OPIOID RECEPTORS AND EXCITATORY AMINO ACIDS IN OPIOID DEPENDENCE

I. K. Ho, Y. Z. Feng, S. Tokuyama, S. P. Jaw, H. Wakabayashi, M. Makimura, K. W. Oh., T. Zhang, R. W. Rockhold and B. Hoskins
Department of Pharmacology and Toxicology
University of Mississippi Medical Center
2500 North State Street
Jackson, Mississippi 39216-4505 USA

INTRODUCTION

Our laboratories seek to understand the mechanism by which glutamatergic neurobiology influences the development of tolerance to, and dependence upon, opioid analgesic agents. Data generated by ourselves, and by others, suggest that critical aspects of this interaction take place within the pontine locus coeruleus, and it is upon this brain stem region that we have focused our attention. Additionally, as we will outline below, we have chosen to use the opioid mixed agonist/antagonist, butorphanol, as a model drug. The principal reasons for investigation of butorphanol can be found in a growing awareness of the abuse liability of this agent, and in its particularly robust binding to the κ-opioid receptor subtype. The ensuing discussion will detail our state of knowledge about these issues and their relationship to opioid dependence.

Data generated over the last few years from our studies have demonstrated that physical dependence upon butorphanol can be produced readily in the rat, and that κ-opioid receptor stimulation is a feature of butorphanol that contributes uniquely to the development of dependence and withdrawal. The locus coeruleus (LC) is a neuroanatomical substrate mediating signs and symptoms of precipitated withdrawal from butorphanol, as others have demonstrated for morphine (Aghajanian, 1978; Maldonado *et al.*, 1992; Maldonado and Koob, 1993; Rasmussen, 1991). Moreover, withdrawal from butorphanol dependence is associated with increases in extracellular excitatory amino acid levels within the LC (Feng *et al.*, 1995), again similar to effects observed with morphine (Aghajanian *et al.*, 1994; Zhang *et al.*, 1994). However, our data demonstrate that κ-opioid receptor stimulation exerts an influence over coerulear excitatory amino acid levels during withdrawal from butorphanol dependence that is not evident during withdrawal from morphine dependence.

As an analgesic agent, butorphanol is seven times as potent as morphine (Dobkin *et al.*, 1976), and differs further from morphine in that a ceiling effect on respiratory depression and miosis is evident (Heel *et al.*, 1978). At present, butorphanol is recommended for the relief of moderate to severe pain, for preoperative or preanesthetic medication, as a supplement to balanced anesthesia, and for the relief of postpartum pain (Wilkinson, 1987). Butorphanol is considered to have a low abuse potential when used within the therapeutic dose range. However, higher doses carry a clear abuse potential. For example, it has been reported that teenagers have abused the drug via intravenous injection of butorphanol with diphenhydramine (Smith and Davis, 1984). Evans *et al.* (1985) reported that after eight months of butorphanol use, a patient exhibited opioid withdrawal symptoms including tachycardia, diaphoresis, generalized malaise, myalgia, rhinorrhea, nausea, abdominal cramping, and diarrhea. Brown (1985) reported that a second case of butorphanol dependence which involved use of the drug

intramuscularly. Hoover and Williams (1985) carried out a survey of butorphanol diversion in U.S. hospitals and concluded that the diversion of butorphanol for purposes of abuse may be a more serious problem than is generally known. Finally, Jasinski *et al.* (1976) administered butorphanol (48 mg/day) subcutaneously to former narcotic addicts who subsequently developed typical opiate-like withdrawal symptoms. Since abuse of this drug has been documented, studies on mechanisms of butorphanol dependence are warranted and particularly relevant.

KAPPA-OPIOID RECEPTORS IN PHYSICAL DEPENDENCE UPON BUTORPHANOL

Our data indicate that no differences can be detected in either the behavioral or neurochemical responses to naloxone-precipitated withdrawal from butorphanol, when peripheral and icv routes of administration are compared (Feng *et al.*, 1995). An earlier report from our laboratories found also that there were no significant differences, between rats made dependent upon equimolar doses of morphine and butorphanol, in behavioral signs of withdrawal such as teeth chattering, escape responses and wet-dog shakes (Horan and Ho, 1991; Feng *et al.*, 1995). However, the receptor selectivities of morphine and butorphanol are known to differ, with morphine stimulating primarily μ-, and possibly δ-opioid receptors (Abdelhamid *et al.*, 1991; Gulya *et al.*, 1988; Miyamoto *et al.*, 1993) while butorphanol activates μ-, δ-, and κ-opioid receptors (Horan and Ho, 1989a; Lahti *et al.*, 1985). In addition, butorphanol has been shown to be an opioid agonist/antagonist, and can precipitate withdrawal in morphine-dependent animals (Horan and Ho, 1989b; Leander, 1983). High densities of several opioid receptors, particularly of the μ- and κ-opioid receptors (Atweh and Kuhar, 1977), have been found in the LC.

The involvement of κ-opioid receptors in butorphanol dependence has been studied systematically in our laboratories (Jaw *et al.*, 1993; 1994). Our findings indicate that nor-binaltorphimine (nor-BNI; a κ-opioid receptor antagonist) precipitated withdrawal behaviors similar to those precipitated by naloxone in butorphanol-dependent rats. Furthermore, when κ-opioid receptors were masked by nor-BNI before and during the induction of butorphanol dependence in rats, naloxone-precipitated withdrawal signs were blocked (Jaw *et al.*, 1993). These results demonstrate clearly that κ-opioid receptors are involved in mediating symptoms in butorphanol-dependent rats undergoing withdrawal.

Our most recent data identified the opioid receptor subtypes involved in regulation of brain glutamate levels during opioid withdrawal. This was examined using *in vivo* microdialysis of the pontine LC following precipitation of withdrawal using nor-BNI in conscious butorphanol- or morphine-dependent rats. Our findings showed that behavioral withdrawal was detected following intracerebroventricular (icv) administration of nor-BNI in butorphanol-infused rats, but not in morphine-infused animals. Significant increases in LC levels of glutamate were observed only in butorphanol-dependent rats. These results provide direct evidence to support the role of κ-opioid receptors in butorphanol withdrawal, but not in morphine withdrawal. Nor-BNI binds to both high affinity κ1-opioid receptor sites (Horan *et al.*, 1991; Zukin *et al.*, 1988) and U-69,593-insensitive, low-affinity κ2-opioid receptor sites. κ2-Opioid receptor sites are known to predominate in the rat brain (Zukin *et al.*, 1988). The finding that nor-BNI-precipitated withdrawal is similar to that produced by naloxone suggests that κ1-

opioid receptor sites, κ2-opioid receptor sites, or both are important in the induction of butorphanol dependence despite the fact that in the rat, there are more κ2-opioid receptor sites than κ1-opioid receptor sites (Unterwald *et al.*, 1991). When the subtypes of κ-opioid receptors are fully characterized and as specific antagonists become available, the identity of the κ-opioid receptor subtype(s) involved in opioid dependence can be determined.

Our preliminary study of κ-opioid receptor binding revealed that three days of butorphanol infusion significantly increased K_D values (in the cortex and striatum), decreased B_{max} (in the cortex only) of [^{3}H]U-69,593 binding, and shifted the K_i of nor-BNI against [^{3}H]U-69,593 binding in the cortex by more than 10-fold. Concurrent administration of nor-BNI and butorphanol blocked these effects of butorphanol on κ-opioid receptors. These results indicate that in butorphanol-dependent rats, κ-opioid receptors become down-regulated to agonists and supersensitive to antagonists. The mechanisms by which continuous infusion of butorphanol altered the binding characteristics of κ-opioid receptors are not clear.

Both the supersensitivity to antagonist and up-regulation of antagonist binding after 3 days of butorphanol infusion may be due to the interconversion of the conformations of κ-opioid receptors from a state of high affinity for agonists/low affinity for antagonists to one of low affinity for agonists/high affinity for antagonists. Sodium, phosphorylation of opioid receptors, or G-protein-uncoupled receptors favors the latter state (Law *et al.*, 1983; Nijssen and Childers, 1984). Therefore, our observations of a desensitization to κ-opioid receptor agonists, down-regulation of κ-opioid receptors, supersensitivity to κ-opioid receptor antagonists, and up-regulation of antagonist binding after chronic activation of κ-opioid receptors by butorphanol support such an interconversion of the κ-opioid receptor conformations.

GLUTAMIC ACID IN OPIOID DEPENDENCE

Microdialysis studies from the laboratories of Aghajanian *et al.* (1994), as well as from our laboratories (Zhang *et al.*, 1994; Feng *et al.*, 1995), have demonstrated that increased extracellular fluid levels of excitatory amino acid neurotransmitters within the LC occur contemporaneously with acutely-precipitated withdrawal from opioid dependence. However, only morphine, which acts preferentially as a μ-opioid receptor agonist, had been used to elicit dependence. The results of our studies (Feng *et al.*, 1995) demonstrated that extracellular levels of glutamate are elevated in the LC during naloxone-precipitated withdrawal from dependence upon butorphanol. This provided direct evidence that increases in brain levels of excitatory amino acids may represent a general phenomenon of opioid antagonist-precipitated withdrawal from opioid dependence. The contention that the LC participates in morphine withdrawal is supported by an abundance of electrophysiological data (Rasmussen, 1991; Rasmussen and Aghajanian, 1989; Rasmussen *et al.*, 1990, 1991b). In this regard, the hallmark of withdrawal from morphine dependence is hyperactivity of noradrenergic neurons within the LC (Aghajanian, 1978), a response that has been correlated with behavioral symptoms of opioid withdrawal (Gold *et al.*, 1980; Redmond and Krystal, 1984). Several lines of evidence are available which suggest the participation of excitatory amino acids in both the hyperactivity of coerulear neurons and the behavioral symptoms that accompany acutely-precipitated withdrawal from narcotic analgesics. Specifically, the withdrawal-induced activation of coerulear neurons has been shown to be blunted by icv pretreatment with kynurenic acid, a nonspecific antagonist

at excitatory amino acid receptors (Rasmussen and Aghajanian, 1989; Tung *et al.,* 1990). Additional investigations by Akaoka and Aston-Jones (1991) determined that coerulear neuron hyperactivity could be suppressed, although not totally abolished, by local administration of kynurenic acid or antagonists selective for NMDA and non-NMDA glutamate receptor subtypes. Importantly, those investigations established that local coerulear application of opioid antagonists was ineffective in precipitating neuron hyperactivity, which indicated that coerulear hyperactivity does not result from local opioid-mediated events. However, it must be stated that withdrawal-induced hyperactivity of coerulear neurons could not be blocked by peripheral (sc) administration of MK-801 ((+)-5-methyl-10,11-dihydro-5H-dibenzo[a,d]cyclohepten-5,10-imine), a noncompetitive NMDA receptor antagonist, in a similar study, although the behavioral responses to withdrawal were effectively inhibited (Rasmussen *et al.*, 1991a). As these latter results suggest, the behavioral responses observed during acutely precipitated withdrawal also appear to involve excitatory amino acid mechanisms. For example, the subcutaneous administration of MK-801 effectively suppressed the behavioral symptoms produced during acute withdrawal from morphine dependence, not only in guinea pigs and mice (Tanganelli *et al.*, 1991), but also in rats (Rasmussen *et al.,* 1991a). In addition, administration of MK-801 has been demonstrated to inhibit the development of both tolerance to and dependence upon morphine in the rat (Trujillo and Akil, 1991). Based on these data, it is not surprising that microdialysis studies have demonstrated consistent increases in extracellular concentrations of glutamate within the LC during withdrawal from morphine dependence (Aghajanian *et al.*, 1994; Zhang *et al.*, 1994). Such an increase in glutamate efflux is consistent with the tentative conclusion that acutely precipitated withdrawal stimulates glutamate release from excitatory amino acid-containing nerve terminals within the LC. This conclusion is supported further by the results of our recent study (Feng *et al.*, 1995) which demonstrated that withdrawal from butorphanol elicits effects similar to those seen in morphine withdrawal. Nevertheless, the mechanism which underlies the association between opioid withdrawal and increased coerulear levels of excitatory amino acids remains unclear. Glutamatergic projections to the LC are known to originate from the n. paragigantocellularis of the rostral medulla oblongata (Rasmussen and Aghajanian, 1989; Rasmussen *et al.*, 1991b). Lesions of the n. paragigantocellularis have been shown to attenuate the hyperactivity of coerulear neurons associated with opioid withdrawal (Rasmussen and Aghajanian, 1989), a response that is believed to be mediated by an excitatory amino acid pathway from the n. paragigantocellularis to the LC. These data have led investigators to suggest that the source of the increased glutamate originates from activation of glutamatergic nerve projections from the n. paragigantocellularis to the LC (Ennis *et al.*, 1992; Zhang *et al.*, 1994). This hypothesis is supported by the time course of the increases of glutamate within the LC during acutely-precipitated withdrawal from both morphine (Zhang *et al.*, 1994) and butorphanol (Feng *et al.*, 1995). It is possible also that opioid dependence/withdrawal may alter the quantal release of glutamate at excitatory amino acid-containing terminals within the LC. Aside from our data, no studies have examined interactions between butorphanol and excitatory amino acid systems.

CONCLUSIONS

Butorphanol is a potent mixed 'agonist/antagonist' opioid analgesic belonging to the group of opiate derivatives known as morphinans. The pharmacology of this compound, in

terms of its actions on the opioid receptor systems, is complex and may be due to its apparent multiplicity of actions on the opioid receptor systems. Comparative studies between morphine and butorphanol would suggest this to be the case and illustrate some similarities as well as differences in their pharmacological actions.

Results obtained from our laboratory have demonstrated that κ-opioid receptors are the major opioid receptor subtype involved in mediating butorphanol dependence and withdrawal, in contrast to a former belief that the κ-opioid receptor is minimally associated with opioid physical dependence in rats (Cowan *et al.*, 1988). Our studies further provide direct evidence that glutamate in the LC is essential for the expression of opioid withdrawal signs. The findings also suggest that the NMDA subtype of glutamate receptors may be related closely to the expression of withdrawal signs from opioids.

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