist—which has some of the features of the drugs of abuse and yet, by virtue of its selectivity and pharmacokinetic profile, much more attenuated effects—is highly effective in management of opiate dependency. Therefore, we suggest that a D_1 agonist or partial agonist might be effective in managing cocaine dependency. Such a ligand would act not as an antagonist but rather as a selectively directed agonist or partial agonist, allowing some dopamine-like action, yet at the same time it would give an attenuated dopaminergic surge (as contrasted with that evoked by a stimulus such as cocaine) and thus a more stabilized dopaminergic responsive system that potentially would allow normalization of disrupted physiology.

ENDOGENOUS OPIOID SYSTEM EFFECTS OF "BINGE" PATTERN COCAINE ADMINISTRATION

These changes in the dopaminergic system that we have observed during chronic cocaine administration are, for the most part, relatively modest. Therefore, we have asked the question of whether or not disruption of the dopaminergic systems alone may be involved following chronic exposure to "binge" pattern cocaine administration and thus potentially in the phenomenon of "drug hunger" or craving, which may lead to self-administration both in animal models and in humans.

Quantitative Autoradiography Studies of Effects of "Binge" Pattern Cocaine Administration on Opioid Receptor Density

We have thus specifically asked whether or not cocaine may also disrupt the endogenous opioid system. Of great interest, in studies conducted by Dr. E. Unterwald and colleagues, we have found that chronic "binge" pattern cocaine administration causes a significant increase in the density of mu-type opioid receptors, very specifically in brain regions that have abundant dopaminergic terminals (i.e., the caudate putamen of the nigrostriatal system; the nucleus accumbens, a site of the primary reinforcing effects of drugs of abuse; the cingulate cortex; and the basolateral amygdala—all regions of the mesocortical–mesolimbic dopaminergic system) (129,131). Chronic cocaine administration using the "binge" pattern also was found to alter both basal and opioid-regulated adenylyl cyclase activity, in a series of related studies conducted in collaboration with the laboratory of Dr. B. Cox (38,130).

In further studies, we have shown that chronic "binge" pattern cocaine administration caused an increase in density of the kappa-type opioid receptors, along with the mu-type; again, this increased density of kappa opioid receptors was seen selectively in regions abundant with dopaminergic terminals, including the caudate putamen, the nucleus accumbens, the cingulate cortex, and the olfactory tubercle (131). However, no changes were seen in density of delta-type opioid receptors following chronic cocaine administration (131).

Quantitative Molecular Biological Studies of Effects of "Binge" Pattern Cocaine Administration on Opioid Receptors and Receptor Gene Expression

In early studies conducted using the "binge" pattern cocaine administration model and the modified RNase protection assay technique detailed above, Dr. R. Spangler found significant upregulation of prodynorphin gene expression, specifically in the caudate putamen region of the rat brain (118, 119,123). In further studies, we have shown that these changes in dynorphin gene expression occur following acute and subacute cocaine administration by this "binge" pattern and persistently occur following chronic "binge" pattern cocaine administration of at least 14 days duration (118,119,123). Recently, Dr. J. McGinty and colleagues, using a similar model, have confirmed that chronic "binge" pattern cocaine administration upregulates prodynorphin gene expression with mRNA estimated by in situ hybridization (20). Also, other studies from both Dr. McGinty's laboratory and that of Dr. J. Hurd, using different molecular biological techniques, including in situ hybridization or Northern blot analysis, have shown that cocaine self-administration in the rat increases prodynorphin gene expression (19,34). Dr. Hurd also has shown that single-dose cocaine and amphetamine administration increases prodynorphin gene expression (33). It is of considerable importance that essentially identical responses to cocaine with respect to dynorphin gene expression were found using the very different experimental paradigms of investigator "binge" pattern administration vs. self-administration in rat models. In some studies addressing various other questions, very different findings have been made in animals "yoked" to, as opposed to directly in, animals self-administrating reinforcing agents, including several drugs of abuse. Thus, administration of drugs by passive administration by way of a "yoked" model could have very different effects.

Dr. Spangler recently has gone on to show that kappa opioid receptor gene expression is also altered following chronic cocaine administration (122,123). Specifically, we have found that mRNA levels for kappa opioid receptors are significantly reduced in the substantia nigra, a region one could hypothesize would be the site of action of negative feedback control by dynorphin peptides produced and released by the dynorphinergic neurons, which originate in the caudate putamen and extend back, with nerve terminals in the substantia nigra (122,123). This recent study reproduced earlier findings indi-

cating that "binge" pattern cocaine administration enhances prodynorphin gene expression in the caudate putamen, but causes no change in kappa opioid receptor gene expression in that region. However, a positive correlation was found between the levels of dynorphin gene expression and kappa opioid receptor gene expression in the caudate putamen of each individual animal, whether treated with saline or treated with cocaine; despite the changes in dynorphin gene expression caused by cocaine, this relationship pertains in both groups (122).

RECENT BASIC CLINICAL RESEARCH STUDIES OF THE DYNORPHINERGIC KAPPA OPIOID RECEPTOR SYSTEM

Based on these early findings, we formulated the hypothesis that dynorphin A may act directly or indirectly to lower dopaminergic tone. We went on to specifically hypothesize that because prolactin release in humans is almost exclusively under tonic inhibition by dopamine, administration of dynorphin A to humans would result in a rapid increase in serum prolactin levels by an action in the hypothalamus to reduce dopaminergic tone. Pilot studies have been conducted and reported by our group; further rigorously controlled studies are currently in progress (85). In these studies, we used the synthetic dynorphin $A_{1–13}$, which has the natural sequence of dynorphin $A_{1–17}$ but is four amino acid residues shorter. Using the technique developed by Drs. J. Chou and J. Yu in my laboratory in collaboration with Dr. B. Chait, head of the Laboratory of Extended Range Mass Spectrometry at the Rockefeller University, we have developed techniques to study ex vivo the processing of both dynorphin $A_{1–13}$ and $A_{1–17}$ in human blood (as well as rat brain) (10–13,15). In our pilot studies, dynorphin $A_{1–13}$ was administered intravenously to healthy normal volunteer subjects without any history of chemical dependency or drug abuse. Two doses of dynorphin, 120 mg/kg and 500 mg/kg, were used. In the pilot study, we found a dose-dependent prompt rise in serum prolactin levels, with the levels returning to baseline in a dose-dependent fashion after 90–120 min (85). In additional studies, conducted both in healthy subjects and subsequently in patients with defined addictive diseases, we will further document this impact of dynorphin on normal physiological regulation of prolactin release, with all studies conducted in the stress-minimized, basic clinical research environment of the NIH-supported General Clinical Research Hospital at the Rockefeller University. We will then go on to determine whether or not there is any difference in response in defined patient populations as contrasted to healthy volunteer subjects. We will also determine in our healthy volunteer subjects whether or not this is primarily an opioid receptor-mediated event, and if so, if it is primarily mediated by the kappa or, alternatively, the mu opioid receptor system (85).

POSSIBLE ROLE OF DYNORPHIN IN MODULATING DOPAMINERGIC TONE

These findings have given further support for our hypothesis that dynorphin may serve to modulate dopaminergic tone. Additional studies are ongoing to investigate our hypothesis that such action may also pertain to specific regions of the brain that are central to the reinforcing and locomotor effects of drugs of abuse. These studies combine laser desorption mass spectrometry (for analysis of biotransformation of dynorphin peptides in specific brain regions) and microdialysis techniques with quantitative measurements of dopamine levels in the ECF to determine the impact of dynorphin $A_{1–17}$ (or its active biotransformation products) on basal dopamine concentrations, as well as effects on the levels of dopamine in ECF during various defined perturbations (12,14,15). All of these findings have suggested that indeed dynorphin, or presumably any highly selective kappa receptor-preferring ligand that is a synthetic analogue of dynorphin, might be effective in modulating dopaminergic tone in the setting of a specific addictive disease and, in particular, might prove to be useful in the management of cocaine dependency.

PATTERNS OF CONCOMITANT COCAINE AND HEROIN USE IN HUMANS

Several patterns of cocaine abuse have been noted in humans; two are of particular interest with respect to any consideration of developing a pharmacotherapy for cocaine abuse. First, it has been known for years that some drug abusers use combined self-administration of cocaine plus an opiate, usually heroin, in a so-called "speedball" to achieve an effect that is putatively different qualitatively, as well as quantitatively, from use of either agent alone (58). However, of potentially more interest in consideration of the topic under discussion herein is the sequential use of cocaine followed by heroin, a pattern we described in the mid-1980s in our observations of heroin addicts seeking entry into methadone maintenance treatment. We found that many persons who had begun their drug abuse career by use of cocaine alone, upon the development of increasing side effects of chronic cocaine administration and all the stigmata of a "crash" (including nervousness, jitteriness, hypervigilance, and insomnia), would attempt to self-medicate their cocaine dependency problems. First attempts to self-medicate usually included use of licit drugs, such as benzodiazepines, or the licit agent alcohol. However, we found that increasing numbers of cocaine addicts were turning to use of short-acting opiates, primarily heroin, to self-medicate their problems. This raised the question early on about some of the potential commonalities on a neurobiologic basis, as well as a behavioral basis, of the effects of the sequential use of cocaine and heroin. More recent findings from my laboratory and several others that cocaine may disrupt the specific components of the endogenous opioid system have given further support to the possible amelioration of cocaine's effects by an opioid.

HISTORICAL PERSPECTIVE ON DEVELOPMENT OF PHARMACOTHERAPIES FOR HEROIN ADDICTION: POSSIBLE IMPLICATIONS FOR DEVELOPMENT OF PHARMACOTHERAPIES FOR COCAINE ADDICTION

It may be helpful in considering the strategy and specific needs in the development of a pharmacotherapy for cocaine addiction to recapitulate some of the very early experiences and scientific findings that were part of our early research in developing the long-term methadone maintenance treatment for heroin addiction. I had the pleasure of joining Professor Vincent P. Dole at the time of inception of his work at the Rockefeller Institute for Medical Research (now the Rockefeller University) in 1963–1964; at the same time he was joined by the late Dr. Marie Nyswander, who became our mentor, because she had been working extensively with opiate addicts both in New York and in Lexington, Kentucky. Our first task was to reexamine the lifestyle and drug self-administration patterns of the heroin addict. What we learned was that the addict self-administers heroin three to six times each day, first to simply achieve a "high" or euphoric effect; in time, with development of increasing tolerance, the addict needs to continually escalate doses of heroin self-administered simply to prevent the appearance of opiate withdrawal symptoms. If no heroin is self-administered to a tolerant and

physically dependent individual, opiate withdrawal symptoms begin within 3–6 h after the last dose of heroin. Therefore, most heroin addicts, because of the short-acting pharmacokinetic profile of heroin, undergo opiate withdrawal or are "sick" several times each day (25,46,47,124,125) (Fig. 3). The negative impact on normal physiology that is effected by chronic heroin use may be due in part to opiate effects (i.e., peak action of high levels of opiate at receptor sites), but it is also due to the profound effects of opiate withdrawal, including all the signs and symptoms thereof and the neurochemical changes, which include activation of the hypothalamic–pituitary–adrenal (HPA) axis (48,57,124,125).

In recent studies from my laboratory, as well as from my laboratory in collaboration with the laboratory of Drs. T. Kosten and M. Rosen at Yale University, we have found that the activation of the HPA axis occurs by the same time that the first subjective and objective identification of signs and symptoms of opiate withdrawal occurs and, in fact, before the appearance of many of the other signs and symptoms of withdrawal (16,39,57,111,112). Before those findings, we nevertheless knew that we wanted to develop a medication that would not yield a rapid rise to a high peak plasma level (or high level at the then-hypothesized specific opioid receptors) or a rapid fall to a low nadir, but rather a medication with a slow onset and a prolonged duration of action that would produce a sustained plasma level over a dosing interval (25,64,65,124,125). Thus, the physiological effects, in theory (and this has turned out to be correct), should be able to be normalized with steady-state perfusion of (the then-hypothesized) opioid receptors (25,58, 61,63,64).

Although at that time there were no analytical techniques to measure levels of opiates (including morphine, heroin, or any potential treatment agent) in plasma or even urine, we were seeking a medication with a long-acting pharmacokinetic profile in humans. The clinical research had to be based on careful clinical observations alone. All of our early studies were also carried out in the protective environment of the NIH-supported General Clinical Research Center of the Rockefeller University Hospital (25). A brief series of early studies was conducted in 1964 to confirm the findings of many earlier studies, primarily conducted at the USPHS Resource at Lexington, Kentucky. These studies again showed that when morphine was administered in repeated doses adequate to prevent withdrawal symptoms, the patients, all former heroin addicts in a medication-free state, were rendered at first "high" or euphoric and then sleepy or somnolent for a period of 3–6 h, followed by the onset of opiate withdrawal symptoms (Fig. 3). Also, continuously escalating doses of morphine were needed to prevent opiate withdrawal when morphine was administered in this intermittent fashion.

We therefore sought a medication that would have a long-acting pharmacokinetic profile and long-acting properties. Based on earlier work, primarily from pain research groups, it had been found that the synthetic opioid methadone, when administered acutely, would relieve pain for a limited duration of time, much like morphine, producing around 3–6 h of analgesia. However, when repeated doses of methadone were given in attempts to get a sustained analgesic effect, it was noted that these relatively opiate-naive subjects would develop mild to moderate respiratory depression, a major and ominous sign of impending narcotic overdose. Methadone had also been used to a limited extent both in the USPHS facility in Lexington, Kentucky, and in at least two detoxification resources in New York, Bellevue Hospital and Manhattan General Hospital. At these sites, methadone was usually prescribed to be administered three or four times a day, although some observations had been made suggesting that such frequent administration might not be necessary to prevent withdrawal symptoms (69).

Chronic Management of Heroin Addiction with a Long-Acting Opioid Agonist

In our first research attempts to develop a pharmacotherapy for the long-term treatment of heroin addiction, the decision was made to study this synthetic opioid, methadone, in a maintenance mode, because it was known to be orally effective and possibly longer-acting than any other opioid available at the time. The initial oral doses of methadone administered were 20–40 mg/day, similar to those used then and now in detoxification units. (The recommended starting dose of methadone to date for most patients is 20–40 mg, although with the increasing purity of heroin, a higher starting dose may be needed in some patients.) Although our very first studies at the Rockefeller Hospital were performed using methadone in divided doses, by the end of the first month of research, we progressed to administering it by the oral route in one dose each day. After initial stabilization on 20–40 mg/day, which prevented withdrawal signs and symptoms, the dose was slowly escalated (at a rate that we now recommend to not exceed 10 mg/week) up to a full treatment dose of usually 80–120 mg/day. Once patients were stabilized on such a dose, there were no narcotic-like effects and no symptoms of narcotic withdrawal, and we found that "drug hunger" or craving had also abated and then disappeared in most patients (25) (Fig. 4).

Role of Opioid Tolerance, Cross-Tolerance, and Classical Deconditioning in Effectiveness of Opioid Agonist Treatment of Addiction

We hypothesized that such a dose would "block" the effects of any superimposed heroin through the mechanism of cross-tolerance; therefore, we conducted a series of studies to determine whether or not such a blockade would occur (25,64,65,69,70). These studies, which were conducted to determine some of the actions and part of the mechanism of action of methadone both in preventing withdrawal symptoms and, possibly more importantly, in preventing or reducing "drug hunger" or drug craving, showed that the steady-state opioid effects per se are augmented by a very powerful addi-

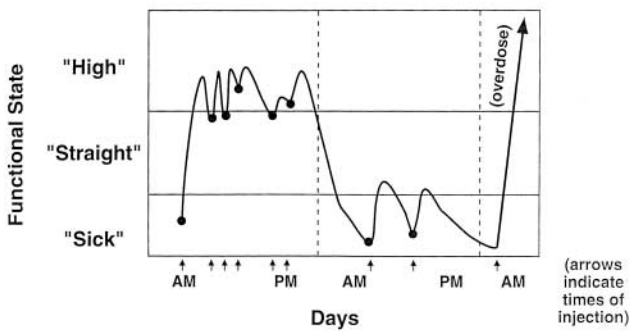

FIG. 3. Diagrammatic summary of functional state of typical "mainline" heroin user. Arrows show the repetitive injection of heroin in uncertain dose, usually 10–30 mg but sometimes much more. Note that addict is hardly ever in a state of normal function ("straight"). [Originally published in and modified from Dole et al. (25), *Arch. Intern. Med.* 118:304–309, copyright © 1966 American Medical Association.]

tional effect that could be considered to be one of classical conditioning and deconditioning. These clinical research studies have been replicated innumerable times in heroin addicts during the early induction period into methadone maintenance treatment. In each case, the addict, achieving no euphoric effects from superimposed self-administered illicit heroin, has to make an important decision: whether to stop methadone treatment to be able to get the "high" or euphoric effects from heroin, or to accept the stabilizing effects of methadone maintenance treatment, with its prevention of opiate withdrawal and reduction or elimination of drug craving. Numerous studies have shown that the majority of patients in early treatment will conduct such self-experimentation during the first 3–6 months of treatment and then will elect to stay in methadone maintenance treatment. This so-called "blockading effect," through the mechanism of tolerance and cross-tolerance, which develops with adequately high treatment doses of methadone, provides an additional mechanism for the efficacy of methadone (in addition to its long-acting pharmacokinetic and pharmacodynamic profile and resultant prevention of withdrawal symptoms, as well as its reduction of drug craving over a 24-h dosing interval).

Pharmacokinetic Studies determining the Long-Acting Pharmacokinetic Profile of Opioid Agonist Pharmacotherapy for Heroin Addiction

By the early 1970s, two groups then working independently (and now united through our NIDA-supported Research Center), Dr. C. Inturrisi working at Cornell University Medical College and my laboratory at the Rockefeller University, had each developed a technique using gas liquid chromatography for the quantitation of methadone levels in plasma (24,36,37,47,74). These studies showed that methadone given in a single dose has an apparent short-acting pharmacokinetic profile because of its wide distribution into tissue stores (24,31,36). However, during chronic methadone administration, as in maintenance treatment, it was found that methadone had a half-life of approximately 24 h (37,47). Further studies, using an isolated perfused rabbit liver preparation, have shown that the liver is one of the primary sites of early distribution and extensive storage of methadone for subsequent release in unchanged form (24,31,75).

In collaboration with the laboratory of Dr. P. Klein, then at the Argonne National Laboratories, we used deuterium to label the separate enantiomers of methadone, yielding three unique stable isotope-labeled species of methadone included in the racemic mixture, all of which could be detected by gas chromatography–chemical ionization mass spectrometry with selected ion monitoring (29,76,94). By using these techniques, we reconfirmed that the half-life of the racemic methadone as used in therapeutics is approximately 24 h; we also found that the inactive *d*(*S*) enantiomer has a shorter half-life of approximately 16 h, whereas the active *l*(*R*) enantiomer has a half-life of approximately 48 h, further supporting the long-acting properties of racemic *dl*(*S*R) methadone in humans (76,94). [In rodent models, methadone has a short-acting half-life of 60 min in the mouse and around 90 min in the rat (5,52).] From numerous studies, we now know that heroin has a half-life of only a few minutes in humans, and its major monoacetylyl metabolite has a half-life of approximately 30 min to 1 h. Its major, final metabolite, morphine, has a half-life of 3–6 h in humans. In sharp contrast, methadone has a half-life of 24–36 h in humans when used in the usual racemic mixture form on a chronic basis. The longer acting congener of methadone, *l*-alpha-acetylmethadol (LAAM), has a half-life of around 48 h, with an even longer acting half-life for its major (and in contrast to methadone) biologically active *N*-demethylated metabolites.

Effectiveness of Management of Heroin Addiction with a Long-Acting Opioid Agonist Agent

By use of long-term pharmacotherapy utilizing adequate doses of methadone (60–120 mg/day for most patients), in conjunction with effective on-site counseling and optimally coupled with direct access to medical and psychiatric care as needed (possibly on-site), it has been shown in the 1960s and in every decade up through the 1990s that there can be a voluntary 1-year retention rate in treatment of 70–85% (2,60, 61,64,66). Also, it has been shown that after the first 6 months of stabilization in methadone maintenance treatment, continuing use of illicit opiates can drop to less than 15% in such effective programs. Prospective and retrospective studies have shown that methadone is medically safe when administered on a long-term basis (45,46,51,61,99).

EARLY STUDIES RELATED TO RESEARCH ON OPIATE ADDICTION AND EXISTENCE OF SPECIFIC OPIOID RECEPTORS

The action of methadone is to provide a steady plasma level and, thus, a receptor level of medication. It is now known that methadone is highly mu-opioid receptor selective. This long-acting narcotic provides steady levels of opioid at the specific mu-opioid receptor sites. In 1963–1964, when our research was begun, we only could hypothesize the existence of opioid receptors. Subsequent seminal work was performed by many groups, including the early work by Dr. Dole and Dr. A. Goldstein, who both hypothesized the existence of specific opiate receptors and provided a research protocol using stereoselective ligands with which to identify those specific receptors (23,28,35). However, it was not until tritiated ligands of high specific activity became available that three groups, those of Drs. S. Snyder, E. Simon, and L. Terenius, each working independently, were able to report essentially simultaneously the existence of

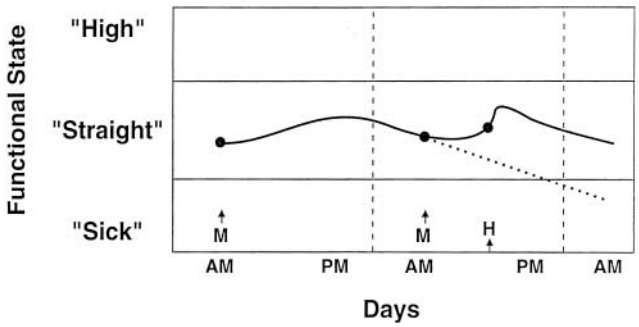

FIG. 4. Stabilization of a patient in a state of normal function by blockade treatment. A single daily oral dose of methadone prevents him from feeling symptoms of abstinence ("sick") or euphoria ("high"), even if he takes a shot of heroin. Dotted line indicates course if methadone is omitted. [Originally published in and modified from Dole et al. (25), *Arch. Intern. Med.* 118:304–309, copyright © 1966 American Medical Association.]

specific opioid receptors by use of the experimental technique of stereoselective vs. nonspecific binding (103, 115,126).

Subsequent studies, a few of which will be briefly mentioned below, have shown that, in addition to preventing withdrawal symptoms and significantly reducing both drug craving or "hunger" and self-administration of illicit opiates, long-term methadone maintenance treatment can bring about normalization of many of the physiological systems disrupted by chronic illicit use of a short-acting opiate such as heroin (46, 51,53,56,62,99).

EFFECTIVENESS OF MANAGEMENT OF HEROIN ADDICTION WITH A LONG-ACTING OPIOID AGONIST IN THE PREVENTION OF AIDS: PUBLIC HEALTH IMPACT

Because of the decrease in drug hunger that occurs during long-term methadone maintenance treatment, self-administration of illicit drugs, which often involves use of nonsterile needles, is greatly reduced or eliminated; this effects a highly significant reduction in exposure to infectious diseases. From a public, as well as individual, health standpoint, it is of great importance that initial HIV-1 or AIDS infection is significantly reduced in patients receiving effective methadone maintenance treatment. In 1983–1984, in collaboration with the Centers for Disease Control, studies were performed in my laboratory using sera that we had banked prospectively from 1969 onward; these samples had been obtained from heroin addicts seeking entry into pharmacotherapy research or into other research protocols, as well as from some patients already stabilized in the early methadone maintenance treatment programs. We found that, by 1983–1984, between 50 and 60% of those heroin addicts in New York City who were not in any treatment program were already HIV-1 antibody positive, that is, infected with the AIDS virus (21,22,95,96). In sharp contrast, in the same study we found that of those patients who had entered an effective methadone maintenance treatment program prior to the advent of the AIDS epidemic in New York City (which we were able to define as having happened in 1978, with rapid escalation after that time), and who had remained in treatment up to the time of the study in 1983–1984, only 9% were HIV-1 positive (21,22,95,96). Also, of great importance, in this first study we found that the 9% who were HIV-1 positive were all methadone-maintained patients who were continuing to use cocaine by parenteral routes of self-administration (95,96,98). Subsequent studies from my laboratory, with our collaborators at the Beth Israel Medical Center, further documented these findings: (a) the protective value of being in an effective methadone maintenance treatment program, with the resulting significant reduction in use of nonsterile needles, and, in contrast, (b) the increased risk for HIV-1 infection for those persons, whether heroin addicts or methadone-maintained patients, who were using cocaine by a parenteral route.

PROBLEM OF COMORBIDITY OF COCAINE ADDICTION WITH OPIATE ADDICTION

One major problem that continues in methadone maintenance treatment, and is very relevant for this discussion, is the concomitant cocaine abuse and cocaine addiction present in a high prevalence of untreated heroin addicts coming into treatment (58). In a study very recently completed by Dr. L. Borg and colleagues, the progression of the magnitude of the cocaine epidemic among heroin addicts in the greater New York area was demonstrated. In the study patients in a single methadone maintenance treatment program were divided into subsets according to time of admission; thus, number of months of treatment duration was determined, along with the impact on several outcome variables (2). Over a period of 5 years, it was found that the percentage of heroin addicts seeking treatment who were also chronic cocaine abusers rose from 40% to almost 80%. It is important to determine the prevalence of cocaine use at the time of entry into methadone maintenance treatment before attempting to determine whether methadone treatment has had a positive or negative impact on cocaine self-administration. In this study, it was found that less than 20–35% of those in methadone maintenance treatment for 6–53 months continued chronic use of cocaine; thus, after 6 months in methadone maintenance treatment, there was a reduction in prevalence of cocaine use from nearly 80% to around 30%. For those who had been in treatment for more than 53 months, continuing cocaine abuse persisted in less than 10% of the patients. These findings, and similar findings from other clinical research groups, have been attributed primarily to successful counseling and other rehabilitation efforts (using the same techniques used in a medication-free treatment) directed specifically against cocaine abuse, as well as concomitant alcohol abuse, in methadone maintenance patients. However, our recent research findings, and some from other laboratories, suggesting disruption of specific components of the endogenous opioid system by cocaine—disruption that may be, in part, normalized by steady-state treatment with high doses of a selective mu agonist such as methadone—indicate that there may also be a beneficial, though probably limited, pharmacological effect of methadone with respect to reducing cocaine dependency. All of these findings provide clues revealing the possible utility of an opioid ligand, preferably one that does not itself create a state of tolerance or physical dependence, as adjunctive treatment for cocaine dependency.

POSSIBLE ROLE OF ATYPICAL RESPONSIVITY TO STRESSORS IN THE NEUROBIOLOGY OF ADDICTIONS

Since the early 1970s, my laboratory has been addressing various aspects of our hypothesis that opiate, and more recently cocaine, addiction may be due, in part, to an inherent or drug-induced atypical responsivity to stress and stressors, which may include significant alterations of the endogenous opioid system and related aspects of neuroendocrine–neurotransmitter function (46,50,59,63,70,148). We have hypothesized that these alterations may persist long after cessation of drug abuse, and may, in fact, in some cases even precede drug abuse, and that they may contribute to drug craving and, thus, to relapse.

Glucocorticoids and Opioids as Modulatory Components of the Stress-Responsive Hypothalamic–Pituitary–Adrenal Axis Function

Since the discovery that a single gene, proopiomelanocortin, encodes for the long-acting opioid peptide beta-endorphin and also yields equimolar amounts of ACTH (both of which circulate peripherally after release from the anterior pituitary, as well as being produced within various brain regions and presumably acting within the brain), it has been very provocative to determine opioid-related events in the classical stress-responsive system, the hypothalamic–pituitary–adrenal axis, and to study the possible role of the endogenous opioids in certain aspects of the derangements of physiology seen in addictive diseases. In humans, the primary regulator of the hypothalamic–pituitary–adrenal axis is the peptide corticotropin releasing factor (CRF), which is produced and released

from specific sites in the hypothalamus and then acts on the anterior pituitary to effect processing and release of the proopiomelanocortin (POMC) peptides, including ACTH and beta-endorphin, which then circulate in blood peripherally (82). ACTH acts on the adrenal cortex to cause release of cortisol in humans (corticosterone in rats and mice), as well as, to a lesser extent, other steroids. The glucocorticoid cortisol (or corticosterone) then acts in a negative feedback control mode, presumably at both hypothalamic and anterior pituitary sites of action, to effect a reduction in release of both CRF and POMC peptides. The negative feedback role has been defined in humans primarily by indirect studies through the measurement of the levels and the responsivity of release of hormones into the peripheral blood, as well as cerebrospinal fluid in limited studies (43).

By conducting studies in animal models, several groups have further documented that the negative feedback by glucocorticoids occurs both at the hypothalamic and pituitary sites of CRF and POMC peptide release, respectively. In extending those studies by using a sensitive and quantitative solution hybridization RNase protection assay to quantitate mRNAs, Dr. Y. Zhou in my laboratory has directly determined that the synthetic glucocorticoid dexamethasone will inhibit hypothalamic CRF mRNA levels significantly (149–151). However, he also found that there is no effect on the levels of mRNA for CRF in other regions of the brain where it is abundantly expressed, including the cerebral cortex, the brain stem, and the amygdala (150). It was also found that both dexamethasone and CRF regulate, in a negative and positive way, respectively, mRNA levels of proopiomelanocortin in the pituitary. POMC mRNA levels are significantly reduced by dexamethasone in the anterior and also in the intermediate lobe of the pituitary of the rat (150). POMC mRNA is significantly increased by CRF treatment only in the anterior pituitary. However, the levels of POMC mRNA are not altered after CRF treatment in other locations, including the posterior lobe of the pituitary, the hypothalamus, and the amygdala. In these studies, it was also found that CRF-R_1 mRNA levels are modulated by dexamethasone (150). As has been previously reported by many other groups, presumably because of these changes in mRNA levels (and thus presumably changes in CRF and POMC peptides, particularly ACTH), it was found that dexamethasone effects a highly significant reduction of plasma corticosterone levels, whereas CRF causes a significant increase in corticosterone levels.

In earlier studies from my laboratory, we determined that short-acting opiates have a profound, persistent, and disrupting effect on hormones of the HPA axis and, therefore, on stress responsivity. Acute administration of either a short-acting or a long-acting opioid in humans causes suppression of the HPA axis; chronic use of a short-acting opioid, such as heroin, which provides regular and intermittent high levels of opiate with profound opiate effects, also continues to provide suppression of this axis (49,51,55,124,125). In sharp contrast, during opiate withdrawal in a tolerant and dependent individual, activation of this axis may be seen and, as discussed earlier, this activation may occur concomitantly with, or even precede, many of the signs and symptoms of opiate withdrawal. These observations suggest that activation of this important stress-responsive axis is not simply due to the stress of withdrawal and the abstinent syndrome per se, but rather may contribute to the abstinence syndrome and be directly due to a rapid relative decline in opioid tone at potentially both the hypothalamic and pituitary sites of action (16,41,42,44,111,112).

We have also shown in healthy normal volunteer subjects, building on the earlier work of Dr. J. Volavka, that specific opioid antagonists including naloxone, naltrexone, and nalmefene cause activation of the HPA axis, with increases in levels of ACTH, beta-endorphin, and cortisol after administration of these antagonists (16,63,68,84,113,136). Further studies are ongoing in my laboratory at this time to determine the role of responsivity to different opioid antagonists, all of which are primarily directed at the mu-type opioid receptor, but each of which has slightly different degrees of selectivity at the delta and kappa opioid receptor systems. Thus, the effects of a specific opioid antagonist in healthy nonopioid-dependent human beings, and the effects of abrupt withdrawal of opiates in an opiate-tolerant and dependent individual, both cause a similar activation of the hypothalamic–pituitary–adrenal axis.

Clinical Research Studies of Stress-Responsive Hypothalamic–Pituitary–Adrenal Function in Opiate and Cocaine Addiction

In extensive studies that we initiated back in 1967, we have explored the integrity of the stress-responsive hypothalamic–pituitary–adrenal axis in heroin addicts who are patients entering into methadone maintenance treatment; these studies are still ongoing, and they have been expanded, with new approaches made possible by advances in sensitive and specific technology, new clinical research strategies developed by our own group, and new types of challenge studies that are now possible. We have found that during the first 2–3 months of methadone treatment, when the initial starting dose of methadone is stabilized and then slowly increased to the full treatment, or "blockading," dose, followed by stabilization at higher dose levels, there is a continuation of modest suppression of HPA axis function (46,51). However, after 3 months or more of steady treatment on moderate to high doses of methadone, complete normalization of this axis occurs, involving normalization of levels of all hormones of the axis, including ACTH, beta-endorphin, and cortisol, and reestablishment of the normal circadian rhythm of release of these hormones (i.e., a classical diurnal pattern, with the highest levels of both ACTH and beta-endorphin and, shortly following, of cortisol, in the early morning and a decline through the day into the early evening hours) (18,46,67,77,78,81).

We have also addressed the question of the response, of heroin addicts entering treatment and former addicts on methadone maintenance, to a chemically induced stressor. Metyrapone is a compound that blocks 11-betahydroxylation in the adrenal cortex, the last step in the biosynthesis of cortisol in humans. By blocking its synthesis, there is a rapid reduction in plasma levels of cortisol, which in turn, temporarily cuts off the normal negative feedback control by cortisol at both hypothalamic and pituitary sites of action. This test has been used for many years by neuroendocrinologists as a test of "hypothalamic–pituitary reserve." The earlier test that was used, prior to the advent of radioimmunoassays to directly measure neuropeptides in peripheral blood, required the administration of multiple doses of metyrapone over at least a 1-day period, followed by 24-h urine collections for 3 days and measurement in urine of the immediate precursors (the "Porter Silber chromogens") of cortisol that accumulate during blockade. A normal response was a significant increase in the amount of precursors, which should appear in urine in response to the increased levels of ACTH circulating after the cutoff of the negative feedback control (18,45,46,48,51). The more recent test, developed after radioimmunoassays to determine the levels of ACTH and beta-endorphin became

available, includes administration of a single oral dose of metyrapone, preceded and followed by the collection of multiple blood samples for up to 8 h after metyrapone administration. Normal response is defined as a 2–4-fold increase in plasma levels of beta-endorphin and ACTH. We have performed both forms of this test, using the early form in our basic clinical research studies from 1967 onward and, since the early 1980s, the single oral dose form.

We have found that there is a reduced hypothalamic–pituitary reserve response to metyrapone challenge in heroin addicts. We have also observed that the test is abnormal during the first 1 or 2 months of methadone treatment, when the dose of methadone is being initially elevated and, then, stabilized (45,46,48,51,53). However, the metyrapone test yields normal responses in former heroin addicts stabilized in methadone maintenance treatment for 3 months or more (45,46, 48,51,53). Thus, whereas in heroin addicts there is a reduced hypothalamic–pituitary reserve, in long-term steady-dose methadone-maintained patients, this response to a chemically induced stressor becomes normal. We have found, in studies that are still ongoing, an apparent hyperresponsivity to this chemically induced stress in medication-free, illicit drug-free, former methadone-maintained patients or former heroin addicts, and we have observed that this hyperresponsivity may persist for many months following the last treatment with or exposure to opiates (39,81). These findings suggest atypical responsivity at hypothalamic and/or pituitary sites of central release of those peptides that regulate the stress-responsive axis. Further, the findings suggest that the role of the exogenous opioids in the negative feedback control of these hormones (which has been enhanced by either heroin addiction or methadone treatment), along with the inhibition by glucocorticoids (which may be elevated in periods of relative or absolute opiate withdrawal, a situation that occurs three to six times a day in most heroin addicts between self-administered doses of heroin), may contribute to this atypical hyperresponsivity following the abrupt reduction in glucocorticoid levels by metyrapone (39,81). Very provocatively, we have recently found a similar hyperresponsivity to the metyrapone test in cocaine addicts during early abstinence from cocaine, including cocaine addicts who are former heroin addicts successfully maintained on methadone with respect to treating illicit opiate use, but also in cocaine addicts who have no other drug abuse problem (6,32,58,59,63). This hyperresponsivity in cocaine addicts, which is currently under further study in my laboratory, suggests that atypical responsivity to stressors may also pertain in this addiction, a phenomenon we hypothesized some time ago; indeed, this hypothesis is supported by increasing laboratory research evidence from many groups, including from members of my laboratory, as well as several very important studies by the group of Dr. P. Piazza in Bordeaux, France (104–107,148).

In other basic clinical research studies, we have found that in addition to normalization of the hypothalamic–pituitary–adrenal axis, there is also normalization of the hypothalamic–pituitary–gonadal axis, findings that have been extensively studied by the groups of Mello, Mendelson, Santen, and others (17,46,51,56,58,67).

Clinical Research Studies of Immune Function as Related, in Part, to Neuroendocrine Function in Opiate Addiction Before and During Pharmacotherapy

In addition in other studies, we have found that there is normalization of specific indices of immune function in patients during long-term, stable-dose methadone maintenance treatment. Some of our very earliest studies have shown disruption of immune function during cycles of heroin addiction (46,51,72,83). More recently, since the advent of the AIDS epidemic, it has been more difficult to study immune status in methadone maintenance patients. It is essential to clearly define whether or not patients are HIV-1 positive before considering any possible role of drugs of abuse per se on disruption of immune function. Similarly, in our earlier work, it was essential to consider the possible role of immune dysfunction from hepatitis B, an infection that until very recently afflicted over 90% of all heroin addicts in treatment and that, along with hepatitis C and delta, and alcohol abuse, can also disrupt immune function (54,72,83,97,100–102).

In many of these studies, including all of our own, the most dramatic findings have been highly significant reductions in natural killer cell activity in untreated heroin addicts (97). We have addressed the question of whether or not opiates such as morphine, the major metabolite of heroin, or the long-acting opiate methadone may alter natural killer cell activity through in vitro studies using human cells, and we have also investigated whether or not opiate antagonists may alter natural killer cell activity in vitro (100,101). In these studies, Ochshorn and colleagues have found no changes in natural killer cell activity, whether studies are conducted using the cells of normal healthy individuals or of former heroin addicts in methadone maintenance treatment (100,101). In contrast, we have found that ethanol causes a dose-dependent reduction of natural killer cell activity when studied in vitro; this result is of potential importance because ethanol abuse is a problem in 20–50% of all heroin addicts entering methadone maintenance treatment and in 20–30% of patients in methadone maintenance treatment (102). With many findings from my laboratory and others concerning the possible effects of opioid agonists on specific components of the immune system, we have conducted a rigorous study to determine whether long-term treatment with the long-acting opioid methadone (which, in fact, exposes the individual in treatment to much higher and more sustained levels of opioids than pertain during cycles of heroin addiction) would cause any abnormalities of immune function (97). What we have learned in a carefully controlled study conducted by Novick and colleagues comparing long-term methadone-maintained patients (who have been in treatment with moderate to high doses of methadone continuously for 11 years or more) and normal healthy subjects with no history of opiate dependence is that natural killer cell activity has become normal in the long-term methadone-maintained patients (97). In addition, absolute numbers of T-cell subsets have become normal, and levels of immunoglobulins IgG and IgM have returned toward normal during long-term methadone maintenance treatment (97). These findings may be of great importance with respect to the ability of the methadone-maintained former heroin addict to have normal immune responses to a variety of diseases, including those that contribute to the progression of HIV-1 infection to AIDS (21,22, 95,96,98).

SUMMARY OF DESIRABLE OUTCOMES OF AN EFFECTIVE PHARMACOTHERAPY FOR OPIATE ADDICTION

These studies and others have shown that normalization of physiological functions that have been disrupted by illicit opiate use can be achieved, along with prevention of opiate withdrawal symptoms and highly significant reduction in "drug craving" or drug hunger. Also, a highly significant reduction

of illicit use of opiates occurs during successful treatment. Additionally, indirect but certainly desirable outcome measures also include a highly significant reduction of criminal activity, as measured by arrests and imprisonments, and a highly significant increase in adaptation to society, including increased vocational pursuits and an increase of conventional employment (61,62,64–67). All of the studies regarding the management of opiate addiction—many of which undoubtedly will be replicated as studies are conducted involving patients receiving long-term treatment with the longer acting opioid agonist 1-alpha-acetylmethadol (LAAM)—support the concept of the need for appropriate pharmacotherapy for an addiction. However, all of these studies also emphasize the need for concomitant counseling of an appropriate intensity, by compassionate, trained health care workers, as well as access to medical and psychiatric care as needed, preferably at a single location, which has been shown to be both effective for delivery of health care services and also cost-effective.

SUMMARY OF SOME PERSPECTIVES AND HYPOTHESES: CHALLENGE FOR DEVELOPING A PHARMACOTHERAPY FOR COCAINE ADDICTION AND FURTHER PHARMACOTHERAPIES FOR HEROIN ADDICTS

In summary, from our extensive (now 32-year) experience with first developing a pharmacotherapy for opiate addiction, one that has turned out to be both safe and highly effective when appropriately used, and also from our continuing studies both on the medical status of heroin addicts entering treatment and on the physiological effects of short-acting opiates (such as heroin and morphine) and, later, of long-acting opiates (such as methadone), we have learned a great deal about what may be the effects of chronic illicit use of short-acting opiates on normal physiology. We have also learned which of these effects or drug-induced abnormalities may be persistent or long-acting, as well as which effects may be reversed by successful pharmacotherapy with a long-acting opioid such as methadone and probably also LAAM (67). Our research findings have taught us a great deal about what may be the role of the endogenous opioids or endorphins in normal human physiology, and how the endogenous opioid system may be perturbed by chronic use of drugs of abuse.

Basic clinical research, as well as bench research from my laboratory and many others, has supported our early hypothesis that an atypical responsivity to stress and stressors may contribute to the acquisition and persistence of and relapse to opiate addiction. More recently, there is increasing evidence that such atypical responsivity to stressors possibly also contributes to the pathophysiology of cocaine addiction and alcoholism. We have confirmed our early concept that a successful pharmacotherapeutic agent should be targeted to a specific site of action or receptor or, alternatively, to some aspect of normal physiology that is chronically perturbed or altered and made persistently abnormal by illicit use of drugs.

We also have further confirmed our early concepts that ideally a pharmacotherapeutic agent for an addictive disease would be orally effective, to prevent any need for parenteral use and to avoid medication preparations with abuse liability. Such a medication should have a slow onset of action to prevent any "rewarding" or reinforcing effects, a long duration of action to permit a dosing pattern of once a day or once every other day, and a gradual offset of action to prevent any rapid onset of usual or atypical withdrawal symptoms. We have learned through innumerable studies that for most unselected opiate addicts, an opiate agonist such as methadone or LAAM, or possibly a partial agonist such as buprenorphine, is far more successful than an opioid antagonist such as naltrexone. Data from my laboratory and more recently others have suggested that long-acting opioids such as methadone may allow normalization of physiology that has been significantly altered by chronic use of short-acting opiates. These aspects of physiological function may have been abnormal, or alternatively vulnerable to the induction of abnormalities, on a genetic basis, before any exposure to the illicit drug use, a topic under intense investigation at this time in many laboratories, including our own.

Possible Usefulness of a Sustained-Release or Long-Acting Opioid Antagonist for Management of Early Heroin Addiction

Normalization of several aspects of physiology apparently does not occur fully during use of the relatively short-acting opioid antagonists, primarily naltrexone and naloxone, that have been available for study or use in therapeutics to date. Whether or not the persistent derangement in physiology contributes to the very high rate of dropouts from naltrexone treatment in unselected populations of heroin addicts, and to the general lack of patient acceptance of this type of medication by heroin addicts, has yet to be fully determined (41,42, 44,63,68,71). However, many studies have shown that less than 15% of unselected heroin addicts voluntarily accept and remain in chronic naltrexone treatment for 1 year or more (68). During such treatment, certain aspects of physiology, including abnormalities of the hypothalamic–pituitary–adrenal stress-responsive axis, continue to pertain. These persistent abnormalities may or may not contribute to the very low patient acceptance of antagonist treatment in unselected populations of heroin addicts under no coercion (such as loss of probation status or loss of professional license). Future studies using opioid antagonists with longer pharmacokinetic profiles, or using sustained-release formulations of currently available antagonists, could address part of this issue. It has been shown that steady-state intravenous infusion of the very short-acting opioid antagonist naloxone does not result in activation of the hypothalamic–pituitary–adrenal axis, at least in healthy non-opioid-dependent volunteers with no history of addiction (30,79,80). Use of a sustained-release or long-acting opioid antagonist could be of special utility in the management of short-term illicit opiate users or early addicts not meeting the current federal guideline criteria for entry into methadone or LAAM maintenance treatment.

Possible Usefulness of a Dopamine Transporter-Directed Agent for Management of Cocaine Abuse or Addiction or in Relapse Prevention

With respect to the specific enormous needs at this time to develop pharmacotherapeutic agents for cocaine addiction, many research findings that have been made by other investigators, but also highlighted by certain research findings of our own work, are now available to provide clues as to what could be ideal sites of action for such medications. Studies have been conducted by many laboratories addressing the possible therapeutic approach of blocking the dopamine transporter. To date, no pharmacotherapeutic agent of this type used on an experimental basis has been shown to be effective, although the data with respect to use of methylphenidate, are, in part, inconclusive. Also, many efforts are under way to attempt to develop a chemical agent that would be safe to use in humans and would be directed solely at the cocaine binding site on the dopamine transporter, thus permitting normal function of the dopamine transporter while preventing any action of cocaine. Such agents will be extremely interesting to

study in various appropriate animal models, and ultimately in humans, when they are available and when appropriate preclinical toxicity studies are completed.

Possible Usefulness of D_1 Agonists or Partial Agonists in the Management of Cocaine Abuse or Addiction or in Relapse Prevention

Data from many laboratories suggest that chronic disruption of D_1 receptor density, binding potential, and/or activity, especially as related to the putative role of the D_1 receptor in modulating dynorphin peptide production, may play a major role in cocaine addiction (114,119,120,122,133). Many studies suggest that, although both D_2 and D_1 receptor types, as well as possibly other subtypes of dopamine receptors, may be altered by chronic use of cocaine, the D_1 receptor system may be predominant and important from a pathophysiological standpoint (114,119,120,122,133). We have suggested for several years now that a D_1 agonist, or possibly preferably a D_1 partial agonist, with a slow onset of action and a long duration of action, and one that has modest intrinsic activity, providing an attenuated effect, but that has effects in the same direction as cocaine in its reinforcing effects, could be potentially effective in its management of cocaine addiction. Such a medication might allow enhancement of dopamine activity, possibly (by medication formulation) on an intermittent basis, thus emulating a major neurochemical response to many pleasurable stimuli (including certainly not only illicit use of drugs, but also normal pleasures such as ingestion of desired food) or the expectation of such a pleasurable response.

At this time, only relatively modestly selective D_1 agonists or partial agonists are available for use in humans (FDA approved for safety and efficacy); several compounds directed at other sites within the dopaminergic system are available, but most of these are not selective. When such a highly selective D_1 agonist or partial agonist agent becomes available, it will be of interest to conduct a variety of neurobiological and efficacy studies, using the medication not only in a conventional formulation but also in studies in which small doses of the compound are released for intermittent activity over an appropriate dosing interval. Certainly the experience with the management of opioid addiction suggests that a compound that blocks all of the so-called desired effect of an opiate (i.e., a pure, specific opioid antagonist such as naltrexone) is not effective in the majority of unselected long-term heroin addicts. However, clearly one does not wish to emulate the enormous surge of effects, in large part due to the surges in dopamine concentrations in the extracellular fluid, that are seen after cocaine self-administration. An attenuated activity dopamine agonist, one possibly directed to the D_1 receptor, would be of considerable interest and could become possibly an accepted short-term or even long-term therapeutic agent for cocaine and possibly other stimulant addictions.

Possible Usefulness of a Dynorphin-Like Kappa Opioid Receptor-Directed Agonist or Partial Agonist in the Management of Cocaine Abuse or Addiction or in Relapse Prevention

Another neurotransmitter neuropeptide system that has been documented by several groups, including our own, to become markedly deranged during cocaine addiction, is the dynorphinergic system and, in more recent work from my laboratory, the dynorphin-linked kappa opioid receptor system. It is of great interest, of course, that many laboratories have linked D_1 receptor activity with dynorphinergic tone, especially in the nigrostriatal system and also in the limbic system. More basic studies will be needed to determine the relative role of derangement of the dynorphinergic systems, in the nigrostriatal system, as contrasted with the excessive activation of the mesolimbic–mesocortical dopaminergic systems. Also, more investigation is needed of the direct relevance of such dynorphinergic derangements to the development by cocaine of continual drug craving and self-administration, and to the reinforcing effects of cocaine in the development of cocaine addiction. It is of interest that studies from my laboratory, as well as from the laboratories of Drs. J. McGinty and J. Hurd, have clearly shown that dynorphin gene expression is similarly significantly enhanced following both "binge" pattern cocaine administration, as in our model, and self-administration of cocaine, in other animal models. More recently, we have also found that kappa opioid receptor gene expression is reduced in what would be the putative feedback area of control, that is, in the substantia nigra. Other work in my laboratory, using the techniques of quantitative autoradiography, has shown upregulation in density of the kappa opioid receptors in the caudate putamen, as well as in the nucleus accumbens and in the olfactory tubercle sites, all regions where dopaminergic terminals are abundant. Studies done by our group, along with earlier work from the group of Drs. A. Herz with T. Shippenberg and R. Spanagel, have all suggested that synthetic kappa opioid receptor ligands, with varying degrees of selectivity, may attenuate dopaminergic levels in some specific brain regions, investigated using the technique of microdialysis. Recent ongoing microdialysis studies from my laboratory suggest that dynorphin $A_{1–17}$ itself may attenuate dopaminergic levels in the nucleus accumbens (15).

In our human research, several years ago we reported pilot studies (and a rigorously controlled long study is currently under way) to determine whether in healthy humans there is any evidence that dynorphin peptides may attenuate dopaminergic tone (85). In our pilot studies, we found that intravenous administration of the shortened, but natural, sequence dynorphin $A_{1–13}$ will cause an abrupt and modestly sustained rise in serum prolactin levels (85). Because, in humans, prolactin release is essentially completely under tonic inhibition by dopamine, these findings suggest that the intravenous administration of dynorphin successfully achieves an abrupt decrease in dopaminergic tone in the critical hypothalamic–pituitary regions governing prolactin release into the systemic circulation. The fact that the prolactin increasing effects were observed to occur in a dose-dependent fashion and to have a dose-dependent duration (ranging from 90 to 120 min with the doses studied to date) provides evidence that, in humans, dynorphin may modulate dopaminergic tone.

CHALLENGE FOR THE FUTURE

These various research findings from my laboratory, as well as many other findings from numerous other laboratories, point the way to possible sites of action of potential pharmacotherapeutic agents for cocaine dependency. It should be underscored that any pharmacotherapeutic agent, to be effective, will have to be specifically tailored with respect to the targeted site of action, mode of formulation, and route of administration, as well as the intrinsic or fabricated pharmacokinetic profile, to provide the desired sustained duration of action (or intermittent action, if that indeed turns out to be more appropriate for cocaine dependency). At the same time, however, it will be essential to configure a pharmacothera-

peutic program that provides not only the pharmacotherapeutic agent, but also appropriate access to medical care, psychiatric care, other behavioral care and intensive counseling as needed. It has been repeatedly shown that methadone maintenance programs may be highly effective when such a combined approach is used. However, because of stigmatization of treatment, unfortunately, decreasing numbers of treatment programs have been appropriately configured. This stigmatization presents a major challenge for us all, as basic scientists as well as clinical scientists and clinicians (1). We all have an ongoing obligation to interact with, to educate, and thereby to help guide our policymakers, who will, in large part, determine the extent of appropriate utilization of any new (or existing) pharmacotherapeutic agent and, thus, the success of such agents in treating specific addictive diseases.

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