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MOLECULAR AND CELLULAR NEUROBIOLOGY AND PATHOPHYSIOLOGY OF OPIATE ADDICTION

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In the years 1994 to 2001 (since the last edition of this book went to press), innumerable and important advances have been made in our understanding of the molecular and cellular neurobiology, as well as pathophysiology, of opiate addiction. Clearly, the greatest advances have come about ultimately because of the first successful cloning of a specific opiate receptor, the δ receptor, achieved in late 1992 by two groups working independently, using expression cloning in a cell line that is known to express the δ-opioid receptor (1,2). The reports of the groups of Evans and colleagues from Los Angeles and Kieffer and colleagues from Strasbourg, France, followed by the cloning of μ- and κ-opioid receptors of rodents and in humans by Yu, Uhl, and others, opened new doors for both animal and basic clinical research studies, as well as human molecular genetics studies (1–5). Other notable technologic advances have been made recently and are continuing to be made. Possibly the most dramatic of these, from which we will undoubtedly see novel and unexpected findings over the next few years, is the development of microarray technology, to determine the changes in levels of gene expression of literally thousands of genes simultaneously (although not yet with the sensitivity required to detect changes in mRNA levels reflecting gene expression of many neuropeptides and most neuroreceptors), and also even newer microarray technology for identification and screening for human polymorphisms, including single nucleotide polymorphisms (SNPs) (6,7). By using these new findings and technologies, as well as by building on earlier and current best techniques, profound advances have been made in each of three areas, of which only a few may be covered briefly herein.

Many of these varied advances have been collated and placed in perspectives of our earlier knowledge in several thoughtful reviews, such as selected reviews of preclinical research (6,8–26), basic clinical research (27–31), and molecular biology and genetics (32–35). All these advances have and will continue to make further revelations concerning each of the addictions, and in particular, for this discussion, opiate addiction.

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PRECLINICAL STUDIES OF CHRONIC ADMINISTRATION AND WITHDRAWAL EFFECTS OF OPIATES IN DIVERSE AND NOVEL ANIMAL MODELS

Neuropeptide and Neurotransmitter Systems Primarily Affected

Opioid Peptides and Receptors: Molecular, Cell Biological, and Signal Transduction Alterations, and Possible Implications for Pathophysiology of Opiate Addiction

After the definitive discovery of specific opioid receptors in 1973, research began to address what had been a long-standing hypothesis, later apparently to be disproved. The hypothesis was that tolerance to opioids depended on down-regulation or decreased availability of, and thus access to, μ-opioid receptors after chronic μ-opioid agonist (e.g., heroin or morphine) exposure. Later, this could be considered to result from "desensitization" of μ-opioid receptors while still on the cell surface (i.e., phosphorylation or uncoupling of the receptors from their G-protein–coupled signal transduction mechanisms essential for the effects after binding), or, alternatively, to result from a decrease in numbers of receptors on the cell surface (i.e., actual down-regulation), which could be caused either by a long-hypothesized, but only recently documented phenomenon, that of endocytosis or internalization of receptors, or by a decrease in the reap-

pearance of receptors at the cell surface once internalized (36–52). Moreover, a significant decrease in production of new receptors could contribute to a so-called down-regulation. Although the terms *down-regulation* and *up-regulation* have been used loosely with inadequate definitions, the overall concept that chronic μ-opioid agonist administration may cause reduced capacity to bind, or increased capacity to bind, or to have no effect, but each alternative with reduced capacity of activated receptors to have an effect (or "tolerance"), has persisted, and repeatedly studied, with conflicting results. The earliest studies to address this issue of impact of chronic opioid administration effects on binding were conducted to elucidate the well-documented and accepted phenomenon of tolerance, both in cell systems and in whole animals. Morphine was the most common opiate used to determine whether opioids down-regulated or otherwise altered opioid-receptor–binding sites.

Later, the effects of specific opioid antagonists on opioid-receptor binding or density were also conducted, primarily using naltrexone. The binding of opioid antagonists, of course, does not involve coupling; that is, no $G_{i/o}$-protein–coupled signal transduction mechanisms are involved, because the opioid antagonists (according to most current theories) do not activate receptors, but rather prevent activation by endogenous or exogenous opioids. The effect of chronic administration of primarily μ-opioid-receptor antagonists to up-regulate the binding capacity, or density, of μ-opioid receptors has been well established. There is now essentially a consensus from many and diverse studies that the chronic administration of opioid antagonists, primarily naltrexone, will cause a significant up-regulation or increase in density of μ-opioid receptors (53,54). There has, however, been some controversy regarding whether opioid antagonist treatment and the resultant up-regulation of μ-opioid receptors leads to a sensitized state, that is, a state in which an opioid agonist would have a greater than usual effect on any system. This has been addressed both in animal models and in humans, with some conflicting results.

From the very beginning of the documentation of the existence of specific opiate receptors, in 1973, although numerous studies have used several different opioid agonists, primarily morphine, given by different regimens, ranging from intermittent injections to repeated pellet implantation, to a few studies using chronic administration by pump, there have been conflicting study results and no consensus on the effects on μ-opioid-receptor binding or density. The results reported from studies conducted in living adult animals, for the most part, have shown no overall net changes in μ-opioid-receptor–binding capacity, that is, no overall changes in opioid-receptor density, as measured by quantitative autoradiography or by classic homogenate binding assay studies, and, more recently, no overall changes in μ-opioid-receptor mRNA levels. The original studies, conducted in living adult animals by the groups that included those who first defined opioid receptors, showed no alterations of opioid receptors during chronic morphine exposure, and this finding altered their initial hypothesis, that such chronic exposure to an opioid agonist would cause down-regulation of receptors (55–57). Subsequent studies using diverse ligands and dosing regimens continued to give varied results, with up-regulation of μ-opioid receptors, down-regulation of μ-opioid receptors, and no change of μ-opioid-receptor density or binding after chronic μ-opioid-agonist administration all reported. The prevailing concept for receptor-agonist ligands and, in this case, specifically agonists for the μ-opioid-receptor system, has been that persistent activation of receptors would generally lead to down-regulation, and conversely, the persistent deprivation of receptors of specific ligands would generally lead to persistent lack of activation of receptors and thus to up-regulation. However, the results are complex and conflicting.

From 1996 to 2000, several intriguing articles appeared concerning the effects of opioid-agonist administration on receptor internalization (44–52). In addition, further and relevant studies on signal transduction, primarily through G-protein–coupling mechanisms, but also alternative mechanisms, appeared and extended our earlier knowledge and may ultimately explain some of the apparently conflicting results concerning receptor binding and density (12–15, 18–21,23,24,34,36–43).

Starting with the early seminal work of Aghajanian in the late 1970s, chronic morphine administration to whole animals was shown to alter, at a cellular level, the function of neurons, specifically in the locus ceruleus, and also to lead to tolerance and physical dependence (12,18,20,24). These changes included initially the well-established acute inhibition by morphine of adenylyl cyclase, as well as resultant inhibition of the cyclic adenosine monophosphate (cAMP)–dependent cascade, followed by, during chronic morphine treatment, a compensatory increase of activity of adenylyl cyclase, with an increase in the cAMP-dependent cascade, including increases in protein kinase A and increases in phosphorylated proteins, as well as of the cAMP-dependent response element binding protein, or CREB (18, 20,24). Nestler and Aghajanian hypothesized that this up-regulation of the entire cAMP pathway in the locus ceruleus represents a compensatory change to oppose or offset the initial inhibitory effects of morphine and thus could be considered to be one component of tolerance (18,20). They also suggested that these increases in the cAMP pathway components could contribute to opiate dependence and thus withdrawal, because these changes could be involved in a variety of functions once no longer opposed by morphine (18,20). This concept is of particular relevance because this up-regulation of the entire cAMP pathway during chronic morphine exposure has been shown to occur predictably in the locus ceruleus of all strains and species of rodents studied to date. Because the locus ceruleus is the major noradrenergic nucleus of the brain, diverse noradrenergic functions that are known to be activated in opiate

withdrawal could be affected. Nestler and other groups showed that although these changes occur uniformly in the locus ceruleus neurons, and in a few other brain regions, particularly in the nucleus accumbens, this type of change in the nucleus accumbens is strain dependent, and also such changes do not occur in many other brain regions in any strain or species (12,18,20,24). They also do not occur in the gastrointestinal tract. Nestler and others did not find a down-regulation of μ-opioid receptors during chronic morphine treatment in the locus ceruleus (18,20,24). They did, however, report an uncoupling of the μ-opioid receptor from its G-protein–coupled inwardly rectifying potassium channels during chronic morphine exposure, with a resultant reduction in the maximal outward current and documented decreased efficiency along with decreased potency of the opioid. This is intriguing in the context of findings of the laboratories of Yu and Kreek, who reported that after binding of the long, 31-residue, endogenous opioid β-endorphin to the variant μ-opioid receptor coded by the very common SNP, A118G, there is enhancement of activity of these G-protein–coupled inwardly rectifying potassium channels (58).

Almost all groups, again starting with the earliest work of Aghajanian, as well as more recent work of Nestler and others, have suggested that the locus ceruleus may be primarily involved in expression of opioid physical dependence and thus in opioid withdrawal (20). Selley and Childers et al. studied the effects of chronic morphine treatment on opioid-receptor–coupled G-protein activity in membranes from the locus ceruleus and showed that chronic morphine treatment decreased the inhibitory G-protein activity in the locus ceruleus and yet did not produce any detectable desensitization, a finding suggesting a potential adaptation at that level (40). Chronic morphine treatment decreased both basal and opioid stimulated guanosine triphosphatase (GTPase) activity and yet caused no changes in the percentage of stimulation by an opioid agonist. All these results were extended by binding assays using [^{35}S]GTPγS (40). In further studies, it was found that long-term heroin self-administration also similarly altered the opioid-receptor–activated G proteins in specific brain regions, primarily in specific brainstem nuclei (42). Decreased μ-opioid-agonist–stimulated [^{35}S]GTPγS binding was observed in the locus ceruleus and in a few related regions during long-term heroin self-administration. These findings were similar to those previously described in animals treated with morphine on a long-term basis (40). Moreover, the decreased μ-opioid-stimulated [^{35}S]GTPγS binding was found in two additional regions, the thalamus and the amygdala, which may be of importance for the reinforcing effects of drugs of abuse and thus self-administration (42).

All these scientists mention that the neuronal and molecular basis of opioid tolerance and dependence remains unclear. The opioid receptors involved have all been cloned and have been documented to be part of the G-protein–coupled family of seven transmembrane receptors; there has been further documentation of receptor phosphorylation, desensitization, and uncoupling from G proteins, as well as new studies documenting internalization (endocytosis) of opioid receptors (36–52). However, studies in animals continue to produce very conflicting results concerning the effects of chronic opiate administration on opioid-receptor binding and density or number. Similarly, despite documentation by many groups that cellular adaptations may be directly involved in the development of tolerance and dependence, the mechanisms have yet to be fully elucidated. Moreover, multiple other neurotransmitter systems have been implicated, in particular the *N*-methyl-D-aspartate (NMDA)–receptor complex and its ligands.

Chronic morphine administration, with resultant changes in G-protein–coupled signal transduction mechanisms and changes in downstream effectors, such as increases in CREB and phosphorylated CREB, and also other changes such as increases in and accumulation of chronic FRAs (Fos-related antigens), are all nonspecific. For instance, diverse stimuli such as cocaine, opiates, opiate withdrawal, nicotine, other drugs of abuse, and stress have been shown to cause increases in chronic FRAs (12). CREB is one component of the enhanced cAMP response and is a transcription factor; chronic FRAs have now been identified as isoforms of ΔFosB, which is a splice variant of the *fosB* gene. Each of these enhanced or altered transcription factors can change the levels of expression of many specific genes and in specific brain regions. These increases in CREB and in chronic FRAs, both results of chronic morphine administration, may yield enhanced or altered gene transcription elements and, in turn, changes in levels of expression of specific genes and in specific brain regions. The increases in chronic FRAs after long-term morphine administration occur exclusively in the striatum, whereas the increases in chronic FRAs seen after stress occur in the prefrontal cortex (20). However, these components are also transient. For instance, Nestler found that chronic FRAs probably persist for only a few weeks after accumulation during chronic opiate or cocaine administration and thus require repeated exposure to a drug of abuse for reappearance or for persistence. These increased and accumulated amounts of CREB and chronic FRAs are tangible examples of neuroplasticity of the brain and document one type of change that may occur and persist with chronic exposure to a drug of abuse. As Nestler warned, however, they are but two of probably many such changes and, although related to specific addiction related phenomena, are not the sole cause of any of the three distinct and separable phenomena of tolerance, physical dependence, or addiction. Moreover, all the resultant gene expression changes that occur, related to CREB and ΔFosB, and that may contribute to an atypical activator protein 1–type transcription factor, are nonspecific changes, with respect to the causative agent. In addition, these transient, but gene-specific, changes in gene expression can

occur in specific brain regions after specific times of exposure to, or withdrawal from exposure to, a drug of abuse such as morphine (but also cocaine and other drugs of abuse).

Direct effects of these transcription factors have been studied only to a limited extent. Nestler hypothesized that the documented increases in amounts and phosphorylation of CREB in the locus ceruleus caused by chronic morphine administration may be directly involved in the regulation of the entire cAMP pathway through the CREB effects as a transcription factor on gene expression (18). His group showed that application of CREB antisense oligonucleotides applied to the locus ceruleus of opiate-dependent rats decreases the opiate withdrawal-induced increases in neuronal firing that are usually seen (18). Further, the laboratory of Nestler showed that accumulation of chronic FRAs during chronic morphine treatment, related to the transient early gene protein products Fos and Jun, which, in turn, join to form a major gene transcription factor, activator protein 1, may play a role in the effects of morphine and also of stimulants (18). For instance, Nestler's group showed in *fosB* knockout mice, in which chronic FRAs are presumed not to be formed or accumulate, an enhanced locomotor response to cocaine (12). Using an effective construct involving both a tetracycline transactivator gene to allow regulation of gene expression and the gene encoding ΔFosB that then can be delivered to a specific brain region, Nestler's group conducted further studies of the effects of drugs of abuse. This gene construct allows overproduction of ΔFosB by the gene insertion, the overexpression of which can be prevented by administration of a tetracycline congener, but it be started again by stopping treatment with the tetracycline congener. The overexpression can be both brain region specific and time specific (12). To date, enhancement of ΔFosB in the striatum has been shown to alter the behavioral response to cocaine (12). Using a different transgenic approach, a viral vector may be used to deliver a desired gene to a specific brain region to yield overexpression. Carlezon and Nestler and their colleagues used such a delivery system to achieve overexpression of the NMDA-receptor component, $GluR_1$, with overexpression in different specific brain regions; in some brain regions, and with this region-specific overexpression, they were able to document increases in opiate reward and, in other brain regions, increases of aversion to opiates (59).

There is an increasing consensus that the reinforcing effects of drugs of abuse, along with possibly physical dependence, are not directly related to tolerance, and they also may not be directly related to any changes in receptor density, number, desensitization, internalization, G-protein uncoupling, or other effects on signal transduction mechanisms. These findings are further supported by the report of Bohn, Lefkowitz, Caron, and colleagues that, in studies in β-arrestin knockout mice, one sees enhancement and persistence of the antinociceptive effects of morphine (60). Furthermore, Bohn and colleagues reported that no tolerance develops to the antinociceptive effects of morphine during chronic administration, but they also said that there apparently is no impact on physical dependence in the β-arrestin knockout mice (61).

Dopamine, Other Neurotransmitters, Neuropeptides, and Their Receptors: Molecular, Cell Biological and Signal Transduction Alterations, and Possible Implications for Pathophysiology of Opiate Addiction

The early work of many groups showed that opiates, like most other drugs of abuse, appear to act to enhance dopaminergic tone and through that enhancement achieve some, most, or all of their reinforcing effects. Moreover, through a variety of studies, primarily conducted in animals using either surgical lesions or specifically directed neurotoxins, and also other specific chemicals to enhance or decrease dopaminergic function, along with ultimately microdialysis techniques, researchers showed that enhancement of dopamine tone in the mesolimbic-mesocortical dopaminergic system in particular is associated with the rewarding or reinforcing effects of most or all drugs of abuse. The seminal work by Johnson and North documented unequivocally that one action of μ-opioid agonists, exerted through μ-opioid receptors localized in the ventral tegmental area, is on inhibitory GABAergic interneurons and is one of inhibition of those neurons (62). Thus, by inhibiting these inhibitory neurons, which normally put a brake on the dopaminergic neurons in the ventral tegmental area, the result is activation of the dopaminergic neurons, with enhanced release of dopamine in the nucleus accumbens, as well as in the amygdala and probably in all other regions of the mesolimbic-mesocortical dopaminergic fields (62).

Although many investigators have attributed the reinforcing effects of all drugs of abuse, including heroin and morphine, to actual or presumed enhanced dopamine levels in the nucleus accumbens, through this indirect action for opiates, and for cocaine, through a direct blockade of the dopamine reuptake transporter, there is increasing evidence that dopamine not only is not essential for the reinforcing effects of heroin and morphine, but also does not play a central role in the reinforcing and rewarding effects of opiates.

Studies have been conducted in animals with deletion of the dopamine transporter gene, which many researchers had hypothesized would eliminate cocaine self-administration because of the very high constant levels of dopamine and the lack of further effects by superimposed cocaine (63). This was found to be not the case (63). The dopamine transporter knockout mice were found unequivocally to self-administer cocaine, although the acquisition of that behavior was slower than in the wild-type mice (64). Thus, even for cocaine, dopamine clearly plays a role in the reward-

ing effects, but it is not the sole component, nor are changes in dopamine levels essential for the reinforcing effects of cocaine (63,64). In that same animal model, the dopamine reuptake transporter knockout mice, it has been found, however, that morphine is more avidly self-administered than in wild-type mice, a finding suggesting a positive interaction between the persistently elevated levels of dopamine and morphine to enhance reward (65). Hemby and Smith and their colleagues also found a synergistic elevation of extracellular dopamine when cocaine was added to heroin in self-administration studies (66). These findings may explain, in part, the common co-dependency in humans of both heroin and cocaine addictions.

With respect to opiates, two very early studies showed that when animals were lesioned to delete the dopaminergic neurons completely in discrete brain regions by use of a neurotoxin, 6-hydroxydopamine, self-administration of morphine proceeded normally as in unlesioned animals. However, in such animals, cocaine self-administration was eliminated.

There have been conflicting results in other studies. For instance, in one study using the technique of *in vivo* fast cyclic voltammetry, it was found that heroin caused a dose-dependent increase in dopamine in the nucleus accumbens during heroin self-administration, and co-administration of a κ-agonist (U-50,488 H) with the heroin, or alternatively, intracerebroventricular administration of dynorphin A, significantly depressed the heroin-stimulated dopamine release (67). Moreover, installation of the κ-synthetic compound or natural ligand dynorphin A alone decreased basal dopamine release, as had also been shown by Claye and others (68). Studies by Xi, Fuller, and Stein thus suggested that the μ-agonist morphine activates the mesolimbic-mesocortical dopaminergic pathway and that κ-opioid-receptor activation offsets, or counterregulates, that activation (67).

However, another set of studies by Hemby, Smith and colleagues showed that systemic self-administration of heroin alone does not cause any elevation in dopamine as determined by *in vivo* microdialysis with the probes in the nucleus accumbens (69). In related studies, these investigators found, as have numerous others, that cocaine caused a striking increase in extracellular dopamine concentrations in the nucleus accumbens, and, moreover, the combination of cocaine and heroin caused a synergistic elevation (66). Their finding that heroin alone failed to cause an increase in dopamine in the nucleus accumbens complemented several earlier findings that heroin self-administration is not attenuated by administration of dopamine antagonists, as well as even earlier studies showing that integrity of dopamine pathways in the nucleus accumbens is not essential for heroin self-administration. These findings document further the early hypothesis of the Kreek laboratory, and many others, that the reinforcing properties of heroin are mediated primarily by dopamine-independent mechanisms and probably by the μ-opioid receptor itself. This hypothesis has been ultimately supported by the findings that μ-opioid-receptor deletion knockout mice have no self-administration of opiates and no rewarding effects of opiates (reviewed in ref. 33).

In another study, which used morphine pellet implantation to develop opioid tolerance and dependence, a reduction in dopamine D2-receptor mRNA levels, but no change in dopamine D1 mRNA levels, was found at the end of the 6 days of morphine exposure (70). The mRNA levels for both dopamine D1 and D2 receptors was reduced after 1 day of withdrawal, and both returned toward normal by the third day after drug withdrawal. These findings may be related to the reduction in dopamine D2-receptor binding, which has been seen in human heroin addicts, by using positron emission tomography. However, curiously in this study, but not in other studies, reductions of mRNA levels for dynorphin and enkephalin genes were found during morphine exposure. In contrast, enhanced dynorphin mRNA levels have been found at least after acute single and multiple intermittent-dose morphine administrations (71, 72). Further studies will be needed to determine the time course of dynorphin mRNA level changes during morphine exposure. Trujillo, Akil, and their colleagues showed that chronic injection or infusion of morphine caused increases in levels of dynorphin peptides in the dorsal striatum (caudate putamen) but not in the ventral striatum (nucleus accumbens) (73).

Intriguingly, Lee, Henriksen, and colleagues found that only a few (approximately 20%) nucleus accumbens neurons seem to exhibit an inhibitory response after heroin self-administration, along with about 40% of prefrontal cortex neurons showing such inhibition (74). Thus, the multiple changes in signal transduction observed and discussed earlier, including the effects of chronic morphine administration on μ-opioid-receptor–stimulated [^{35}S]GTPγS binding changes, with reduction of [^{35}S]GTPγS binding specifically in the brainstem nuclei, including the dorsal raphe nucleus, the locus ceruleus, the lateral and medial parabrachial nuclei, and the commissural nucleus tractus solitarius, may result from a direct opiate effect or an indirect effect by alteration of the dopaminergic system (40,42). Similar findings were made by the group of Sim-Selley, Selley, Childers, and colleagues after chronic heroin self-administration, with the greatest decrease in μ-opioid-receptor–stimulated [^{35}S]GTPγS binding in the brainstem and the lowest alterations in binding in the striatum and cortex (42). Because the changes of dopamine D1-receptor activation would act in one direction and dopamine D2-receptor activation would act in the opposite direction on adenylyl cyclase activity, the effects on these receptors could also influence the effects of μ-opioid-receptor activation, and the changes that have been observed may result exclusively from the opioid effects acting at the μ-opioid receptors or also secondary indirect effects on dopamine receptors.

These and other findings suggest that opiates may act

directly to alter dopaminergic systems both in the ventromedial striatum, that is, the core and shell of the nucleus accumbens, and in the dorsolateral striatum, that is, in the caudate putamen region. Clearly, there are abundant μ-opioid receptors as well as κ-opioid receptors in those regions (26,75–77). Work from the Kreek laboratory showed that another drug of abuse, cocaine, when delivered in a binge pattern, which markedly enhances dopaminergic tone, causes an increase in density of μ-opioid receptors and also κ-opioid receptors in those brain regions, and it also alters basal and opioid-regulated adenylyl cyclase activity in these regions (75–77). There have been no similar findings with respect to increasing μ-opioid-receptor density after chronic opioid administration, however. It is not really known to what extent reinforcement or reward resulting from heroin and morphine occurs because of activation directly in these areas, especially the nucleus accumbens and possibly also the amygdala, as contrasted to indirect effects on the ventral tegmental area. The effects on dopamine in each of these different locations and also the different mechanisms involved have not yet been fully elucidated using a model of chronic, high-dose, intermittent but evenly spaced opiate administration, mimicking the human pattern of heroin or morphine abuse and addiction, and also after withdrawal, as well as during reexposure after such opiate administration. During chronic binge pattern cocaine administration, a pattern mimicking the human condition, there is a progressive lowering of basal, as well as cocaine-stimulated, dopamine levels in the extracellular fluid of the caudate putamen and in the nucleus accumbens (78). Noble and Cox clearly defined a role of the dopaminergic system in opioid-receptor desensitization in these brain regions during chronic morphine administration (39).

After chronic opiate administration, Nestler and colleagues found increases in tyrosine hydroxylase in the ventral tegmental area. This is a rate-limiting enzyme in the biosynthesis of dopamine. They also found a reduction in mean size of the ventral tegmental area dopaminergic neurons and decreased axonal transport to the nucleus accumbens (24, 79). However, there were no changes in numbers of dopaminergic neurons and no changes in the size of nondopaminergic neurons (79). Within ventral tegmental area, infusion of brain-derived neurotrophic factor prevented this morphine-induced reduction in size of dopaminergic neurons (79). Their group also found that chronic morphine administration resulted in an increase of other components related to signal transduction, including the extracellular signal regulated kinases (ERKs), which are effectors for brain-derived neurotrophic factor in the ventral tegmental area (24). However, the time course of these changes and their persistence after morphine withdrawal have yet to be elucidated, and their relation to both physiology and the behaviors of addiction also have not yet been fully explored, although the findings suggest that neurotrophic factors may act in response to the opiate-induced changes in neural integrity, that is, the neuroplasticity after chronic opiate administration that results in impairment of normal neural integrity. Both the chronic morphine-induced injury and the counterregulatory events may alter neural growth, development, and synapse formation, signal transduction, and overall system integrity (24,79).

Similarly, the findings that acute and chronic morphine administration and withdrawal may enhance dynorphin gene expression and dynorphin peptides, undoubtedly events mediated in part through action of dopamine D1 receptors, often co-localized on cells with dynorphin gene expression, as well as more direct effects of enhanced transcription factors on dynorphin gene expression, may be again important counterregulatory events, which also represent examples of profound neuroplasticity of the brain. Such findings have also been made during binge pattern cocaine administration (80,81). Enhanced dynorphin peptides, in turn, acting at κ-opioid receptors, may reduce dopaminergic tone in many brain regions, including those involved in reward and also locomotor activity, and they may also attenuate opioid withdrawal in dependent animals or humans (6,8,9–11,16). Again, these events must be considered to be a direct result of neuroplasticity and are counterregulatory, the attempt to attenuate, modulate, or even brake the events caused by the rapid changes in dopaminergic tone brought about especially by stimulants such as cocaine, but also to a lesser extent also by opiates.

The changes in signal transduction mechanisms after chronic heroin or morphine administration are undoubtedly primarily the result of the effects of chronic opioid administration. However, because there are also significant changes in dopaminergic tone with enhanced signaling through the dopaminergic pathways, owing to indirect or direct activation of dopamine release, the changes in signal transduction observed may also result from enhanced activation of the dopaminergic neurons, as stated earlier. D1- and D5-type dopaminergic receptors enhance adenylyl cyclase activity, an effect similar to that occurring in the locus ceruleus after chronic, but not acute, morphine administration, in most strains of rodents studied, and also in the nucleus accumbens in some strains of some species. In contrast, activation of the dopaminergic D2 receptors causes a reduction in adenylyl cyclase activity, such as observed during acute morphine administration in all brain regions of strains and species of rodents studied, as well as in all cell systems studied, and an effect that continues to pertain in some specific regions of the brain and other parts of the body during chronic opioid administration. Thus, the observations of alterations in the downstream events of the adenylyl cyclase changes may be the cumulative response of chronic morphine administration on μ-opioid-receptor activation and also of dopamine on dopaminergic D1- and D2-receptor systems.

More recently, the findings of Crain and Shen showed the ability of very small amounts of specific opioid antago-

nists, in fact, to enhance the analgesic effects of the μ-opioid-receptor agonists and to prolong the opioid-agonist effects both in animal models and in humans (82). Crain and Shen hypothesized that, although most μ-opioid receptors are coupled with inhibitory $G_{i/o}$ protein, a small proportion may be coupled at the stimulatory G_s protein, which can be suppressed with small amounts of opioid antagonists. These findings of enhanced morphine analgesia are, in part, very similar to the findings of Bohn, Caron, Lefkowitz, and colleagues, in mice with deletion of β-arrestin (60). These researchers also showed that β-arrestin is important in several distinct functions, including events leading to the internalization of an agonist bound μ-opioid receptor, which, after the phosphorylation of the bound form, binds to β-arrestin, along with binding by G-protein–receptor kinases (60). This event of β-arrestin binding has been described as potentially part of the process that desensitizes, that is, leads to G-protein uncoupling of the μ-opioid receptors, as well as being actually involved in the internalization of endogenous and some exogenous agonist-bound μ-opioid receptors (44–52,60). The role of internalization in the development of tolerance and the independent process of dependence remain unclear because there are many conflicting results, including the finding that most exogenous opioid ligands, including morphine, that do not induce prompt internalization of μ-opioid receptors once bound, clearly lead to the development of both tolerance and physical dependence (44–52). In sharp contrast, methadone and etorphine do lead to prompt internalization of μ-opioid receptors, just as do all the natural endogenous opioid peptides such as Met-enkephalin and β-endorphin (44–52). Intriguingly, in the mice with deletion at the β-arrestin-2 gene, enhanced morphine analgesia was seen, and further studies revealed that tolerance does not develop to morphine effects, and yet objective signs reflecting the development of physical dependence are present after chronic morphine administration (60). These studies again dissociated the development of tolerance from the development of physical dependence. The studies of the group of Crain, as well as the studies of the group of Caron and Lefkowitz, suggested that either deletion of β-arrestin or suppression, by opioid antagonists in very small doses, of opioid receptor coupled to G_s, the stimulatory G-protein pathway, will enhance opioid analgesia and also may attenuate or prevent development of tolerance. It is not known whether blocking of the $G_s$coupling alters the development of physical dependence, however. In possibly related studies, Jeziorski and White showed that the NMDA antagonist, MK-801, prevents development of behavioral sensitization during chronic morphine administration, whereas dopamine-receptor antagonists prevent expression, but not development, of sensitization (83). Sensitization has been suggested to be related to drug reward or craving. Possibly in contrast, Churchill, Roques, and Kalivas found that dopamine depletion, such as may happen during chronic opiate, as well as chronic cocaine, administration, augments opioid-induced locomotion (84).

There have been only limited studies of the time course of all these dopaminergic responses during investigator-administered morphine or heroin on an intermittent basis, mimicking the human pattern of heroin abuse, or during chronic self-administration of opiates. It would be assumed that possibly, as with cocaine, one sees a progressive diminution of the responsivity, with a resultant lowering of basal level and stimulant-induced rise of absolute levels of dopamine (78). Numerous human studies suggest this may indeed happen. It has been repeatedly shown in heroin addicts that the short acting μ-opioid agonist heroin will cause a prompt increase in serum prolactin levels, resulting directly from an abrupt lowering of dopamine levels in the tuberoinfundibular dopaminergic systems (85). In humans, and to a greater extent than in rodents, prolactin release is essentially solely under tonic inhibition by dopaminergic tone in the tuberoinfundibular dopaminergic system. However, it was found that during chronic methadone treatment, there is adaptation or tolerance to this phenomenon, an attenuation, but not a complete removal or ablation of this response caused by dopamine lowering and resulting in elevation of serum prolactin levels (85). Even during long-term methadone maintenance treatment, as reported in 1978, it was found that peak plasma levels of the μ-opiate agonist methadone are related temporally to the peak plasma levels of prolactin (85). These findings suggest that the long-acting opioid methadone administered orally continues to have an impact at least on the tuberoinfundibular dopaminergic system, with a lowering of dopaminergic tone, resulting in a modest increase of prolactin levels, although not exceeding upper levels of normal. However, that attenuation occurs suggests that there may be either a lowering of dopaminergic levels and tone in the turberoinfundibular dopaminergic system of that region or, alternatively, an attenuation of responsivity of the μ-opiate-receptor system. It has been shown that the κ-opiate-receptor system similarly plays a role in modulating prolactin levels in humans (86). In normal healthy volunteers, dynorphin A causes a prompt rise in serum prolactin levels, resulting again presumably from a lowering of dopaminergic tone in the tuberoinfundibular system (86). This is a μ, but also a κ-opioid-receptor effect, as documented by use of two different opioid antagonists with different receptor selectivity (86). In preliminary studies, the Kreek laboratory showed that there is altered responsivity both in former heroin addicts and in former cocaine addicts, as well as those with combined heroin opioid and cocaine dependency (87).

Acute morphine administration has been shown to have a variety of profound effects on many other neurotransmitters; this group comprises fast-acting neurotransmitters including excitatory amino acids such as glutamate and slower-acting neurotransmitters such as norepinephrine, epinephrine, and serotonin, as well as dopamine, and a vari-

ety of neuropeptides. Very few studies have been conducted in models using chronic heroin or morphine administration, or self-administration, using long-term, high-dose, regularly spaced intermittent administration or by long-access, high-dose, self-administration, mimicking the human pattern of heroin abuse. Further work will be central to detail the long-term effects and, also of special interest, the effects of the withdrawal and reexposure to mimic relapse. However, qualitatively and quantitatively different changes have been found during chronic morphine or heroin administration by different patterns, dose, and routes of administration.

Physiologic Systems and Behaviors Primarily Altered

Stress Responsivity: Possible Implications for Opiate Addiction

An atypical responsivity to stress and stressors existing on a drug-induced basis or possibly *a priori,* on a genetic or environmental basis, as one component of the "metabolic basis" of heroin addiction was a concept that was hypothesized by the Kreek group in 1964, and it was therefore addressed directly in our prospective studies started at that time and completed in 1972, as well as in other early basic clinical research studies (6,85,88–92). Several laboratories went on to study, in humans, the impact of drugs of abuse and specifically heroin, but also morphine, (as used in a single dose or on a chronic basis in the pharmacotherapy of pain), on one component of stress response, the hypothalamic-pituitary-adrenal (HPA) axis (6,93–108). Long-term studies in animal models came later, however, and were performed by many different groups (6,8,109–118). The initiation of these studies was predicated not only on the clinical research, which clearly documented that opiates suppress the HPA axis in humans and continue to do so during the long-term self-administration of short-acting opiates such as heroin, but also, and very importantly, that normalization of this HPA axis occurs during steady-dose long-term methadone maintenance treatment, findings that were made in rigorous studies and reported as early as 1972 (6,89,90). Studies reported from the late 1970s onward have documented that the endogenous opioids clearly play a tonic modulatory role of inhibiting the hypothalamic-pituitary part of the HPA axis (reviewed in ref. 9). Further more recent studies in humans have shown that this modulation is effected by both μ- and κ-selective opioid ligands (108).

In the middle to late 1980s, several groups began to study the concept that stress and the response to stress, as well as novelty and risk-seeking, may contribute to self-administration of drugs of abuse, including opiates, and parallel studies showed that drugs of abuse including opiates, cocaine, and alcohol perturb components of the stress-responsive systems in animal models. The initial studies measured primarily specific behaviors after assessment of the relative response to novelty or to risk and used different strains of rats, as well as mice. Similarly, more recent studies looked not simply at the acute effects of drugs of abuse, but also at the subacute and chronic effects of drugs of abuse and the impact of withdrawal from such drugs on components of the stress-responsive axis. Even more recent studies went on to study levels of gene expression and the impact of exposure to drugs of abuse over a defined time course of exposure on gene expression, first on "early gene response" and then, more recently, on changes of expression of many other specific genes, in particular, components of stress-responsive axis (6, 8,9,109–113).

The interactions of the dopaminergic system on the HPA axis as well as the effects of catecholamines on this axis have been studied in both animal models and in humans. It is clear that opiates, like cocaine but to a much lesser extent, cause an elevation in dopaminergic tone, especially in the mesolimbic-mesocortical dopaminergic system. However, as discussed earlier, several groups have shown that although this is a reproducible phenomenon, the mesolimbic-mesocortical dopaminergic system is not essential for heroin or morphine self-administration, and animals that have received lesions abolishing this mesolimbic-mesocortical dopaminergic system readily self-administer opiates such as morphine. This finding is in sharp contrast to that which pertains for cocaine self-administration in which lesions of the mesolimbic-mesocortical dopaminergic system abolish cocaine self-administration. Thus, the role of dopamine in the well-established acute morphine activation of the HPA axis in rodents is of interest, but it may be a related, but not central, component of the mechanism underlying self-administration. More recent studies performed in transgenic mice have had a deletion or knockout of DARPP-32, an obligatory component of the signal transduction mechanisms after activation of primarily dopaminergic D1 receptors; a profound attenuation of the well-established cocaine effect of enhancing hormones of the HPA axis, including adrenocorticotropic hormone (ACTH) and corticosterone levels, was found (110). Parallel studies using this model to explore the impact of this deletion on the well-established acute morphine activation of this axis have yet to be conducted.

Of great interest for many years, and not always recognized by research groups, has been the finding that rodents have the opposite response to acute opiate administration than do humans; that is, activation of the HPA axis occurs. Studies in drug-naive healthy humans, as well as in formerly opiate-dependent healthy humans, and in active heroin addicts have shown that the first, or initial, acute administration of a short-acting opiate, such as morphine or heroin, as well as the first or initial acute administration of a long-acting opioid, such as methadone, will cause suppression of the stress-response systems. Further, in humans, chronic self-administration of short-acting opiates, such as heroin, leads to a continuing suppression of this HPA axis. In con-

trast, many rigorous studies have shown with chronic administration of a long-acting opioid, such as methadone, which allows steady-state profusion of μ-opioid receptors in humans and which is provided during methadone maintenance treatment of heroin addiction, one sees normalization of this axis (6,8,9,85,89–95,100–101).

Zhou et al. modeled this phenomenon in rodents and found that whereas acute intermittent morphine administration causes activation of the HPA axis, delivery of methadone by pump to achieve a steady state, paralleling the situation in humans receiving chronic methadone treatment for management of opiate addiction or chronic pain (with pump delivery essential because methadone has a half-life of 90 minutes in the rat), yields neither alterations in any component of the HPA axis nor alterations in ACTH or corticosterone levels seen (111,112). When administered on a chronic basis in humans or in rodents, short-acting opiates such as heroin and morphine cause suppression of the HPA axis and with no sustained activation in rodents. During either spontaneous or naloxone-precipitated withdrawal, one sees activation of the hormones of the HPA axis in all species studied.

Recently, an animal model was designed to mimic more closely the human pattern of heroin administration, with multiple short-acting opiate (morphine) administrations given at evenly spaced intervals over a single day; activation of the HPA axis with elevation of levels of ACTH and corticosterone was found (111). In addition, as part of this initial study of the effects of acute intermittent morphine, but given in a mode more closely similar to that in the human heroin addict, the impact of a superimposed stress on the effects of morphine was also studied (111). A modest stress of water restriction was applied that, like acute morphine, also significantly increased the ACTH levels. However, when morphine was concomitantly administered to the animals undergoing modest water restriction, morphine attenuated the stress-induced elevation of ACTH and corticosterone levels of this axis (111). These findings may have enormous implications for the human condition, in which morphine or heroin may act immediately to attenuate any activation of the HPA axis caused by any one of numerous types of environmental stressors. Rigorous studies have now been conducted showing that another drug of abuse, cocaine, not only causes elevation of ACTH and corticosterone levels, but also initially enhances corticotropin-releasing factor mRNA levels; however, it was also found that chronic binge pattern cocaine administration led ultimately to an attenuation of the still elevated plasma levels of ACTH and corticosterone by 14 days, and at that time corticotropin-releasing factor mRNA levels were significantly lower than basal levels (109). Recently, Zhou and colleagues made similar findings with respect to acute versus chronic ethanol treatment (113).

Various studies in humans, from the Kreek laboratory, and in animal models, by Stewart, Shaham, and Erb and many other investigators, further documented that stress and stressors, in addition to cues of drug use, and "priming," or reexposure to a drug, may play an important role in relapse to self-administration of drugs of abuse (99,103, 104,114–118). Moreover, various studies (99,103,104, 114–116), such as the work of Piazza and LeMoal, showed that animals with a greater response to novelty or stress and also animals with higher basal levels of the stress-responsive hormone, corticosterone, may more readily begin to acquire self-administration of a drug of abuse, at least of low-dose psychostimulants such as cocaine (reviewed in ref. 116).

Other studies have documented unequivocally that each of the major drugs of abuse highly significantly not only alter the hormone levels of the HPA axis, but also causes alterations of levels of expression of genes of that axis, as well as of similar stress-responsive genes in other parts of the brain, not directly involved in the HPA axis (109,112, 113). Corticotropin-releasing factor, indirectly and directly measured, for instance in the work of Weiss and Koob, was shown to play a potentially very important role in particular aspects of withdrawal from drugs of abuse and in relapse (6,8,9,89,90,95,99,103,104,117,118).

Studies in Novel Animal Models

Since the mid-1990s, investigators have increasingly developed and used animal models that more closely mimic human patterns of drug abuse and emulate the pharmacokinetic situation that pertains during treatment of addictions, such as the pharmacotherapy of heroin addiction, which has been successful primarily by using long-acting, specific μ-opioid-receptor–directed agonists, and also a partial agonist, including methadone, L-α-acelytmethadol (LAAM), and more recently buprenorphine (with its abuse potential minimized by the addition of the non–orally bioavailable antagonist naloxone).

One of the earliest of these animal models that closely parallels a human pattern of addiction was the development of the binge pattern cocaine (investigator) administration model. This model mimics the most common pattern of human abuse, that is, multiple self-administrations of cocaine either by the intravenous route of administration or by inhalation (smoking) of the freebase form, known as crack (75–78,80,81,119,120) This model has uniquely allowed identification of molecular neurobiological changes, including increases in μ-opioid-receptor density that has subsequently been identified in human cocaine addicts (75, 77,121). Animal models mimicking the most common human pattern of heroin addiction have really just begun to be used (111). Heretofore, most of the subacute and chronic models used morphine, the major metabolite of heroin, not heroin itself, and they also used morphine pellet implantation, to develop tolerance and dependence with ease and predictability (with such morphine pellets usually implanted every 1, 2, or 3 days, and most commonly using

the NIH-NIDA developed 75-mg morphine pellets developed by the National Institutes of Health and National Institute of Drug Abuse). Although extremely useful and convenient for many studies, this pellet (prolonged exposure, followed by slow withdrawal) approach does not give the features that have been shown in many studies to be profoundly different from when "steady-state" (pump) or "on-off" (intermittent injections) are used. Thus, increasingly, investigator administration models are being developed in which the human pattern of heroin addiction may be mimicked, that is, with heroin or morphine administered at equally spaced intervals during the animal's awake period, three to six times every day, and with opiate withdrawal over the sleep period, which is most common for the heroin addict.

Similarly, because methadone, the most widely used and efficacious of the μ-opioid agonist treatments for heroin addiction, has, in fact, a very short-acting pharmacokinetic profile in rodents (90 minute half-life in the rat and 60 minutes half-life in the mouse, as contrasted with a 24 hour half-life in humans, and with an even long-acting half-life of the active l(R) enantiomer in humans), to mimic the human situation for treatment in rodents, one must administer methadone in a steady state, using pump technology (122–126). When this has been done, very different findings may have been made than when methadone has been administered intermittently, and thus it behaves in the rodent as a short-acting μ-agonist (112).

Over the past several years, it is has also been recognized that whereas opiates and also other drugs of abuse may cause innumerable acute effects, ranging from enhanced early gene expression (e.g., cfos and related Fos peptide changes) to later changes in other gene expressions and resultant neurochemical and behavioral changes, most of these changes disappear, become attenuated, or are altered by opposing or counterregulatory events after subacute or chronic short-acting opiate administration in an on-off pattern, in which setting, for instance, both dynorphin expression and κ-opioid-receptor gene expression become elevated (71,72). Increasing numbers of basic laboratory investigators are therefore focusing on studies of subacute and chronic effects of opiates, as well as other drugs of abuse, and then are proceeding to study those effects that persist during and after withdrawal of opiates (and other drugs of abuse) and into the abstinence period, to determine the point of no return or very slow return to normal status and thus the critical turning point in the development of relentless drug self-administration or addiction. Thus, models also have been developed and studies conducted to attempt to model human craving and relapse (or resumption of drug exposure or self-exposure), including the use of cue-induced, stress-induced, and small amounts of drugs of abuse-induced (priming) challenges, as well as in investigator-administered drug. Relevant molecular and neurobiological effects also are being conducted.

Most of these models mentioned earlier are investigator-administered models. There have also been several parallel studies attempting to modify long-existing, self-administration models to more closely parallel the human pattern of drug abuse and addiction (8,127–130). For various important and valid research reasons, self-administration studies, which use rats, mice, or nonhuman primates, primarily have been conducted using short sessions (usually, 1 to 3 hours in length) and in special cages to which each animal is moved for such studies, to provide the repeated cueing of a novel drug-related environment. Some studies, notably by the groups of Koob and Ahmed, Miczek and Tornatzky, and Mantsch et al., are starting to use much longer sessions of self-administration and also with very different unit doses of drug to be self-administered, with the resultant findings of different patterns of acquisition, extinction, and relapse that are probably more relevant to the human disorders of addictions (127–130).

Most studies since the mid-1980s years also have used relatively low to very low unit doses of the drug to be self-administered (although much higher unit doses were used effectively in some very early studies). These low doses have been used to allow evaluation of the reinforcing or rewarding properties of the drug by measurement of the number of responses, or work performed, and thus willingness to work to receive a unit dose of drug and also thereby to evaluate perturbations, either pharmacologic or behavioral, that may reduce that level of work. However, in human drug abuse and addiction, much larger unit doses of drugs of abuse (heroin or cocaine) are self-administered, and for opiates especially, with longer intervals between self-administrations. Thus, the bolus effect of a very rapid onset of action of a large amount of a short-acting drug such as heroin (or cocaine), self-administered either intravenously or by inhalation with sublimation of freebase drug, is achieved. It has been shown that the rapid rate of rise of amount of drug at a specific site of action, such as the μ-opioid receptor for heroin, is more closely related to the reinforcing effects, and also the rapid offset of drug action is related to the withdrawal or abstinence effects of a drug of abuse. Thus, higher unit dosages of drugs, such as are self-administered in the human situation, will have greater positive and negative reinforcing effects than small doses (8). Numerous small doses may, in fact, more closely begin to model a maintenance or steady-state mode, although the sessions are often too short to be analogous to desired treatment. A few groups are now using much longer sessions of self-administration and also, in some studies, higher unit doses of drug (primarily cocaine, but also heroin or morphine), with the expectation of longer self-administration dosing intervals and much larger total doses self-administered, thus probably with greater impact on molecular, cellular, and neurobiological features and, importantly, a greater magnitude and also qualitatively different and relevant behavioral changes (127–130).

BASIC CLINICAL RESEARCH

From the mid-1960s, the Kreek group hypothesized that there is a metabolic basis to addictive diseases, and an atypical responsivity to stress and stressors may contribute to the persistence of and relapse to addiction to heroin and also addictions to other specific drugs of abuse. Furthermore, it was hypothesized that such an atypical responsivity to stress and stressors may exist *a priori* on either a genetic or an early environmentally induced basis and may contribute to the initial acquisition of an addiction (6,85,88–91). Therefore, prospective studies, which were started in 1964 at the beginning of research on use of the long-acting opioid methadone in the pharmacotherapy of heroin addiction linked with behavioral treatment, included studies to assess the HPA axis component of the stress-responsive system, because this is one critically important component and one that can be evaluated in living humans; additional special studies were also conducted (6,85,89–91). Those very early studies documented an atypical responsivity of the stress-responsive HPA axis in heroin addicts, with suppression of all aspects of this axis by chronic self-administration of the short-acting opiate heroin, including reduction of plasma levels of hormones and alterations in the feedback control mechanisms, and also abnormal gonadal function with an impact on reproductive biology (6,85,89–92). Further, those early prospective studies on the effects of the long-acting opioid methadone, contrasted to the physiologic and pharmacologic effects of short-acting opiates, such as heroin, showed normalization during chronic methadone treatment of diverse physiologic functions disrupted by chronic heroin abuse, with gradual normalization of the stress-responsive HPA axis function over a 3- to 4-month period during steady moderate to high-dose treatment with the long-acting opioid methadone (6,8,9,10,85,89,90, 93–102).

Studies also showed reduced responsivity to a chemically induced stress during cycles of heroin addiction and normalized neuroendocrine function of the HPA axis including a normal response to a chemically induced stressor during methadone maintenance treatment (6,85,89–91). However, further studies that we initially performed in 1983 and 1984 (95,99) showed a hyperresponsivity to a chemically induced stressor in medication-free and illicit drug-free former heroin addicts. A hyperresponsivity to an induced stressor, not only in drug- and medication-free former heroin addicts, but also in active cocaine addicts, who were using cocaine alone, and in methadone-maintained persons who continue to be addicted to cocaine was subsequently documented (95,98,99,103,104).

Other very important clinical research studies that have been conducted have shown, for instance, that activation of the stress-responsive HPA axis may precede, rather than follow, the signs and symptoms of opiate withdrawal (105–107). The activation of the HPA axis may drive the onset and may contribute to the severity of withdrawal symptoms, rather than result from the unpleasant or noxious qualities of these signs and symptoms (105–107). In addition, further studies of the continuing disruption of the HPA axis during naltrexone treatment and the lack of normalization of the assumed disruptions by heroin of HPA axis function during short-term buprenorphine treatment have been reported (10,28,96,97,102).

The Kreek laboratory hypothesized that natural sequence, but shorter, dynorphin $A_{1\text{-}13}$ administered intravenously would result in prompt induced elevation of serum prolactin levels in normal healthy volunteer subjects. This was hypothesized because of two sets of previous findings. First, it is well-known that μ-opioid-receptor agonists will effect a rise in serum prolactin levels. Moreover, it has been shown that even during long-term methadone treatment, tolerance or adaptation is not fully developed to this prolactin-releasing effect of methadone (85). The mechanism for this is also known. In humans, prolactin release is essentially completely under tonic inhibition by dopamine. Therefore, an elevation in prolactin levels indicates a spontaneous or induced reduction in dopaminergic tone in the tuberoinfundubilar dopaminergic system. Other studies by several groups showed that synthetic small compounds that are κ agonists may reduce dopaminergic tone in rodents, and Claye et al. showed that the natural peptide dynorphin $A_{1\text{-}17}$ instilled into the nucleus accumbens results in a reduction of dopaminergic tone in rats (68). In a study of healthy human volunteers, it was shown that a dose-dependent elevation of serum prolactin levels occurs in response to intravenous administration of dynorphin $A_{1\text{-}13}$ (86). Further studies using two different opioid antagonists documented that this effect was mediated by the κ- as well as μ-opioid receptors. It was also shown that females, who have significantly higher basal prolactin levels, responded to a significantly greater extent to this natural peptide κ-opioid-receptor agonist challenge with respect to elevations in serum prolactin levels (86).

In other studies, Specker and Pentel and colleagues found attenuation of opiate withdrawal symptoms in heroin addicts given dynorphin $A_{1\text{-}13}$ (131). These studies build onto much earlier studies, which were not well controlled but which suggested that dynorphin peptides may attenuate some of the signs and symptoms of opiate withdrawal. In studies conducted in patients with chronic pain, dynorphin $A_{1\text{-}13}$ was shown to augment the analgesia provided by the usual μ-opioid agonists (morphine or methadone), a finding suggesting a positive interaction between the μ- and κ-opioid-receptor agonists and a possible novel approach for providing pain relief (132). All these findings suggest that one could consider clinical research studies using a κ-opioid agonist along with a μ-opioid agonist in a pharmacotherapy of opiate addiction (11).

In another area of basic clinical research related to the neurobiology of heroin addiction or its treatment, imaging

techniques, using positron emission tomography or the related technique of single photon emission computed tomography, with studies of glucose metabolism or blood flow to assess activation or depression of activity of specific brain regions, as well as some studies using ligands directed toward specific types of receptors, including recently the opiate receptors, have been conducted in humans and reported (133, 134). In addition, some studies using magnetic resonance imaging and functional magnetic resonance imaging have begun to contribute to our information about withdrawal from heroin addiction (135). We hope that, in the future, such imaging studies will contribute even further to our understanding of the neurobiology of the development of and relapse to opiate addiction and will also potentially be able to be related to the apparent normalization of function that can occur during long-acting μ-opioid receptor agonist treatment with methadone, or alternatively with l-α-acelytylmethadol (LAAM) and possibly also (but yet to be studied) with the buprenorphine-naloxone combination. The implications of all of this basic clinical research for treatment have been considered further in reviews (10,11,27–31).

Finally, the first successful cloning of the genes of the specific opiate receptors, starting in late 1992, led to studies to identify polymorphisms of the human opioid receptor and peptide genes and as well as of other genes that have been shown to be affected by drugs of abuse, and specifically for this discussion, by short-acting opiates used illicitly. Many such polymorphisms, including primarily SNPs of the μ-opioid receptor as well as of related genes, have been identified recently (6,7,32,58,136–138). Studies of potentially functional changes resulting from those polymorphisms, especially SNPs in the coding region of the genes resulting in amino acid changes, and thus in resultant peptide differences, have been initiated (32,58). In addition, a few groups are now studying human molecular genetics of the specific addictive diseases, including heroin addiction. In fact, an epidemiologic study by Tsuang et al. suggested that heroin addiction may have an even greater relative risk attributable to heritable factors than any other addiction, including alcoholism (139).

MOLECULAR BIOLOGY AND GENETICS

The completion of cloning of the genes of the endogenous opioid system, following the first reports of the cloning of the δ-opioid receptor in late 1992, allowed the expansion of many types of studies, as well as the initiation of new studies. All the genes of the endogenous opioid system in rodents, as well as in humans, now may be included in molecular neurobiological studies, such as studies of quantification of levels of gene expression (mRNA levels). It is now also possible to look for polymorphisms, including SNPs, in human genes of the endogenous opioid system, as well as genes of related neurotransmitter, neuropeptide, and receptor systems (as discussed earlier). Since 1994, new technologies for such studies have been developed, and during the next decade, undoubtedly, they will be able to be used for novel discoveries of heretofore unrecognized genes and gene products involved in the acquisition, persistence, and relapse to addiction (6,7,32,58,136–138). These include use of microarray technologies for measuring gene expression (although to date, these arrays are relatively insensitive and cannot yet detect, let alone measure with precision and accuracy, the small changes, usually less than 50% to 100% increase or decrease, that may be expected in integrated neurobiology for genes of low-abundance encoding neuropeptides and their receptors, such as those of the endogenous opioid system). Nevertheless, use of microarray technology, and with the developing informatics to analyze the vast amounts of the expected resultant data, will undoubtedly reveal novel gene systems involved in the specific addictive diseases. In addition, microarray technology and other new approaches are beginning to be used for the identification of already recognized polymorphisms, including SNPs, and they may be able to be used in the future for the identification of novel polymorphisms (6,7,32).

The completion of the cloning of the endogenous opioid system has permitted the development of appropriate gene deletion, so-called knockout mouse models (reviewed in refs. 33–35). The single most important and relevant finding with respect to opiate addiction has been the documentation, first by Kieffer and colleagues, that there is no opiate-induced reward, as measured by conditioned place preference in the μ-opioid-receptor knockout mice; in addition, these mice show essentially no self-administration of ethanol (33,140). All investigations have shown that μ-opioid-receptor knockout mice have no analgesic response to conventional μ-opioid-receptor agonists such as morphine (reviewed in refs. 33–35). Cloning of these genes also permitted the further use of knock-down, or antisense modeling, as well as gene enhancement using appropriate constructs for gene delivery. Many laboratories have initiated work for conditional knockout or knock-in enhancement of gene expression, with control of time of onset of the deletion or enhancement, as well as in some models, specific brain region–dependent changes.

SUMMARY

Many exciting developments stemmed from the initial cloning of each of the three opioid receptors—δ, μ, and κ—in 1992 and 1993, and the subsequent cloning of each of those genes in humans in 1994. Subsequently, many studies have been and can be conducted, using classic techniques, as well as other new modern techniques, such as microarray technology. Various studies on the impact of opiates on gene expression as well as signal transduction systems and integrated physiologic function have been conducted.

Moreover, novel animal models have been developed. Possibly most excitingly of all, further basic clinical research studies have been performed, including studies identifying many polymorphisms of human genes of the endogenous opioid systems. These studies have already given, and will continue to give, increased insights into the pathophysiology as well as molecular and cellular neurobiology and related behavioral changes of opiate addiction, and all these studies have continued to teach us about the enormous capability of the brain to change through neural plasticity.

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