REVIEW

The Role of Glutamate in Physical Dependence on Opioids

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ABSTRACT—The present review will evaluate the interactions between κ-opioid receptors and glutamate within the locus coeruleus (LC) during the development of opioid dependence and on expression of withdrawal from dependence on opioids. Hyperactivity of noradrenergic neurons in the LC has been proposed to play a critical role in the physiological and behavioral responses that comprise opioid withdrawal. Several studies indicate that the excitatory amino acid system, in particular, glutamate and its receptors, participate in both the withdrawal-associated increase in LC neuronal activity and the expression of opioid withdrawal behaviors. Most studies on opioid dependence have focused on the prototypical opioid morphine, which produces its physical dependence through agonist actions at the μ-opioid receptor. Butorphanol (Stadol®), which exhibits a markedly different profile of opioid receptor activity than does morphine, produces its physical dependence primarily through actions at the κ-opioid receptor. Studies from our laboratories using a rodent model in which butorphanol administration induces dependence indicate further that the κ-opioid receptor is an important regulator of glutamate release within the LC. Glutamate exerts actions within the LC that mediate expression of behavioral symptoms of butorphanol withdrawal.

Keywords: Glutamate, Physical dependence, Withdrawal, Locus coeruleus, *N*-Methyl-D-aspartate receptor, Morphine, Butorphanol

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Abbreviations used are (in alphabetical order): AMPA, α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid; AP-5, 2-amino-5-phosphonopentanoic acid; D-APH (AP-7), 2-amino-7-phosphonoheptanoic acid; Butorphanol, 17-cyclobutylmethyl-3,14-dihydroxymorphinan (Stadol®); CPP, 3-((+)-2-carboxypiperazin-4-yl)propyl-1-phosphonic acid; 5,7-DCKA, 5,7-dichloro-kynurenic acid; EAA, excitatory amino acid; H-7, 1-(5-cyclic isoquinolinesulfonyl)-2-methylpiperazine; i.c.v., intracerebroventricular; LC, locus coeruleus; LY274614, (+)-6-phosphonomethyl-decahydroisoquinoline-3-carboxylic acid; MK-801, (+)-5-methyl-10,11-dihydro-5*H*-dibenzo(*a,d*)cyclohepten-5,10-imine; NMDA, *N*-methyl-D-aspartate; Nor-BNI, nor-binaltorphimine; PCP, phencyclidine; PGi, nucleus paragigantocellularis; PKA, cAMP-dependent protein kinase; PKC, protein kinase C; U-50,488, *trans*-(+)-3,4-dichloro-*N*-methyl-[2-(1-pyrrolidinyl)-cyclohexyl] benzeneacetamide methane sulphonate; U-50,488H, 3,4-dichloro-*N*-methyl-*N*-[2-(1-pyrrolidinyl)-cyclohexyl] benzeneacetamine; U-69,593, (5α,7α,8β)-(+)-*N*-methyl-*N*-[7-(1-pyrrolidinyl)-1-oxaspiro[4,5]dec-8-yl]-benzeneacetamine.

I. Introduction

Physical dependence on opioid drugs is identified, both clinically and experimentally, by the expression of excitatory physiological responses. These responses, which become evident when the influence of the offending opioid is removed abruptly, constitute the phenomenon of withdrawal. During the last decade, hyperactivity of noradrenergic neurons within the locus coeruleus (LC) has become a focal point for investigation into genesis of the excitation associated with opioid withdrawal (1, 2). Excitatory amino acids (EAA), in particular, glutamate and, to a lesser extent, aspartate have been hypothesized to mediate at least some component of this increase in neuronal activity (3–7). One potential source for excitatory glutamatergic projections to the LC is the medullary nucleus paragigantocellularis (PGi). The recent review by Christie et al. (8), while emphasizing the overall neuroanatomical complexity underlying opioid dependence /withdrawal, continued to recognize a pivotal role for excitatory events within the LC.

This review will focus upon discussion of the participation of glutamatergic neurotransmission in expression of neurochemical and behavioral aspects of opioid withdrawal. However, in so doing, it must be acknowledged that the preponderance of studies on opioid dependence have used the prototypical opioid analgesic morphine to induce physical dependence. A specific caveat must accompany recognition of this unitary direction for research. Clinical use of opioid analgesics, other than morphine, which often have actions at opioid receptor subtypes that differ markedly from those of morphine, is increasing (9). Of particular interest in this regard are recent data indicating that the κ-opioid receptor subtype is an important regulator of neuronal activity and glutamate release within the LC (10–13). Thus, experimental models that test only morphine may have neglected or underestimated certain important aspects of EAA involvement in opioid dependence/withdrawal. This caveat is uniquely relevant when considering the mechanism of action of the mixed agonist/antagonist class of opioids. Indeed, butorphanol (17-cyclobutylmethyl-3,14-dihydroxymorphinan) tartrate (Stadol®), one member of that class, has become an increasingly important opioid analgesic agent (14). Originally believed to have been lacking in addiction liability, butorphanol has since been associated with numerous reports of diversion (15), abuse (16) and addiction (17–19). Considerable public media attention has been devoted to documentation of the addiction liability of the recently introduced intranasal formulation for butorphanol (20). Thus, the growing awareness of the κ-opioid receptor subtype as a mediator of EAA release within the LC will provide an additional focus for this review.

II. Opioid receptor subtypes and physical dependence/withdrawal

Physical dependence is defined as the need to continue drug administration in order to avoid physical discomfort (withdrawal symptoms, ref. 21). Opioid withdrawal symptoms are generally opposite to the physiological responses observed after acute opioid administration and can be immediately suppressed by readministration of an opioid. The dependence appears to originate from adaptations of cells, particularly within the central nervous system, to repeated or continuous opioid exposure. It is hypothesized that such adaptations attempt to restore a normal neurochemical equilibrium despite the continuing presence of an opioid. The adaptive responses create a new homeostasis that has an absolute requirement for ongoing opioid administration because sudden termination of opioid use or administration of opioid antagonists unmasks the adaptations and results in an aversive withdrawal syndrome. In humans, the opioid withdrawal syndrome is characterized by nausea, vomiting, irritability, restlessness, anxiety, insomnia, hot and cold flashes, muscle aches, increased respiratory and heart rates, sweating and diarrhea. A continuous intracerebroventricular (i.c.v.) infusion method for making animals dependent on opioids has been well-established in rodents (22, 23). In the rat, this opioid withdrawal syndrome is characterized by the appearance of stereotyped jumping, teeth chattering, scratching, wet dog shakes, writhing, ptosis, penis licking, lacrimation, urination and diarrhea. The anxiety and the multitude of autonomic symptoms that accompany opioid withdrawal are presumptively associated with hyperactivity of noradrenergic LC neurons because electrical stimulation of LC neurons increases activity of the sympathetic nervous system (24). Moreover, clonidine, an α_2-adrenoceptor agonist that reduces central sympathetic efferent tone, can attenuate both the withdrawal-induced activation of LC neurons and many of the withdrawal symptoms (1, 25).

Opioid receptors are classified into three main subtypes: μ-, κ- and δ-opioid receptors (26–30). These belong to the family of G-protein associated receptors. Opioids, via coupling to a pertussis toxin sensitive G-protein ($G_{o/i}$), inhibit adenylyl cyclase, activate receptor-regulated K^+ currents, and suppress voltage-gated Ca^{2+} currents (for a review, see ref. 31). As noted above, most studies of opioid physical dependence have focused on morphine, which acts mainly on the μ-opioid receptor and possibly on the δ-opioid receptor (32–34). The role of the κ-opioid receptor in the development of opioid dependence has been much less intensively evaluated. Pasternak et al. (35) have recently summarized data suggesting that the interactions of glutamate neurobiology

which occur during the development of tolerance to an opioid differ between opioids with μ- or δ-agonist activity and those that are κ-opioid receptor agonists.

Butorphanol is a mixed agonist/antagonist that acts on the μ-, δ- and κ-opioid receptors with an affinity ratio of 1:4:25 (36, 37). The physical dependence liability of butorphanol is considered to be minimal when used within the therapeutic dose range. However, a marked physical dependence liability has been observed in animals and humans when larger doses of butorphanol were administered for a prolonged period (17, 38, 39). Studies from our laboratory have shown that in rats, large doses of butorphanol given by i.c.v. infusion can produce physical dependence and that the signs and symptoms of withdrawal from this dependence are similar to those produced during withdrawal from dependence on morphine (23). Distinctions between the mechanisms involved in butorphanol and morphine dependence are evident. Butorphanol produces its physical dependence primarily through κ- and/or δ-opioid receptors because β-funaltrexamine (a μ-opioid receptor selective antagonist) failed to precipitate withdrawal in butorphanol-dependent rats (40), while both naltrindole (a δ-opioid receptor selective antagonist) and nor-binaltorphimine (nor-BNI; a κ-opioid receptor selective antagonist) have been shown to precipitate withdrawal signs similar to those precipitated by the non-selective opioid receptor antagonist, naloxone (41, 42). Furthermore, when κ-opioid receptors were masked by nor-BNI before or during the development of dependence on butorphanol, subsequent naloxone-precipitated withdrawal signs were blocked (41). In contrast, the μ-opioid receptor participates to a significantly greater extent in dependence /withdrawal on/from morphine than on/from butorphanol (33).

III. Regulation of glutamatergic neurotransmission

A. Glutamate release and uptake

Glutamate is the principal EAA neurotransmitter in the central nervous system (43–45). Glutamate is stored in synaptic vesicles and released by exocytosis following nerve terminal depolarization (for a review, see ref. 46). There is a 10,000-fold concentration difference of glutamate across the presynaptic membrane. This concentration difference is maintained by high-affinity, Na^+-dependent glutamate transporters (46, 47). Three glutamate transporters have been cloned: EAAC1 (48), which is selectively localized to neurons (49, 50), and the astroglial transporters GLT-1 and GLAST (49–53). Recently, results from studies using antisense techniques to inhibit the synthesis of each glutamate transporter subtype suggest that the glial glutamate transporters provide most of the functional glutamate transport out of the extracellular space and are essential for synaptic clearance of glutamate (54).

Experimental evidence supports the thesis that opioids modulate glutamate release. Acutely, morphine, a μ-opioid receptor agonist, was shown to prevent the evoked release of endogenous glutamate in the sensorimotor cortex of conscious rats (55) and in both brain slices and synaptosomes from the cerebral cortex (56). Kappa-opioid receptor agonists have been reported to inhibit stimulated glutamate release from guinea pig hippocampal mossy fiber terminals (57), from synaptosomes from rodent and primate striatum (58), and from rat cerebrocortical slices (59). Nerve projections from PGi provide a major excitatory input to the pontine LC (60). Intracellular recordings of LC neurons have revealed that the selective κ-opioid receptor agonist U-50,488 can reduce excitatory synaptic potentials evoked by electrical stimulation afferent to LC neurons without affecting either the input resistance, membrane potential or the calcium action potential recorded from the postsynaptic neurons (61). These results suggest that activation of κ-opioid receptors, located on the presynaptic membrane, inhibits the release of EAAs from presynaptic terminals in the LC. The mechanisms underlying the inhibitory effects of opioids on neurotransmitter release are thought to involve blockade of presynaptic transmembrane Ca^{2+} flux. Parallel inhibition of Ca^{2+} uptake and glutamate release by opioids was observed in synaptosomes and brain slices from cortex (56) and in primary cultures of rat cerebral cortex (62). The selective κ-opioid agonist U-50,488H dose-dependently inhibited the potassium-induced rise in synaptosomal free Ca^{2+} levels (57). In addition, the inhibitory effect of opioids on glutamate release has been suggested to be mediated by adenylyl cyclase since GTP, which inhibits adenylyl cyclase activity, enhanced the inhibitory effect of morphine on Ca^{2+} uptake (56).

B. Glutamate receptors

The glutamate receptors can be classified into two subgroups, termed the ionotropic and metabotropic receptors (for reviews, see refs. 63, 64). Ionotropic glutamate receptors are ligand-gated integral ion channels and are further divided into two major subtypes: those that are responsive to the selective agonist *N*-methyl-D-aspartate (NMDA) and those that respond to either α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA) or kainic acid. Of the ionotropic glutamate receptors, the NMDA subtype has been the most extensively characterized. Analysis of the available data infers that the NMDA receptor is involved in the phenomena of opioid dependence and withdrawal. This subtype is widely distributed

in the mammalian central nervous system (being highly enriched in the hippocampus and the cerebral cortex) and is important in a variety of synaptic plasticity phenomena other than opioid dependence, including long-term potentiation and behavioral sensitization to psychomotor stimulants (65, 66). The NMDA receptor consists of an ion channel and several modulatory sites (67, 68). Glutamate activates the receptor by binding to a stereospecific glutamate recognition site on the complex, opening the ion channel. The NMDA receptor channels are blocked by Mg^{2+} in a voltage-dependent manner (69, 70) and are permeable to Na^+ (71) and K^+ (72). The entry of Ca^{2+} has the ability to participate in numerous intracellular processes, including activation of protein kinases (for a review, see ref. 67). Competitive antagonists for the NMDA receptor, such as LY274614, AP-7 and CPP, selectively block this glutamate recognition site. Activation of the receptor by glutamate is facilitated by the binding of the co-agonist glycine to an allosteric site on the receptor complex. Drugs acting at the polyamine site exert complex effects on the ability of EAAs to activate the receptor (73, 74). Another site, the non-competitive phencyclidine (PCP) site, is located within the channel. Drugs acting at this site, such as PCP or MK-801, block ion movement through the channel, thereby antagonizing the activation of the NMDA receptor. Studies based on molecular cloning have demonstrated that NMDA receptor complexes are made up of various combinations of subunits. All NMDA receptors contain at least one obligatory NMDA-R1 (NR1) subunit that is combined with one or more kinds of NMDA-R2 subunits (NR2A-D) (75, 76). Alternative RNA splicing can generate at least eight distinct NR1 splice variants (NR1a−h) (77). Experiments in vitro have shown that NR1 subunits can form a homo-oligomeric structure that has channel activity (75, 76, 78), whereas NR2 subunits produce functional receptors only when they are coexpressed with NR1. NMDA receptors with different NR1 and NR2 subunit combinations have different electrophysiological and pharmacological properties (76).

The AMPA and kainate receptors gate cation channels that are permeable to Na^+ (79), and some cloned AMPA and kainate receptors also exhibit Ca^{2+} permeability (80, 81). AMPA and kainate receptors mediate rapid excitatory responses to EAA transmitters and play complementary roles to NMDA receptors in synaptic responses (80, 82).

Metabotropic glutamate receptors are G-protein-coupled and are linked to phosphatidylinositol or a cAMP second messenger system (64, 83) and are also involved in synaptic plasticity such as long-term potentiation and long-term depression (84−86).

IV. The LC plays an important role in opioid physical dependence/withdrawal

Many brain regions have been implicated in opioid dependence and withdrawal (for reviews see refs. 8, 87). Some of these brain structures are densely innervated by noradrenergic terminals (88); e.g., the periaqueductal grey matter, amygdala, hippocampus, and hypothalamus. The LC is a pontine nucleus that contains the largest cluster of noradrenergic neuronal soma in the brain (89, 90). These neurons make very broad efferent projections throughout the central nervous system (91, 92). Functionally, the LC has been linked to regulation of anxiety states, vigilance, attention, learning and memory as well as sympathetic efferent circulatory tone (24, 90). Autoradiographic studies have shown that the LC is enriched with opioid receptors, particularly of the μ- and κ-subtypes (93), while the results of a variety of electrophysiological, biochemical and behavioral studies suggest that noradrenergic neuronal activity within the LC plays a critical role in opioid dependence and withdrawal.

Electrophysiological evidence: Numerous electrophysiological studies have documented that modulation of LC neuronal activity contributes to the processes underlying opioid dependence and withdrawal. Acute administration of morphine inhibits firing of LC neurons (1, 94, 95) by opening of K^+ channels (96, 97) and inhibiting a resting Na^+-dependent inward current (98). After chronic treatment, LC neurons become tolerant to and dependent on morphine, since LC neuronal firing rates return to control levels after continued drug exposure (1, 99). It has also been demonstrated that there is a marked increase, above control levels, in the firing rate of LC neurons in vivo during opioid receptor antagonist-precipitated withdrawal from morphine (1−3, 5, 100). This hyperactivity is associated with expression of the behavioral symptoms of withdrawal (2, 101, 102). Moreover, local electrical stimulation of the LC produces both behavioral and physiological signs that are similar to those elicited during opioid withdrawal (103).

Biochemical evidence: Consistent with the results from electrophysiological studies, in chronically morphine-treated rats, naloxone produces a dramatic increase in the catechol oxidation current, which serves as an index of noradrenergic neuronal activity in the LC (104). Decreased release of norepinephrine from the LC is observed after acute morphine treatment (105, 106), while during withdrawal, there is an increase in norepinephrine turnover (107) and release (108) in LC projection areas.

Behavioral evidence: The importance of the LC in opioid dependence is also supported by a study in which bilateral electrolytic lesions of the LC decreased many of the morphine withdrawal signs precipitated by i.c.v. ad-

ministration of the opioid antagonist methylnaloxonium (109). In addition, local injection of naloxone into the LC can precipitate withdrawal signs in morphine-dependent rats (110). A detailed mapping study in which methylnaloxonium, a hydrophilic opioid antagonist, was microinjected into several brain regions in morphine-dependent rats showed that the most sensitive site from which methylnaloxonium could precipitate withdrawal signs was the LC (111).

V. Interactions between glutamate and the LC in opioid dependence/withdrawal

A. The role of excitatory afferent inputs to the LC in opioid dependence/withdrawal

Andrade et al. (112) observed that withdrawal-induced hyperactivity of LC neurons does not occur in isolated brain slices taken from morphine-dependent rats. This indicated that the hyperactivity seen in vivo is dependent on afferents to the LC which are disconnected during preparation of brain slices. Single-cell unit recordings in vivo revealed that i.c.v. injection of kynurenic acid, a nonspecific antagonist at EAA receptors, abolished the activation of LC neurons that is observed during naloxone-precipitated withdrawal (3, 4). In addition, Akaoka and Aston-Jones (5) observed that LC neuron hyperactivity could be suppressed, although not totally abolished, by intracoerulear administration of kynurenic acid or antagonists selective for either NMDA or non-NMDA glutamate receptors. These data reveal that an EAA input to the LC may be critical to generation of the hyperactivity of LC neurons during naloxone-precipitated withdrawal.

One major excitatory input to the LC is the PGi, which is located in the rostral ventro-lateral medulla (60, 113). Lesions of the PGi attenuate morphine withdrawal-induced activation of LC neurons (3), suggesting that an excitatory PGi-LC pathway plays an important role in the withdrawal-induced hyperactivity of LC neurons. Recently, behavioral responses to electrical stimulation of the PGi were examined in conscious, naïve rats. Bilateral stimulation resulted in voltage-dependent stereotyped behaviors that were similar to those observed during withdrawal from butorphanol (114). However, the PGi is not the only source of excitatory input to the LC. Rasmussen and Aghajanian (3) observed that lesions of the PGi attenuate only partially the increase in LC firing during opioid withdrawal, but the attenuation of the LC neuronal activity produced by kynurenic acid was significantly greater than that produced by the PGi lesions. The involvement of another EAA input in the withdrawal-induced activation of LC neurons may account for this phenomenon. This possibility is supported by the fact that somatosensory stimulation that evokes marked increases in LC unit activity can be completely blocked by kynurenic acid, but is unaffected by lesions of the PGi (115). Lamina I of the spinal cord (116) and the medial prefrontal cortex (117) could be the possible sources of such a PGi-independent pathway.

B. Opioid withdrawal induces an increased glutamate release within the LC

There are several putative mechanisms that have been suggested to underlie the hyperactivity of LC neurons during opioid withdrawal. These include increased secretion of excitatory transmitters from presynaptic terminals within the LC, decreased sensitivity or downregulation of postsynaptic μ- and δ-opioid receptors on LC noradrenergic neurons, and alterations in intracellular second messenger systems within LC noradrenergic neurons. At the presynaptic level, an increase in nerve stimulation-induced glutamate release may be responsible for the hyperactivity of LC neurons during the withdrawal.

Recently, direct neurochemical evidence obtained from in vivo microdialysis studies has shown an elevation in extracellular fluid levels of glutamate and, to a lesser degree, aspartate within the LC during naltrexone- or naloxone-precipitated withdrawal in both anesthetized and conscious morphine-dependent rats (6, 7). This increased glutamate release within the LC is associated with the withdrawal-induced behavioral syndrome. The studies from our laboratories have demonstrated that naloxone-precipitated withdrawal from dependence on butorphanol (10) and on the selective κ-opioid receptor antagonist U-69,593 (11) elicit an increase in extracellular levels of glutamate in the LC, similar to that seen in morphine withdrawal. In addition, an increase in glutamate levels in the LC was observed during butorphanol withdrawal induced by the κ-selective antagonist nor-BNI (12, 118). Taken together, these results indicate that increases in the levels of glutamate in the LC may be a common mechanism of opioid antagonist-precipitated withdrawal from opioid dependence.

To investigate further the role of the augmented glutamate release in opioid-dependence and withdrawal, glutamate was directly injected i.c.v. into opioid-dependent animals (119). It is interesting to note that such glutamate injections can dose-dependently precipitate withdrawal signs in morphine- or butorphanol-dependent rats, while similar injection failed to precipitate any withdrawal signs in non-dependent, saline-control groups. The behavioral signs precipitated by glutamate are generally comparable to those precipitated by the opioid receptor antagonist naloxone. There are however, small differences in the duration of withdrawal-induced behavioral excitation and in the total number of behavioral signs elicited. Discrete

microinjection of glutamate into the LC has also been reported to induce withdrawal signs dose-dependently in opioid-dependent rats (120). These unique actions of glutamate suggest that a rapid release of glutamate may be a trigger or key factor for the expression of withdrawal signs from opioids.

The mechanisms that are responsible for the increases in glutamate release in the LC following antagonist-precipitated withdrawal in opioid-dependent animals are not yet fully understood. There are several possibilities. First, chronic opioid treatment may result in adaptations in the glutamatergic neurons. As a result, the activity of the glutamatergic neurons may be increased during withdrawal. It has been reported that expression of c-fos, a nuclear transcription factor that provides a biochemical measurement of neuronal activation, is elevated in the PGi following chronic morphine treatment (121), and an increase in the activity of the cAMP system was observed in the PGi after chronic opioid treatment (122). In addition, afferents to the PGi may also be involved since chronic opioid treatment results in an upregulation of the cAMP system in the dorsal horn of the spinal cord, which provides a major excitatory input to the PGi (122).

Another possibility is that chronic opioid treatment affects the excitatory presynaptic terminals within the LC region. It has been demonstrated that opioids alter Ca^{2+} disposition following either acute or chronic treatment. Acutely, activation of μ-opioid receptor reduces Ca^{2+} influx (56, 123) which is considered to be secondary to activation of the potassium channels (123). In contrast, κ-receptor agonist administration inhibits neurotransmitter release by directly reducing Ca^{2+} entry into the nerve terminals through transmembrane Ca^{2+} channels (124). Chronic administration of morphine produces an effect opposite to that caused by acute opioid treatment; i.e., increased synaptosomal Ca^{2+} levels (125–127). In addition, chronic morphine treatment results in upregulation of L-type Ca^{2+} channels in many regions of the brain including the cortex, hippocampus, hypothalamus, brain stem and striatum (128). Therefore, the increase in glutamate release during opioid withdrawal may result from an increased Ca^{2+} influx during depolarization of the nerve terminals within the LC. Behavioral studies have shown that concomitant infusion of opioids with diltiazem, an L-type Ca^{2+} channel blocker, inhibited the naloxone-precipitated withdrawal from both morphine and butorphanol (129, 130). Although it has been reported that glutamate release from brain synaptosomes might be coupled to P- rather than L- or N-type Ca^{2+} channels (131), microdialysis studies have shown that co-administration of opioids with diltiazem significantly prevented elevation of the LC glutamate release normally observed during withdrawal (130). These results suggest that the L-type Ca^{2+} channels are important in mediation of the increased glutamate release that is noted in the LC during opioid withdrawal.

The increased glutamate release might also be due to alternations in second messenger pathways involved in opioid dependence. It has been shown that chronic treatment with morphine upregulates the cAMP pathway in various brain regions (for a review, see ref. 122). Acutely, opioids inhibit the activity of adenylyl cyclase and cAMP-dependent protein kinase (PKA). After chronic treatment, the neurons become dependent on opioids. Abrupt cessation of chronic opioid treatment, such as precipitation of withdrawal by administration of an opioid antagonist, results in an increased activity of adenylyl cyclase, PKA and several phosphoprotein substrates for the protein kinases. The upregulated cAMP system is viewed as a compensatory homeostatic response of the LC neurons to persistent inhibition by opioids. After removal of the opioid, activity of the upregulated system is unopposed and may induce effects that are responsible for expression of the opioid withdrawal syndrome. Forskolin, which stimulates the activity of PKA, has been shown to increase glutamate release in many preparations (132–134). Protein kinase C (PKC) is also proposed to be involved in opioid dependence because chronic morphine or butorphanol treatment leads to an enhancement of cytosolic PKC activity in the pons /medulla region (129, 135). Recently, Tokuyama et al. (120) reported that H-7, which is an inhibitor of both PKA and PKC, prevented the increase in extracellular levels of glutamate that is normally observed in the LC during withdrawal from morphine or butorphanol. Expression of withdrawal signs precipitated by LC administration of naloxone in these opioid-dependent animals was also completely blocked by concomitant infusion with H-7. Such results indicate that presynaptic enhancement of PKA and/or PKC activity may contribute to the increase in glutamate release within the LC during opioid withdrawal.

VI. NMDA receptors are important in opioid dependence /withdrawal

A. Attenuation of opioid dependence by systemic administration of NMDA-receptor antagonists: behavioral studies

A variety of non-competitive and competitive NMDA-receptor antagonists have been shown to attenuate the expression of opioid withdrawal signs. Ketamine, dextromethorphan (136) and MK-801 (119, 137–140), all non-competitive NMDA receptor antagonists, when administered immediately prior to naloxone-precipitated morphine withdrawal, attenuated signs of withdrawal

syndrome in morphine-dependent rats (119, 136–138), mice (139, 140) and guinea pigs (139). These signs include jumping, teeth-chattering, diarrhea and ptosis. MK-801 not only attenuates withdrawal from morphine, but also blocks the withdrawal from butorphanol (119). LY-274614, a competitive NMDA receptor antagonist at the glutamate antagonist recognition site, also dose-dependently attenuated morphine withdrawal precipitated by naloxone (137). In addition, 5,7-DCKA, an antagonist of the glycine site on the NMDA receptor, has also been reported to inhibit jumping behavior in naloxone-precipitated withdrawal in morphine-dependent mice (140).

Treatment with NMDA receptor antagonists has also been shown to prevent the development of opioid dependence. MK-801 inhibited signs of naloxone-precipitated opioid withdrawal when co-administered with morphine during the development of physical dependence (141, 142). Recently, the involvement of NMDA receptors in the development of opioid dependence was tested by using antisense oligonucleotides to selectively reduce the expression of NMDA receptors. Pretreatment with an antisense oligonucleotide corresponding to the nucleotides 4–21 of the NMDA receptor NR1 subunit (143) significantly attenuated morphine withdrawal signs including escape behavior, rearing, stretching, teeth chattering, vocalization and penis licking (144).

On the other hand, chronic i.c.v. treatment with (*S*)-4-carboxyphenyl-glycine, an antagonist selective for metabotropic receptors, but not AMPA/kainate receptor antagonist, significantly attenuated morphine withdrawal symptoms (142). Thus, both NMDA receptors and metabotropic receptors might be involved in the development of opioid physical dependence.

The mechanism underlying the inhibitory effects of NMDA receptor antagonists on the opioid dependence is unknown. Chronic opioid treatment causes adaptive increases in Ca^{2+} accumulation and activities of PKA and PKC. Activation of NMDA receptors leads to the opening of the receptor-gated ion channels, which allows Ca^{2+} to enter the neuron, and Ca^{2+} participates in numerous intracellular processes, including activation of protein kinases (for a review, see ref. 67). Thus, the effects of NMDA receptor antagonists may be able to prevent changes in second messenger systems associated with chronic morphine treatment.

B. NMDA receptors in the LC play a role in opioid dependence/withdrawal: postsynaptic changes

Immunocytochemical studies have demonstrated that NR1 immunostaining is moderately dense in the LC region (145). *In situ* hybridization studies reveal that LC neurons express mRNAs encoding several NR1 subunit isoforms (4a, 2a > 2b, 4b) as well as NR2 subunits (2B > 2D), indicating that NMDA receptors in the LC are composed of unique combinations of the subunits, e.g., 4a-2B (146).

Although non-NMDA receptor antagonists have been shown to prevent the activation of the LC evoked by stimulation of the PGi or by noxious stimulation (147), NMDA receptors are also involved in excitation of the LC. Direct application of NMDA in vitro or in vivo can activate LC neurons robustly, and the NMDA-evoked excitation can be attenuated by application of NMDA antagonists (147, 148). Intracoerulear infusion of Mg^{2+}-free solution evoked NMDA receptor-mediated synaptic potentials in LC neurons in vivo (149). Moreover, following noxious stimulation (sciatic nerve stimulation), a second slower excitatory response was detected in LC neurons which was sensitive to the NMDA antagonist AP-7 (150).

Several lines of evidence have indicated that the NMDA subtype of glutamate receptors in the LC is involved in opioid dependence and withdrawal. Electrophysiological studies from Akaoka and Aston-Jones (5) determined that withdrawal-induced LC neuron hyperactivity could be suppressed, although not totally abolished, by intracoerulear administration of AP-5, a competitive antagonist selective for the NMDA receptor. Using the technique of in vivo voltammetry, Hong et al. (104) found that in chronic morphine-treated rats, the naloxone-induced increase in the catechol oxidation current signal, which reflects neuronal activity in the LC, was attenuated by pretreatment with the selective NMDA receptor antagonist D-APH (AP-7), indicating that withdrawal-induced increase in LC activity is mediated, at least in part, by NMDA receptors. The involvement of LC NMDA receptors is also supported by the results of behavioral studies. Direct injection of NMDA (0.1 or 1 nmol/rat) into the LC of morphine- or butorphanol-dependent rats induced withdrawal signs (Tokuyama et al., unpublished data), whereas administration of vehicle to morphine- or butorphanol-dependent rats did not precipitate any withdrawal signs.

However, Rasmussen et al. (137) have suggested that ionotropic non-NMDA receptors mediate the withdrawal-induced hyperactivity of LC neurons since systemic (s.c. or i.p.) administration of the NMDA antagonists, MK-801 or LY274614, did not suppress the withdrawal-induced activation of LC neurons, although the behavioral responses to withdrawal were effectively inhibited.

Recent *in situ* hybridization studies have shown that mRNA encoding the NR1 subunit of the NMDA receptors is increased (about 30% above saline controls) in the LC region and in the hypothalamic paraventricular nucleus of morphine-dependent rats (144). This increase in expression of NMDA receptors may be a compensatory

response to the decrease in presynaptic glutamate release mediated by opioid treatment. It is possible that the hyperactivity signs during opioid withdrawal result from not only the overflow of glutamate release in the LC, but also from the upregulation of postsynaptic EAA receptors.

VII. Unique involvement of κ-opioid receptors in glutamate release during butorphanol withdrawal

The possibility that κ-opioid receptors within the LC play a unique role in regulation of presynaptic glutamate release during withdrawal from dependence on butorphanol was evaluated in our laboratories with the use of the κ-opioid receptor antagonist nor-BNI (118). Opioid dependence was produced in rats following i.c.v. infusion continuously of either butorphanol (26 nmol/μl/hr) or morphine (26 nmol/μl/hr) for three days. Upon completion of this period of opioid administration, i.c.v. injections of nor-BNI (48 nmol/rat) were given to precipitate withdrawal. Significant increases in the levels of glutamate were noted in microdialysis perfusates of the LC only in butorphanol-dependent animals. These increases occurred in the first 15 min of microdialysis and returned to baseline within 30 min of treatment with nor-BNI. No significant changes were noted in either morphine-dependent or saline-infused rats. Similarly, the behavioral withdrawal syndrome was obvious following nor-BNI challenge only in butorphanol-treated rats.

Additional comparisons have been made between the responses elicited during naloxone-precipitated withdrawal from dependence on butorphanol and on the highly selective κ-opioid receptor agonist U-69,593 (11). Equimolar infusions (26 nmol/hr, i.c.v.) of either butorphanol or U-69,593 were initiated and maintained for 3 days. Withdrawal was precipitated upon termination of i.c.v. infusion by discrete injection of naloxone (12 or 48 nmol) directly into the LC. Increases in the extracellular fluid levels of glutamate within the LC were noted following treatment with either dose of naloxone in both butorphanol- and U-69,593-dependent rats. A behavioral syndrome typical of opioid withdrawal, including teeth chattering, wet dog shakes and locomotion, was observed following naloxone challenge in both butorphanol- and U-69,593-dependent rats, but not in vehicle-treated rats.

Our most recent studies have examined even more specifically the role played by κ-opioid receptors within the LC in mediation of withdrawal from butorphanol-dependence (12). Comparisons were made between animals made dependent on either equimolar infusions (26 nmol/hr, i.c.v. for 3 days) of butorphanol, U-69593 or morphine. Saline vehicle infusions (1 μl/hr, i.c.v.) were administered to control rats. Discrete, bilateral tissue injections of nor-BNI were made into the LC to precipitate withdrawal responses. Glutamate levels within the LC increased in both the butorphanol- or U-69,593-dependent rats within 60 min of LC injection of nor-BNI. No significant increases in glutamate concentrations were detected in the microdialysate after nor-BNI treatment of either morphine-dependent or saline-treated rats. Behavioral signs of withdrawal were observed following nor-BNI injection in butorphanol- and U-69,593-dependent rats. Significantly lower rates and intensities of withdrawal signs were noted in the morphine-dependent rats and none were observed in the saline-infused controls.

These results indicate conclusively that κ-opioid receptors exert an exaggerated influence in mediation of behavioral and glutamatergic responses to withdrawal from dependence on butorphanol, as opposed to morphine. The use of the highly selective κ-opioid receptor agonist U-69,593 further substantiates the significance of a local involvement of the κ-opioid receptors within the LC in such responses.

A hypothetical role for the κ-opioid receptor in mediation of butorphanol dependence and withdrawal within synapses of the LC is presented in Fig. 1. This model is depicted in four stages of response to opioid administration: Normal, in which no exogenous opioid is present (Fig. 1, upper left panel), following initial exposure to butorphanol (upper right panel), in opioid-tolerant rats following chronic exposure to butorphanol (lower left panel) and during opioid antagonist-precipitated withdrawal (lower right panel).

Under physiological conditions, an endogenous opioid agonist is suggested to exert phasic stimulation of an inhibitory presynaptic, G-protein coupled κ-opioid receptor. This reaction reduces Ca^{2+} entry through L-type Ca^{2+} channels following nerve stimulation of afferent nerve fibers from the PGi and negatively regulates exocytotic glutamate release. Butorphanol dose-dependently stimulates the presynaptic receptor, an action which, if sufficiently intense, can reduce exocytotic glutamate release. Under such conditions, the glutamate-mediated excitation of postsynaptic NMDA receptors is lessened and a reduction in the firing rate of LC noradrenergic neurons ensues. During development of butorphanol dependence, the presynaptic κ-opioid receptor becomes progressively tolerant to butorphanol. A decline in presynaptic glutamate release mediated by butorphanol administration triggers a reactive increase in the number (B_{max}) of postsynaptic NMDA receptors to maintain synaptic homeostasis. However, the development of tolerance to butorphanol is associated with conversion of the presynaptic κ-opioid receptor to an antagonist-preferring conformation. Because the κ-opioid receptor becomes less sensitive to agonists, the inhibition of glutamate

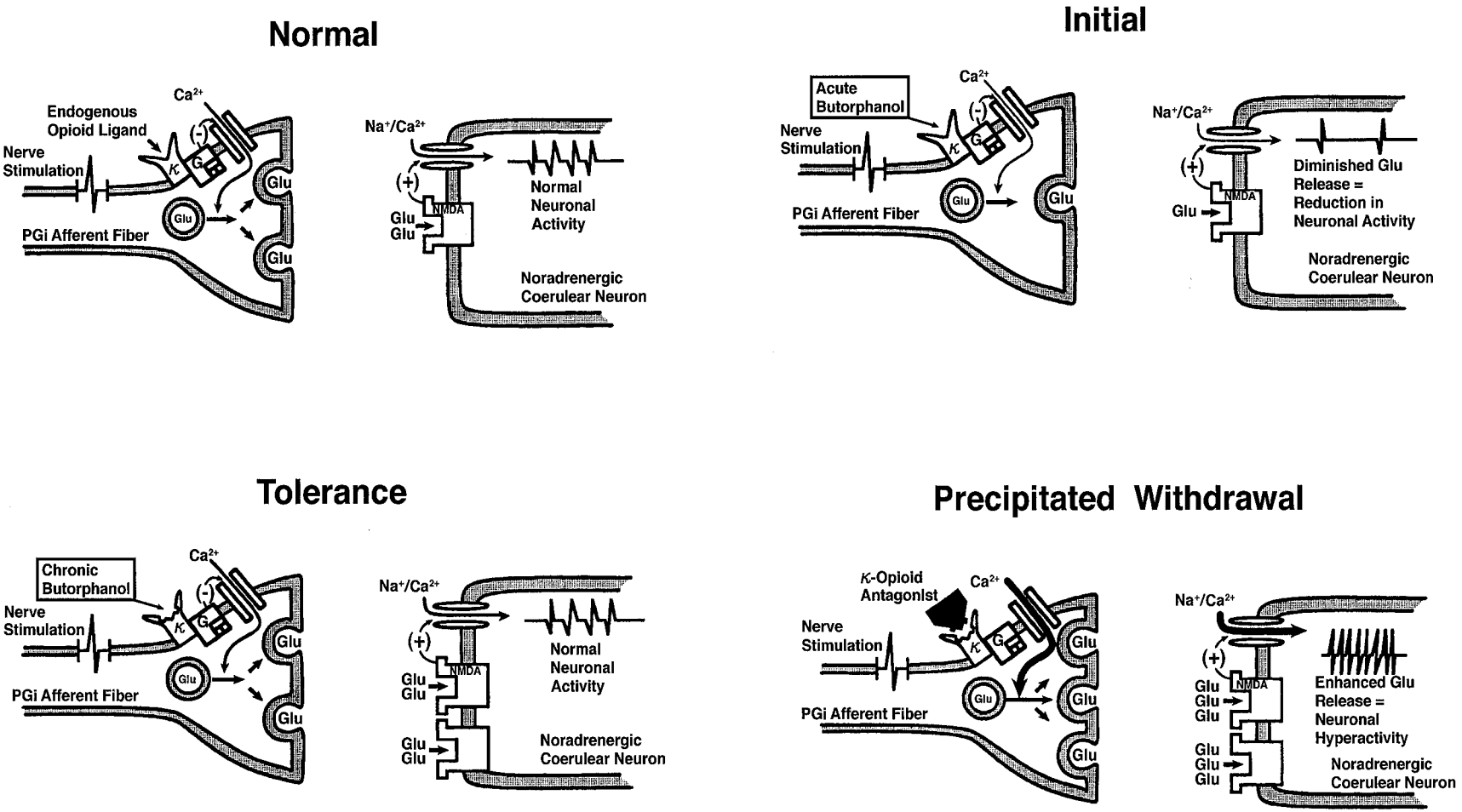

Fig. 1. Diagram of the hypothesized responses of identified neuronal elements within the LC during normal physiological activity and at three specified times during butorphanol administration and following withdrawal. Note that the magnitudes of the responses depicted are illustrative only. LC=locus coeruleus, PGi=n. paragigantocellularis, Glu=glutamate, κ=kappa opioid receptor, G=G-protein, NMDA=*N*-methyl-D-aspartate receptor. See text for details.

release becomes less intense. The increase in NMDA receptor number is hypothesized to outlast conversion of the presynaptic κ-opioid receptor. Reduction of glutamate release and increased NMDA receptor number act in concert to return postsynaptic LC neuronal activity toward normal firing rates. Kappa-opioid antagonist administration eliminates the inhibitory action of the presynaptic receptor on glutamate release, enhancing glutamate levels at postsynaptic NMDA receptors, whose numbers remain elevated. Those two factors mediate the characteristic, intense hyperactivity of LC noradrenergic neurons during withdrawal.

VIII. Conclusions

It is clear that glutamate and EAA receptors are fundamental mediators of the expression of withdrawal from opioid dependence. There is evidence that κ-opioid receptors may participate in presynaptic regulation of nerve stimulation-evoked glutamate release, an involvement which has particular significance for those opioid analgesics with κ-opioid agonist actions. Specifically, the dependence developed by administration of butorphanol tartrate (Stadol®) differs markedly from that of morphine in that interaction with κ-opioid receptors is an essential element of dependence on butorphanol but is not meaningfully involved in morphine dependence. The abuse liability of high doses of butorphanol is now well-established. Further development of κ-opioid receptor agonists as analgesics or for other purposes must recognize this liability. The data suggest that prevention and/or management of butorphanol abuse may be amenable to novel pharmacological strategies.

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