Goals and Rationale for Pharmacotherapeutic Approach in Treating Cocaine Dependence: Insights From Basic and Clinical Research

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The early research conducted in the author's laboratory from 1975 onward stemmed from the even earlier work, beginning in 1964 when the author was a member of the laboratory of Professor Vincent P. Dole at the Rockefeller Institute for Biomedical Research (now the Rockefeller University) (Dole et al. 1966; Kreek 1972, 1973*a*; Kreek et al. 1972). At that time, scientists were challenged to develop a treatment for opiate dependency, a problem that is still being addressed, but for which there are now three different pharmacotherapeutic approaches approved by the Food and Drug Administration (FDA), and a fourth under investigation. This chapter will review briefly some of the early concepts because they are relevant for the current major problem: developing a new medication (and possibly a variety of medications) for treating cocaine dependency.

In 1964, researchers had recognized the need to develop a pharmacotherapy for the treatment of opiate dependency, because the most humane and excellent drug-free approaches were not effective for the majority of patients. It must be emphasized from the outset that any pharmacotherapy for managing any addictive disease must be carried out in concert with excellent psychosocial interventions, with counseling and rehabilitation efforts. The very complex disorders of any one of the addictive diseases can only infrequently be managed with chemotherapy alone. Researchers have to be as realistic with respect to cocaine dependency, as they were 30 years ago with respect to opiate dependency. It should be remembered that even the very best "drug free" psychosocial and rehabilitation approaches alone have been successful for extended periods of time in only a limited number of persons, and have been far too limited to accept these as the only approaches available for treatment. Ultimately, what is needed in most cases of both opiate and cocaine addiction is combination therapy. This chapter, however, will be limited to pharmacotherapy.

Several recent studies, including the high school student and household surveys, have elucidated the current magnitude of the cocaine problem. It is estimated that around 24 million people in the United States have used cocaine at some time; over 3 million are occasional cocaine users; about

1.3 million are current cocaine users, and at least 600,000, and maybe many more, are very frequent users, defined as multiple uses each week, either by "binge" pattern or a regular daily use pattern (Kreek 1996). It should be asked, "For which one of these groups are researchers seeking to develop pharmacotherapy?" This is a question that has not been addressed as potential medications for treatment have been identified and clinical trials for treatment of cocaine addiction have been conducted. Researchers have failed to ask, "For whom is this particular medication targeted?" or more generally, "For what group of persons afflicted with chemical dependency problems, of what type, severity and duration?" These questions are critical. If a nicotine patch is used on someone who smokes only 1 cigarette a day, the investigator or clinician may be dealing with a very different situation, and thus outcome, than when investigating, or attempting to treat someone who smokes 10 cigarettes or 3 packs of cigarettes a day. Results may be different because the neurobiology, as well as the behaviors, are different. Researchers need to develop operational definitions and guidelines, such as in 1964 when the author and her colleagues were forced, over a brief period of time, to define opiate addiction. The original operational definition was multiple daily doses of opiate use, usually heroin, with the development of tolerance, physical dependence, and drug-seeking behavior, and a duration of that pattern of behavior for 3 years or more. In a stepwise manner, with the advice of the author's group and many others, the official definition of "3 years" has been, as of 1983, reduced to 1 year of that pattern of behavior, and thus defined the duration of addiction. The Institute of Medicine has published recommendations on the regulations governing methadone treatment (Rettig and Yarmolinsky 1994); the FDA and the Drug Enforcement Agency (DEA) may be considering the available information supporting the concept that even 1 year of daily illicit opiate use may be too long to demand for entry into a pharmacotherapy using an opioid agonist or, alternatively, partial agonist, such as methadone, l-alpha-acetylmethadyl (LAAM), or in the future, possibly buprenorphine (Rettig and Yarmolinsky 1994). The 3-, then 2-, and now 1-year length of addiction requirement is based on the operational definition first formulated in 1964. It still is an operational, not a medical or neurobiological definition, but it has served clinical scientists, clinicians, and policymakers very well because groups of subjects can be compared with respect to their response to treatment, as well as their neurobiology status. There is a definite need now to define different levels of cocaine dependency at an operational level, which will understandably be imperfect biologically, but at least will allow for more effective comparison of clinical and fundamental studies.

If researchers believed that cocaine dependency was solely a behavior that occurs in a social and environmental context, with no neurobiological ramifications, that is, with both no possible predisposition on a genetic basis, and/or with no persistent or permanent alterations of physiology as a result of its use, then no one would discuss the need for development of a pharmacotherapeutic agent. However, irrespective of the hypotheses that may be formulated and addressed, most agree that there are probably either genetic factors that confer or augment vulnerability to develop each of the specific addictions, and/or persistent or permanent changes effected by the drug of abuse, which may contribute to, or cause, the acquisition and perpetuation of the drug-seeking behavior and also the persistence of "drug hunger" or craving, with the proclivity for relapse. Especially important for cocaine addiction is the profound craving associated with the cocaine-abstinent state. However, any genetic or neurobiological factors must be considered in a contextual setting, including: the individual's stage of development, what other kinds of exposures there have been (including both diseases and drugs), and the individual's response to stressors. Also important is the overall environment, and especially the set and setting of drug exposures and related economic factors.

The goals and rationale for the development of a pharmacotherapy for addictions have evolved in the author's laboratory over the past 20 years, based in part on early conceptualizations 30 years ago with respect to opiate dependency and, more recently, with respect to both cocaine dependency and alcoholism (Dole et al. 1966; Kreek 1972, 1973*a*, 1973*c*, 1978, 1991, 1992*a*, 1992*b*, 1992*c*; Kreek and Hartman 1982).

First, an agent must prevent any physiologically based withdrawal symptoms (Kreek 1992*c*). This is especially important with opiate dependency, though possibly of lesser importance with respect to cocaine dependency. However, there are dramatic histories and presentations in the literature of cocaine withdrawal symptoms, especially in the outpatient setting, where cues and other conditioned factors may play a dominant role. In a quiet, stress-minimized inpatient setting, such as that of the clinical research group at the Addiction Research Center, which is the intramural program of the National Institute on Drug Abuse (NIDA) and the author's laboratory at the Rockefeller University resource at the NIH-supported General Clinical Research Center (GCRC) of the Rockefeller University Hospital, as well as in other clinical investigators' settings, only modest to absent withdrawal symptoms have been described in recently abstinent cocaine addicts (Cambor et al. 1992; Ho et al. 1992).

Secondly, a pharmacotherapeutic agonist needs to reduce drug craving or "hunger." For cocaine dependency, this goal has to be at the top of the list. Long after the cocaine "binge" is over and the cocaine has cleared the body and the major benzoylecgonine (BE) and the other metabolites are gone, craving still persists (Kreek 1992*c*). In fact, relapse may be seen at very distant timepoints and, although cues and conditioning play a role, cravings have arisen in very sterile settings such as a clinical inpatient research unit.

The third goal of any specific pharmacotherapy is normalization of physiological functions disrupted by drug use. Functions that have been disrupted may be epiphenomena. However, it is important to note that some of the disruptions are of the stress-responsive axis, which has been hypothesized to contribute to the perpetuation of drug-seeking behavior.

Finally, any medication ideally should be targeted to a specific site of action, a receptor, or a physiological system, which has been affected or deranged by the drug of abuse in a very specific manner. Therefore, it is imperative that it is clear what the drugs of abuse do, where they act, what the actions are, and what the immediate as well as distant ramifications are, on a biological and neurobiological basis.

After recruitment as a resident in internal medicine at New York Hospital Cornell Medical Center in the autumn of 1963 by Professor Vincent P. Dole, the author had the opportunity to do a research elective at the Rockefeller Institute for Biomedical Research and in early 1964, to join Professor Dole and the late Dr. Marie Nyswander, who also arrived at that time, in the initial research efforts to address the following question: Could a pharmacotherapy for opiate dependency be developed? Some of the criteria for a research pharmacotherapeutic agent then and now are: (1) ideally, the medication should be orally effective; (2) there should be a slow onset of action of that medication to eliminate any reinforcing effects of the agent, so it would not become a primary drug of abuse; and (3) the drug should be long-acting with a gradual offset, as well as onset, of action.

Methadone, which at that time had been studied to a very limited extent, and used in few resources for the "detoxification" of heroin addicts, met all three of these criteria. In 1964, there were no analytical techniques to measure sensitively and specifically any opiate in blood or even in urine; thus clinical observations had to be used to assess the pharmacology of a potential research treatment agent, based on the observed pharmaco-dynamics. In addition, at that time, a medication was sought that could be given in doses that would not cause euphoria or any other kind of opiate effect. This was achieved with methadone. However, in some early

research studies, when doses of methadone were given that exceeded the degree of tolerance developed by the individuals, although true euphoria was not observed, somnolence and sleepiness occurred. If a dose of methadone was selected initially to be less than that for which tolerance has been developed by the individual, no euphoria, no sleepiness, and no other narcotic-like effects would be detected. The dose then could be ascended slowly to achieve ultimately a dose that provides not only tolerance, but cross-tolerance to other opiate drugs. Through the mechanism of cross-tolerance the effects of any super-imposed short-acting opiates are "blockaded" (Dole et al. 1966). Finally, a medication should prevent withdrawal symptoms. This may be of lesser importance with respect to the management of cocaine dependency since in a controlled, stress-minimized environment such as a hospital or clinical research unit, withdrawal symptoms following cessation of cocaine use are minimal (Cambor et al. 1992; Ho et al. 1992). However, each one of these characteristics must be sought in the development of pharmacotherapeutic agents for the management of cocaine dependency. Also, for cocaine dependency, like the case of opiate dependency, where it has been clearly desirable to have more than one therapeutic agent, it would be desirable to have several pharmacotherapeutic agents with different actions and mechanisms of action to manage the diverse populations needing treatment.

By 1972, two groups then working independently (now both part of the NIH-NIDA Research Center) developed techniques for measuring plasma levels of methadone, using gas-liquid chromatography (Dole and Kreek 1973; Inturrisi and Verebely 1972, 1973; Kreek 1973*b*, Kreek et al. 1976). Researchers found precisely what was observed clinically, that is, after a single oral dose of methadone during chronic steady-dose treatment, there is a sustained plasma level over a 24-hour dosing interval (Inturrisi and Verebely 1973; Kreek 1973*b*). Secondly, the rise to peak level is gradual, with a resultant gradual onset of action; the peak levels do not occur until 2 to 4 hours after the oral dose is given. The peak plasma levels are very modest, barely a doubling of the nadir. With this slow rate of rise and low peak levels, thus the very slow onset of action, no reinforcing effects or narcotic-like effects are expected, if the proper dose has been administered. From a kinetic standpoint, whereas heroin has a half-life in humans of 1 to 2 hours, and the major morphine metabolite of heroin is 4 to 6 hours, methadone has a half-life of 24 hours in man (Inturrisi and Verebely 1972, 1973; Kreek 1973*b*). Using stable isotope techniques to label both the active and inactive enantiomer of methadone with different amounts of deuterium at specific nonmetabolically reactive sites, the author and her colleagues were able to define, using chemical ionization mass spectrometry, that the half-life of the active l-enantiomer

was 48 hours (Hachey et al. 1977; Kreek et al. 1979; Nakamura et al. 1982). This long-acting profile of methadone occurs uniquely in humans. In rodents, methadone has a half-life of about 60 to 90 minutes, similar to that of morphine (Kreek 1979). Thus, the pharmacokinetic profile would have been able to be elucidated only in humans, where it would be ultimately determined.

In good treatment programs, steady and adequate doses of methadone are used, 60 to 120 mg/d for the average patient, after slow escalation from initial lower doses, followed by stabilization of dose (Dole et al. 1966; Kreek 1991, 1992*a*, 1992*b*, 1992*c*). Use of stabilized doses in treatment is critical. One should never use changes in methadone dosage to effect a behavioral change, or use dose changes in a contingency contract for behavior modification. Doses must be kept constant. Otherwise, the rationale and proven mechanism of methadone action as used in appropriate pharmacotherapy is impaired and the desired normalization of disrupted physiology is not achieved. In good programs, there should also be concomitant rehabilitation efforts, psychosocial support systems, and access to medical and psychiatric care. Also, in good programs that combine adequate and stable doses of methadone combined with other psychosocial, counseling, and medical services, retention in 1964 and retention in 1994 (as in the two clinics connected with the NIDA Research Center) ranges from 70 percent to 85 percent, and, after the first 6 months of stabilization in treatment, continuing use of heroin drops to below 15 percent. The actions of methadone prevent withdrawal symptoms and also prevent "drug hunger" or craving, that is, the desire to use other illicit opiates (Dole et al. 1966; Kreek 1991, 1992*a*, 1992*b*, 1992*c*; Rettig and Yarmolinsky 1994). However, the blockade of the euphorogenic or other narcotic-like effects of any superimposed short-acting opiates that is achieved by adequate steady-dose methadone treatment also means that, when on methadone, a patient who tries to get a euphoric or "high" sensation from illicit heroin cannot do so unless extraordinarily large and expensive amounts of heroin are used. In the author's original titration studies, over $200 equivalent of illicit heroin purchased on the streets of New York administered intravenously in a single dose was needed to override the cross-tolerance developed by a full blockading treatment dose, i.e., 60 to 120 mg/day of methadone (Dole et al. 1966).

For any pharmacotherapy to be developed for treatment of cocaine addiction, it may be essential both to block the acute reinforcing and euphorogenic effects of cocaine, and also reduce or eliminate the chronic and persistent craving for cocaine which leads to relapse. It may or may not be found that a single pharmacotherapeutic agent can effect both of these desired effects, since there is evidence from the author's laboratory

that more than one neurobiological mechanism may be involved (Branch et al. 1992; Kreek 1987; Maggos et al. 1995; Maisonneuve and Kreek 1994; Maisonneuve et al. 1995; Spangler et al. 1993*a*, 1993*b*, 1994, 1995; Unterwald et al. 1992, 1993, 1994*a*, 1994*b*).

It was also seen that during chronic long-term methadone maintenance treatment, when a stable moderate to high dose of methadone was used, there was normalization of several physiological functions that were critical for normal survival functions as well as for generalized well-being, including behavioral and emotional status, which were functions that were disrupted by chronic use of heroin. Normalization of the stress-responsive hypothalamic-pituitary-gonadal axis, and also normalization of the hypothalamic-pituitary-adrenal axis involved in reproductive behaviors and biology, occur during chronic steady-dose treatment (Cushman and Kreek 1974*a*; Kennedy et al. 1990; Kosten et al. 1987, 1992; Kreek 1972, 1973*a*, 1978, 1992*c*; Kreek and Hartman 1982; Kreek et al. 1981, 1983, 1984*a*, 1984*b*). A moderate extent of normalization to prolactin responsivity occurs, although there is still responsivity of prolactin release as reflected by peak levels of prolactin at the time of peak plasma levels of methadone. However, prolactin levels above normal are not reached (Kreek 1978).

Linked in part to normalization of neuroendocrine function, normalization of immune function indices also occurs during chronic long-term methadone treatment, including normalization of natural killer cell activity, absolute numbers of T cells, T-cell subsets, B cells, and NK cells and, when studied after stabilization for 10 years or more, near normalization of levels of immunoglobulins IgG and IgM, which are profoundly elevated in untreated street heroin addicts (Kreek 1973*a*, 1978, 1994; Kreek et al. 1972; Novick et al. 1989; Ochshorn-Adelson et al. 1994; Ochshorn et al. 1990). Natural killer-cell activity is reduced to a potentially clinically significant level in untreated heroin addicts. This is probably due to multiple factors, including injection of multiple foreign substances and other diseases, but also possibly to indirect or direct opiate effects. It is less clear why there are increased absolute numbers of T cells and B cells in untreated heroin addicts when HIV infection is not present, but that has been a reproducible finding from many studies (Novick et al. 1989; Ochshorn et al. 1990; Ochshorn- Adelson et al. 1994).

By using appropriate doses of methadone, an effective methadone program also prevents drug craving and thus prevents use of dirty needles to inject illicit drugs. When the author carried out a study in which sera that were prospectively banked from 1969 onward from all research subjects entering basic and clinical research in the laboratory were

unbanked and studied in 1983-84, HIV was detectable when the AIDS epidemic appeared in the parenteral-drug-abusing population in New York City (DesJarlais et al. 1984, 1989; Kreek et al. 1990; Novick et al. 1986*a*, 1986*b*). This also allowed the author's group to ask about the impact of effective methadone treatment on acquisition of HIV-1 infection. In the Centers for Disease Control (CDC) Bulletin published in the summer of 1984, the author and her colleagues reported that effective methadone treatment prevents HIV-1 infection by reducing or eliminating use of unsterile needles (DesJarlais et al. 1984). Ten years later, there are still waiting lists for entry into methadone maintenance treatment in many regions, and no access to treatment in many other areas. Also over the past 10 years, the unit funding for each treatment resource has gone down, resulting in too few effective or "good" programs, which by definition, use adequate and stable doses of methadone, and also provide ready access to onsite counseling, medical, and psychiatric care. Whatever efficacious medication for treatment of cocaine dependency might be developed, if there are no appropriate treatment resources in which to deliver it, that is, programs that can combine pharmacotherapy with support services in a proper environment, there will never be a therapy that will be effective and generally accepted by patients and by the community. Researchers must address the need for proper access to treatment and form appropriate, effective programs, while developing new medications and conducting exciting and potentially important neurobiological studies.

In addition to methadone, other medications have been developed for treatment of chronic opiate addiction. A longer-acting congener of methadone, which like methadone is a pure opioid agonist directed at the mu opioid receptor LAAM (for which NIDA is to be credited for gaining prompt FDA approval) is now available for maintenance treatment. Buprenorphine, a partial agonist, or mixed antagonist, is currently under study. Naltrexone, a pure opioid antagonist, was approved by the FDA several years ago. Each medication may be beneficial for some heroin addicts, yet each will require administration by appropriately trained staff and with appropriate monitoring of all patients in treatment, as well as initial administration in a treatment modality that will also provide counseling, rehabilitation efforts, AIDS risk reduction, education, and access to medical and psychiatric care, especially for addicts first entering treatment.

In the "worst" or most limited in terms of ancillary services, of all programs reported, in a controlled study that administered an adequate dose of methadone (often not done in minimal services clinics), as reported by McLellan and O'Brien in their "three levels of treatment

study," it was found that giving out methadone alone has a 45 to 55 percent success rate in terms of stopping illicit opiate use in unselected heroin addicts (McLellan et al. 1993). In contrast, naltrexone, in unselected heroin addicts (not small groups of physicians or nurse addicts or special groups such as those on probation with a 6-month contract), in the best of studies, including the pivotal multicenter trial planned by the National Academy of Sciences group, has been shown to have effectiveness only in 15 percent to 20 percent of patients and then only for a very short time. This low level of effectiveness resulted in stopping this multicenter trial much earlier than planned (National Research Council Committee on Clinical Evaluation of Narcotic Antagonists 1978). Also, drug-free treatment, in the best of approaches and the worst of approaches, results in only 15 to 30 percent success, measured by retention and remaining in an abstinent state for 1 year or more (Cooper et al. 1983).

Clearly, a pharmacotherapy for cocaine as well as for opiate and alcohol addiction is needed, but it should be appropriately delivered in well-staffed and broad services programs, as discussed earlier. Also needed is a definition of cocaine dependency in terms of different stages of duration and severity so that data analogous to the abundant data on treatment of opiate addiction can be presented. Opiate addiction as defined for criteria for entry into methadone maintenance and LAAM treatment, and thus for all studies discussed here is, is at least 1 year of multiple daily dose uses of heroin, with the development of tolerance, physical dependence, and drug-seeking behavior. Studies have yet to be performed on drug abusers with less than 1 year of daily or intermittent heroin use, of the efficacy of naltrexone, for whom naltrexone is the only pharmacotherapy, by law, that can be used. Also necessary are natural history studies on early drug abusers with opiate dependency for less than 1 year, i.e., short-term addicts. Similarly, 1 year of daily or at least weekly "binge pattern" cocaine use might provide a satisfactory definition for long term or "hard-core" cocaine addicts. Researchers could then target a pharmacotherapy for such a long-term or "hard-core" group and correlate data from treatment outcome studies across centers. At the same time, researchers could develop an intervention for cocaine abusers with a shorter history of use.

The author's laboratory has worked on the hypothesis that the endogenous opioid system may be involved to some extent, and possibly to a considerable extent, in each of three major addictive diseases: opiate, cocaine, and alcohol addictions. The endogenous opioid system has three classes of endogenous opioid peptides: the endorphins, the enkephalins, and the dynorphins. Single genes code for each of these three classes of

endogenous peptides. These genes have been cloned, and the biochemical characterization of the several opioid and nonopioid peptides from the single precursor peptide, as well as some of the mechanisms for processing and biotransforming the parent peptides to those various peptides, has been defined. In December 1992, the first opioid receptor gene, the delta opioid receptor gene, was cloned independently by Evans and by Kieffer (Evans et al. 1992; Kieffer et al. 1992). This was soon followed by cloning of both the mu and kappa receptors by Yu, and the mu receptor gene by Uhl, Akil, and Watson, and others (Chen et al. 1993*a*, 1993*b*; Thompson et al. 1993; Wang et al. 1993, 1994*a*, 1994*b*). At this time it seems that there are indeed three genes coding for three types of opioid receptors, as had been predicted through many cell biological and medicinal chemistry studies using primarily selectively synthetic ligands. To date, no separate genes to explain subtypes of each of these three types of opioid receptors, as defined by use of selective chemical ligands, have been found. There is a fourth (or more) "orphan" opioid receptor-like gene(s), with significant homology to the opioid receptor gene, the natural ligand(s), which is still to be determined.

Cocaine acts primarily by inhibiting the synaptic reuptake of dopamine (DA) into presynaptic sites and also inhibits the receptors of serotonin and norepinephrine by acting at their transporters. Of those three neurotransmitters, most studies have focused primarily on DA, since DA has been so closely linked, by many studies in animal models and at the human level, with the reinforcing or the pleasurable effects of drugs of abuse (Koob 1992). DA, normally released into the synapse, primarily acts at postsynaptic DA receptors, now defined as existing in five distinct types with different and opposing effects. DA thereby activates one or more signal transduction systems, including receptor-specific stimulating or inhibiting effects on adenylyl cyclase activity. Similarly, serotonin and norepinephrine released from presynaptic sites may act at many specific receptors with subsequent signal transduction. These neurotransmitters are subsequently transported first back into presynaptic areas by their specific transporter proteins and then back into presynaptic vesicles. There are also presynaptic DA autoreceptors, where DA may act to regulate DA release.

Although the major effect of cocaine that is known is the direct effect of blocking reuptake of the three neurotransmitters at the site of their specific transporters, this effect is transient (Maisonneuve and Kreek 1994; Maisonneuve et al. 1995). Researchers have raised the question of what may be the indirect effects of cocaine on the endogenous opioid peptides and their receptor systems. These may be longer lasting effects, and/or may provide memory that leads to continual self-administration or

relapse to drug use. The reinforcing or reward effects of drugs of abuse, the "pleasurable" or "desirable" effects, are thought to be those that lead to "craving" or "drug hunger," resulting ultimately with spontaneous activity, or work, for drug acquisition and drug self-administration. The primary sites of action of drugs of abuse with respect to their "reward" or reinforcing effects have been identified by many groups as being in specific brain regions, all rich in dopaminergic (DArgic) nerve terminals or alternative cell bodies, including primarily the mesolimbic and mesocortical dopaminergic systems, especially the nucleus accumbens, which receives terminals from the ventral tegmental area. Also, some of the locomotor activity effects of cocaine and other drugs of abuse derive from effects on DA projections from the substantia nigra to the caudate putamen region as well as the mesolimbic-mesocortical system effects. In addition, it has been hypothesized that the hypothalamus may be important with respect to modulating, in part possibly through different DArgic pathways located therein that may, in turn, affect the responsivity of related hormone systems (Kreek 1996). At these sites, altered stress responsivity may be localized which, since 1972, the author had hypothesized may be part of the neurobiological basis of addictive disorders (Kreek 1972, 1992*c*). The question is what are the linkages between the DArgic system and the opioid system within these single brain regions, and what are the feedback loops between these regions. Specifically one of the questions the author has been addressing is whether or not dynorphin plays a significant role in the feedback control of DArgic tone (Chou et al. 1994*a*, 1994*b*; Kreek et al. 1994; Spangler et al. 1993*a*, 1993*b*, 1994, 1995, 1996).

Also especially interesting is the role of the stress-responsive axis in the addictive diseases. A single gene and gene product, pro-opiomelano-cortin (POMC), yields beta-endorphin, an endogenous opioid, in equimolar concentrations with ACTH, long appreciated as the major stress-responsive peptide in mammals, which causes release from the adrenal cortex of glucocorticoids (cortisol in man, corticosterone in rats). Glucocorticoids, in turn, are critical hormones modulating many metabolic and immune functions. Corticotropin-releasing factor (CRF) is released from the hypothalamus and acts on the anterior pituitary to cause production and release of beta-endorphin and ACTH from POMC. Glucocorticoid released from the adrenal cortex act at glucocorticoid receptors both in the hypothalamus and in the anterior pituitary to affect the negative feedback control of CRF and POMC release was reconfirmed as also regulating these hormones encoding mRNA of rat POMC (Zhou et al. 1996*a,* 1996*b*).

The author's work on opiate dependency has shown in humans that opiates will suppress this hypothalamic pituitary adrenal (HPA) axis acutely; in rodents, however, opiates apparently acutely activate hormones of the HPA axis. When used on a chronic basis in man, short-acting opiates, such as heroin, continue to cause suppression of this HPA axis. However, in an opioid-tolerant and opioid-dependent human being who stops all opiate use and thus goes into opiate withdrawal, the opposite is seen—profound activation of this HPA axis (Kreek 1972, 1973*a*, 1973*c;* 1978, 1992*c*). Some data from the author's group, and from ongoing collaborative work with a group at Yale, support that this opioid activation of the HPA axis may actually precede the first measurable or significant signs and symptoms of opiate withdrawal (Culpepper-Morgan and Kreek, in press; Culpepper-Morgan et al. 1992; Rosen et al. 1995, 1996). Opioid antagonists given to opioid-naive individuals, or to former heroin addicts for management of addiction, exert effects on the HPA axis similar to those found in opiate withdrawal (Kosten et al. 1986*a*, 1986*b;* Kreek et al. 1984*b*). They cause activation of the HPA axis with release of ACTH, beta-endorphin, and cortisol. During chronic steady-dose treatment with the long-acting opioid agonist methadone, normalization of this axis occurs with normal plasma levels of hormones and normal release, as well as circadian patterns of release of the HPA axis hormones and normal negative feedback control of that release (Kennedy et al. 1990; Kreek 1972, 1973*a*, 1973*c*, 1978, 1992*c*; Kreek and Hartman 1982; Kreek et al. 1981, 1983, 1984*a*).

A provocative test of chemically induced stress using metyrapone, which blocks the last step of cortisol production in the adrenal cortex, blocks the negative feedback control mechanisms by cortisol at the hypothalamic and anterior pituitary sites and thereby normally yields a twofold to fourfold increase in plasma levels of ACTH and beta-endorphin. It has been used by the author's laboratory to study the responsiveness of the HPA axes in former addicts and addicts in treatment. In active heroin addicts taking heroin, a hyporesponsivity to this blockade of cortisol synthesis is seen; in drug-free former heroin addicts, hyperresponsivity to this chemically induced stress is frequently seen (Cushman and Kreek 1974*b*; Kreek 1972, 1973*a*, 1973*c*, 1978, 1987, 1992*c*; Kreek and Hartman 1982; Kreek et al. 1984*a*). In long-term methadone maintained patients, euresponsivity is seen (Kennedy et al. 1990; Kreek 1973*a,* 1973*c*), 1978, 1992*c*; Kreek et al. 1984*a*).

Cocaine has been shown by several laboratories to activate the HPA axis when cocaine is present, both in animal studies and in humans. Very intriguingly, a hyperresponsivity to this chemically induced stress has been found in some, but not all, recently abstinent cocaine addicts. The

author has recently studied cocaine addicts with long-term and very heavy usage, using the Addiction Severity Index (ASI) to assess the degree of severity, those who have been cocaine addicts for more than 1 year and have a "binge" and/or daily pattern of cocaine use with sustained social disruption, as well as profound weight loss. When such deeply impaired cocaine addicts were admitted to a research unit following continued "refeeding," very rapid weight gain was observed not unexpectedly (Cambor et al. 1992; Ho et al. 1992). However, no dramatic changes in heart rate or blood pressure were found. In ongoing studies of such patients in an NIH-GCRC unit, hyperresponsivity to metyrapone-induced stress in some subjects was seen. The author is continuing to study this phenomenon in such subjects and also has looked at craving in these subjects using two different visual analog scales, "craving now" and "craving sometime during the last 24 hours." Three different patterns of responses have been observed: those who have craving at admission and persisting through 21 days in the hospital; those who had craving when they were first admitted but with the craving gradually decreasing and in some cases disappearing; and those with no craving in the stress-minimized environment with no "cues" until, with no apparent provocation, it suddenly emerges again (Cambor et al. 1992; Ho et al. 1992).

A much less expensive and more easily performed technique for quantitatively measuring BE in urine using a commercialized type of immune assay that can be performed quantitatively has been modified from the vigorous work of Batki, Jones, and colleagues, using gas chromatography (Batki et al. 1993; Peters et al., in press; Reid et al. 1995). This method provides much more pertinent information than simply getting a "positive-negative" result from such testing by immune assays, which only indicate that more or less than 300 ng/mL of BE is present in urine. Studies have been conducted in cocaine addicts admitted to the author's Rockefeller University clinical research ward and studied in the early abstinent state for more than 40 days as inpatients, with no passes allowed. After patients have been in the research unit for an extended time, usually more than 40 days, a limited number of authorized passes are allowed. There are twenty 24-hour urines collected in the hospital daily on all study subjects at all times for a variety of measurements. In a 24-hour urine collection, the authors were able to measure and calculate the total BE metabolite excreted each day as well as the absolute concentration per milliliter. Also, both creatinine concentration and the total amounts of creatinine excreted each 24-hour period were determined. During the first few days of hospitalization, the levels of benzoylcognine slowly declined, but remained above 300 ng/mL for several days (Peters et al., in press; Reid et al. 1995). Thus if the

standard method of designating urine "positive" or "negative" were used, a specimen collected for monitoring purposes could be incorrectly evaluated as "positive." A steady decline in levels of BE (or the ratio to creatinine) documents no further, or more recent, cocaine use. Conversely, an increase in metabolite levels shows recent further use, thus "relapse," with the implied interpretation being continuing or relapse to cocaine use. Like the Batki and Jones group, the author's group has proposed and demonstrated that expressing quantitative data, from both spot urine collections made in the clinic, as well as 24-hour urine collections made in an inpatient research setting, as a ratio of BE to creatinine, may rectify any false-negative data that could result from purposeful or accidental dilution of urine (Batki et al. 1993; Peters et al., in press). Any illicit use of cocaine while out on pass in this group study, which occurred in three study subjects only, was found by the reappearance of BE in the urine after the initial steady decrease and then disappearance of metabolite. The magnitude of the levels of benzylecgonine would reflect the amount of cocaine used.

Several years ago, in the author's laboratory, a rat model was developed to mimic human patterns of cocaine use. Whether daily or weekly, cocaine is most often self-administered in a "binge" pattern, with multiple doses given over a usually 1- to 24-hour timeframe, although sometimes much longer, and then with no cocaine used for a period of time. In animal models, cocaine was administered just before what would be the sleep time in rats, again parallel to the frequent time of human "binge" use by the cocaine addict. In this model, repeated doses of cocaine are given at 9:30, 10:30, and 11:30 in the morning (Branch et al. 1992). Then no cocaine is administered for the next 22 hours. A variety of behavioral, neurochemical, cellular, and molecular biological measurements are made. Food intake was found to be similar in both low dose (2.5 mg/kg, three times per day) and high dose (10 mg/kg, three times per day) cocaine-treated and saline-treated animals. Weight gain, however, was different. An initial slowing of weight gain was found in the late adolescent/early adult rats; the weight gain then became equivalent after 7 to 10 days of cocaine administration.

Locomotor activity monitoring is conducted in the author's laboratory on individual animals in homecages on a 24-hour basis. On day 1 of "binge" pattern cocaine administration, locomotor activity is increased after each of the three administrations of cocaine (15 mg/kg three times per day) (Unterwald et al. 1994*b*). By the last days of 14 days of "binge" pattern cocaine administration, a profound difference is seen in locomotor activity in cocaine-treated versus saline-treated animals. This is "sensitization" described and studied by many other workers, but using

very different experimental protocols. In this study, a regular, although intermittent, "binge" pattern of chronic cocaine administration with a 22-hour interval between cocaine doses caused a hypersensitivity to cocaine with respect to its effects on locomotor activity (Unterwald et al. 1994*b*). Since in this study, 24-hour activity in homecage was measured with no removal of animals for cocaine/saline injections, and no removal for behavioral measurement, the timecourse of development of sensitization has been determined, which seems to be an extraordinarily robust phenomena in rat models. Both acutely and when studied in a chronic basis, this locomotor activation exactly parallels the increases in extracellular fluid concentrations of DA measured directly in microdialysis studies conducted using the same treatment protocol (Maisonneuve and Kreek 1994; Maisonneuve et al. 1995; Unterwald et al. 1994*b*).

Microdialysis studies were performed with probes in the nucleus accumbens (ventromedial striatum) and in the caudate putamen (dorsolateral striatum) (Maisonneuve and Kreek 1994; Maisonneuve et al. 1995). It was found that following each dose of cocaine, levels of DA in the extracellular fluid are elevated. However, following the second and third dose of cocaine on the first day of cocaine administration, a plateau of the rises in DA concentration in extracellular fluid is seen. In contrast, when actual levels of cocaine in the caudate putamen are measured, it was found that the half-life of cocaine in this brain region is around 30 minutes; thus the amounts of cocaine accumulate, with further increases in cocaine levels in the brain regions after each of these three "binge pattern" cocaine administrations (Maisonneuve and Kreek 1994). Thus there is evidence for acute adaptation or tolerance to this particular effect of cocaine on extracellular DA concentrations on this first day of cocaine administration.

After 14 days, there is again a rise in DA levels in the extracellular fluid after each dose of cocaine, paralleling the behavioral data on locomotor activity (Maisonneuve et al. 1995). However, two interesting issues were noted. At every timepoint the actual concentrations of DA in the extracellular fluid in both the caudate putamen and nucleus accumbens regions are lower in the animals that had been receiving the "binge" pattern cocaine administered on a chronic basis, as contrasted to saline-treated animals receiving cocaine for the first time on the last day of the study. Much of the microdialysis study data published by various research groups are presented as percent changes from baseline values. In the author's studies, in which all probes used are precalibrated, the data could also be measured in actual amounts of DA. Significant reductions of extracellular fluid concentrations of DA, both prior to and following

each injection of cocaine, were found on the final 14th day of "binge pattern" cocaine administration. If these data were then expressed in the more conventional units of percent change from baseline, essentially the same responses were observed after the first doses of cocaine in both cocaine and saline-pretreated animals. However, no plateau in DA levels was observed after the second and third cocaine doses in the chronic cocaine-treated rats. This relatively greater rise in extracellular fluid DA after chronic cocaine administration would parallel what was seen with the cocaine-induced activity, which is an enhanced response to the chronic intermittent cocaine administration or sensitization (Maisonneuve et al. 1995).

In related studies using the technique of quantitative autoradiography, the effects of this "binge" pattern cocaine administration on altering D_1 and D_2 DA receptor densities were demonstrated (Unterwald et al. 1994*b*). The D_2 type DA receptors were increased in density significantly at 7 days after "binge" cocaine administration in three areas of the mesolimbic-mesocortical system, including the nucleus accumbens, the caudate putamen, and olfactory tubercle. These changes, however, were transient. By 14 days, no alterations in the density of D_2 DA receptors were found in any brain region (Unterwald et al. 1994*b*). However, D_1-type DA receptors were found to be significantly increased after 14 days in both the nucleus accumbens and the olfactory tubercle. This enhanced density of D_1 DA receptors following chronic "binge pattern" cocaine administration occurred specifically in areas of the brain known to be involved in the rewarding effects of cocaine and other drugs of abuse. These findings are provocative, especially with known linkage between the D_1 DA receptors and dynorphinergic neurons.

What is the answer to the question, "What other changes are present after DA levels have returned to normal in the extracellular fluid or, in fact, to modestly suppressed levels, and if there are enhanced D_1 DA but not D_2 DA receptor densities, what happens to the endogenous opioid system?" What has been found is that the mu opioid receptors are significantly increased in density, as measured by quantitative autoradiography using mu selective opioid ligands in the caudate putamen, the nucleus accumbens, the cingulate cortex, and also in the basolateral amygdala after 14 days of "binge pattern" cocaine administration (Unterwald et al. 1992, 1994*a*).

The effects of "binge" pattern cocaine administration on kappa type opioid receptors have also been studied (Unterwald et al. 1994*a*). Again, when using selective ligands, significant increase in binding capacity in the caudate putamen, the nucleus accumbens, the cingulate cortex and

also in the olfactory tubercle, again all brain regions that are part of the mesolimbic, mesocortical, or nigrostriatal DArgic system, where DArgic terminals are abundant as projections from the substantia nigra and the ventral tegmental area, have been found. No significant changes in mu or kappa opioid receptors were found within other brain regions where they are equally or more dense in the basal state. It is of special interest that the significant changes in D_1 DA receptor density and in kappa receptor density were both found in two areas of central importance for reward mechanisms: the nucleus accumbens and the olfactory tubercle, both important regions of the mesolimbic-mesocortical DArgic system. These findings are of special importance since dynorphinergic activity is known to be linked to activation of the D_1 type DA receptors (Spangler et al., in press-*a*, -*b*, -*c*; Unterwald et al. 1994*b*) and since full-length dynorphin peptides are the natural ligands of the kappa opioid receptors. It has been hypothesized that dynorphin acts to lower DArgic tone, with negative feedback control from the caudate putamen to the substantia nigral site of DArgic neurons, which project to the caudate putamen and which are part of the nigrostriatal DArgic system, and also possibly from the nucleus accumbens to the ventral tegmental area site of DArgic neurons, which project to the nucleus accumbens, amygdala, olfactory tubercle, cingulate cortex, and other brain regions of the mesolimbic-mesocortical-DArgic system. The authors also have hypothesized that there may be action of dynorphinergic peptides to decrease DArgic tone directly within the caudate putamen and within the nucleus accumbens. This hypothesis is supported by the recent finding of DAT (DA transporter) gene message expression within both the caudate putamen and the nucleus accumbens (Maggos et al. 1995, in press).

Several scientists at the NIH-NIDA Research Center have developed a modified technique of solution hybridization RNase protection assay, in which 18S ribosomal RNA is used as an internal standard, and both sense and antisense riboprobes are used to construct calibration curves of internal standards and gene of interest (Branch et al. 1992; Inturrisi et al. 1988). Following gel analysis of hybridization with the use of each new probe or experimental perturbation, the routine procedure for quantitative measurements of the levels of mRNA of genes of interest in specific brain regions of individual animals includes precipitation with trichloracetic acid of hybridized species, followed by filtration and counting. This modified procedure has allowed the study of specific brain regions from individual animals with precise measurements that allow detection of the small, but potentially very significant, changes that impact or perturb integrated physiology in mammalian physiology (Spangler et al. 1993*a*, 1993*b*, 1994, 1995, 1996; in press-*a*, -*b*, -*c*). Using this technique, researchers use ribosomes, usually subcloned from probes provided by

various colleagues for studies of specific genes from specific species. The author prefers using riboprobes that are over 500 bases in length to increase stringency of the solution hybridization RNase, protection assays, a sharp contrast to the very short probes that must be used for in situ hybridization, which usually are 150 to 250 bases in length.
It has been seen that at the end of 14 days of "binge pattern" cocaine administration, there is no change in gene expression, measured as quantities of mRNA levels of the DA transporter gene expression in the substantia nigra or in the ventral tegmental areas, the two areas where this gene is the most highly expressed (Maggos et al. 1995, in press).

Recently, the author used probes for rat genes cloned by Yu and Uhl, and others, following the initial identification of cDNAs of the mouse delta opioid receptor by expression cloning, achieved by Evans and by Kieffer and colleagues, to study the quantitative levels of gene expression of the kappa and mu opioid receptor in specific brain areas (Chen et al. 1993*a*, 1993*b*; Evans et al. 1992; Kieffer et al. 1992; Wang et al. 1993). Researchers are continuing studies to look at the impact that drugs of abuse and treatment agents on these opioid receptors, as well as on signal transduction systems related to these receptors. High levels of abundance of gene expression for both the mu and kappa receptors have been found in the caudate putamen and the nucleus accumbens, and also in the hypothalamus as well as in the substantia nigra, the olfactory tubercle, and the amygdala (Spangler et al. 1994, 1995). The author also has remapped, using this very sensitive technique, the levels of gene expression of opioid peptide genes in various regions (Branch et al. 1992; Spangler et al. 1993*a*, 1993*b*, 1996, in press-*a*, -*b*). Again, the two regions of great abundance of proenkephalin and prodynorphin gene expression are the nucleus accumbens and the caudate putamen, and to a lesser extent the hypothalamus.

The author then studied the effects of binge pattern cocaine administration on opioid peptide gene expression. After 14 days of "binge" cocaine administration, no changes in proenkephalin gene expression were observed in any brain region (Branch et al. 1992). However, following that pattern of chronic cocaine administration in the rat, significant upregulation of prodynorphin mRNA levels in caudate putamen were found (Spangler et al. 1993*a*, 1993*b*).

It has been hypothesized that dynorphin A, one of the major opioid peptides processed from the initial single gene product of prodynorphin gene expression, may act directly or indirectly to lower DArgic tone (Kreek et al. 1994). In humans, DA plays a dominant role in tonically inhibiting prolactin release, acting on the tuberoinfundibular DArgic

system. Such an effect may parallel the effect of dynorphin on the mesolimbic, mesocortical, and nigrostriatal DArgic systems. Thus, an elevation in prolactin levels may reflect a selective or general reduction in DArgic tone on the brain. The question is whether dynorphin A will effect an increase in prolactin levels in humans. Dynorphin A normally has 17 amino acids; the truncated form of dynorphin A (1-13) of natural sequence has been made available to the author for independent clinical research in humans by Neurobiological Technologies, Inc., in Richmond, California. With Dr. B. Chait, Head of the Laboratory of Extended Range Mass Spectrometry, at Rockefeller University, a matrix-assisted, laser desorption mass spectrometry method has been developed to study neuropeptide processing and biotransformation (Chou et al. 1993*a*, 1993*b*, 1993*c*, 1994*a*, 1994*b*, in press; Yu et al., in press). Using this technique, all of the specific products of neuropeptide biotransformation ex vivo can be analyzed simultaneously. By this technique, it has been determined that the most abundant active opioid component of dynorphin A (1-13) is dynorphin A (1-12), along with the nonopioid peptides dynorphin A (2-12), which may have some different activities, and dynorphin A (4-12) (Chou et al. 1993*a*, 1993*b*, 1994*a*, 1994*b*, in press). It has been found that both the opioid peptides dynorphin A (1-13), dynorphin A (1-17), and dynorphin A (1-6), and also the major nonopioid biotransformation products dynorphin A (2-17) and dynorphin A (2-12), inhibit adenylyl cyclase in rat caudate putamen membranes (Claye et al. 1996). In pilot studies, when dynorphin A (1-13) is given to normal volunteers, it causes a significant rise in serum prolactin levels, which persists for around 90 minutes when 120 &g/kg of dynorphin A (1-13) is given intravenously (Kreek et al. 1994). This is dose-responsive effect, with further increases and more prolonged elevations in serum prolactin levels when 500 &g/kg of dynorphin A (1-13) is administered intravenously. Controlled studies in patients with defined addictive diseases are continuing.

In summary, there are at least two medications, methadone and LAAM, both specific opioid agonists, an additional partial agonist under study, buprenorphine, that are highly efficacious in the treatment of opiate addiction, and also, an antagonist, naltrexone, effective in small, well-defined subpopulations. All of these are directed at the opiate system, not surprisingly. But much more surprising, and now well elucidated by the author's group and supported by findings from other groups, is that cocaine disrupts specific aspects of the endogenous opioid system in humans as well as in animal models. Thus, in theory, there may be some pharmacotherapeutic benefit from targeting an opioid agonist or partial agonist in cocaine dependency, at least in the setting of codependency with an opiate such as heroin. Several studies, most recently by Borg and

colleagues (1995), have shown that although some 70 to 90 percent of former heroin addicts have used cocaine heavily prior to admission to methadone maintenance, over 40 to 50 percent stop using cocaine during effective methadone maintenance treatment, and only 20 to 30 percent continue with regular cocaine (Borg et al. 1995). This is an effect that can be attributed primarily to the positive psychosocial intervention; however, it may, in part, be attributable to pharmacological actions of methadone or LAAM or buprenorphine (Borg et al. 1995).

Several groups including that of Volpicelli and O'Brien at the University of Pennsylvania and O'Malley and colleagues at Yale, as well as B. Mason at the University of Miami have shown that specific opiate antagonists such as naltrexone or nalmefene may be useful in the treatment of alcoholism (Mason et al. 1994; O'Malley et al. 1992; Volpicelli et al. 1992). Thus there is increasing evidence that the endogenous opiate systems, as well as the DArgic system, and possibly also the serotonergic system, may be intrinsically involved in each of these three major addictions: heroin, cocaine and alcohol. All of these neurobiological and clinical findings should guide researchers in the exploration for a pharmacotherapy for cocaine addicts.

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