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Direct evidence for a role of glutamate in the expression of the opioid withdrawal syndrome

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Abstract

To investigate the role of glutamate in the expression of the withdrawal signs from opioids, rats were intracerebroventricularly (i.c.v.) infused continuously with morphine (a μ-opioid receptor agonist, 26 nmol/μl per h) or butorphanol (a mixed $\mu/\delta/\kappa$-opioid receptor agonist, 26 nmol/μl per h) through osmotic minipumps for 3 days. An i.c.v. injection of glutamate (5 and 50 nmol/5 μl) dose dependently induced withdrawal signs in morphine- or butorphanol-dependent animals. The withdrawal signs precipitated by the glutamate injection were comparable to those precipitated by an opioid receptor antagonist, naloxone (48 nmol/5 μl), except for the expression of some specific behaviors and the duration of withdrawal signs. Glutamate or naloxone challenge failed to precipitate any withdrawal signs in saline controlled animals. On the other hand, the expression of the withdrawal signs precipitated by glutamate or naloxone in opioid-dependent animals was completely blocked by pretreatment with MK-801 {a NMDA receptor antagonist, (+)-5-methyl-10,11-dihydro-5*H*-dibenzo[*a*,*d*]cycloheptan-5,10-imine}, 0.1 mg/kg, i.p. These unique actions of glutamate in continuously opioid-infused rats suggest that a rapid central release of glutamate may be a key factor in the expression of withdrawal signs from opioids. Furthermore, this effect may be mediated by the NMDA subtype of glutamate receptors.

Keywords: Opioid dependence; Central nervous system; Glutamate; MK-801 (dizocilpine); Morphine; Butorphanol

1. Introduction

The excitatory amino acids such as glutamate and aspartate have been thought to act as the principal excitatory neurotransmitters in the mammalian brain. Recently, Aghajanian et al. (1994) and Zhang et al. (1994) have shown that naloxone (an opioid receptor antagonist)-precipitated withdrawal from morphine (a μ-opioid receptor agonist) is associated with increased extracellular concentrations of glutamate within the pontine locus coeruleus. These data provide evidence to support a role of this excitatory amino acid in the expression of the withdrawal syndrome from morphine. However, it has not been determined whether or not direct application of glutamate to the brain can precipitate withdrawal signs from opioids. Accordingly, it is important to determine the direct effect of glutamate in opioid-dependent animals.

Presently, glutamate receptors are classified into two classes in the mammalian CNS (Watkins et al., 1990). Ionotropic glutamate receptors are ligand-gated integral ion channels that exist as heteromeric protein complexes composed of heterogenous subunit proteins. In contrast, metabotropic glutamate receptors are coupled to cellular effectors via GTP-binding proteins (Schoepp and Conn, 1993). Furthermore, ionotropic glutamate receptors are generally divided into major subtypes of: *N*-methyl-D-aspartate (NMDA), α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA), and kainate, based on activation by selective agonists (Honore et al., 1988; Johnson and Ascher, 1987; Lodge and Collingridge, 1991). The NMDA receptor-mediated mechanism has been noted to be implicated in the expression of withdrawal syndrome from opioids since an NMDA receptor antagonist, MK-801, has been reported to inhibit the expression of morphine withdrawal signs (Tanganelli et al., 1991; Trujillo and Akil, 1991). Therefore, it is also interesting to investigate the

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role of the NMDA receptor in the expression of glutamate-precipitated withdrawal.

Furthermore, dependence on an agonist/antagonist opioid compound, butorphanol, as well as the prototype opioid, morphine, can be produced readily (Horan and Ho, 1989, 1991; Jaw et al., 1993a, c; Oh et al., 1992; Tokuyama et al., 1995a, b). Involvement of coerulear glutamate has also been reported following naloxone-precipitated withdrawal from both opioids (Feng et al., 1995; Zhang et al., 1994). However, the two opioid analgesics differ in their ability to stimulate opioid receptor subtypes. Morphine preferentially stimulates the μ-opioid receptor (Gulya et al., 1988), with additional actions on the δ-opioid receptor (Abdelhamid et al., 1991; Miyamoto et al., 1993), while butorphanol acts not only on μ- and δ-opioid receptors but more likely on the κ-opioid receptor (Horan and Ho, 1989; Lahti et al., 1985; Pircio et al., 1976). Recently, our preliminary findings showed that behavioral withdrawal was detected following intracerebroventricular (i.c.v.) administration of nor-binaltorphimine, a κ-opioid receptor antagonist, in butorphanol-infused rats, but not in morphine-infused animals (Feng et al., 1994). Therefore, as most studies of opioid dependence have focused on morphine, the use of butorphanol should be valuable in studies to evaluate the mechanism of development of dependence on opioids.

For these reasons, the present study was designed to determine the role of glutamate in the expression of withdrawal signs from opioids. The behavioral changes produced by direct i.c.v. injection of glutamate in comparison with that of naloxone in animals rendered dependent on morphine or butorphanol were observed. In addition, we also examined the effect of MK-801 on the action of glutamate in opioid-dependent animals.

2. Materials and methods

2.1. Animals

Male Sprague-Dawley rats weighing 230–250 g (Charles River, Wilmington, MA) were purchased and housed to a group of three or four animals in a cage. They were kept in a room maintained at 21 ± 2°C and a 12 h light-dark cycle with free access to food and tap water. After reaching 280–300 g, they were used for experiments.

2.2. Surgical procedures

Rats were anesthetized with Equithensin (4.25 g chloral hydrate, 2.23 g $MgSO_4 \cdot 7H_2O$, 0.972 g sodium pentobarbital, 44.4 ml propylene glycol, 10 ml 95% ethanol, and distilled water to make a final volume of 100 ml), 0.3 ml/100 g body weight, i.p., and then placed in a stereotaxic instrument. An indwelling stainless steel guide cannula (26 gauge, 10 mm long) was implanted in the right lateral cerebral ventricle (AP: −0.5 mm, LAT: +1.3 mm, and DV: −4.5 mm) with the bregma chosen as the stereotaxic reference point (Paxinos and Watson, 1986). Dental acrylic cement (Lang Dental MFG Co., Wheeling, IL) was applied to the surface of the skull, and a protective cap was placed around the cannula. After the acrylic had hardened, the animal was removed from the stereotaxic frame. A stylet (32 gauge stainless steel tubing) was placed into the guide cannula to maintain patency. The presence of cerebrospinal fluid in the guide cannula was examined to assure proper placement. After surgery, rats were given 300 000 units of procaine penicillin G (Pfizerpen-AS, Pfizer Corp., New York, NY), s.c., to prevent infection and were allowed at least 1 week to recover before commencing the infusion of morphine-HCl (Sigma Chemical Corp., St. Louis, MO) or butorphanol-tartrate (17-cyclobutylmethyl-3,14-dihydroxy morphinan; a generous gift from Bristol-Myers-Squibb Corp., Evansville, IN).

2.3. Administration schedule and induction of morphine and butorphanol dependence

Animals were infused i.c.v. continuously with saline (1 μl/h), morphine (26 nmol/μl per h), or butorphanol (26 nmol/μl per h) for 3 days via osmotic minipumps (Alzet 2001, Alza Corp., Palo Alto, CA). This infusion period and dose paradigm were determined to be optimal from our previous experiments (Jaw et al., 1993a, c). Under ether anesthesia, animals were implanted s.c. with minipumps between the scapulae. A 4 cm piece of tygon tubing (0.38 mm inner diameter, Cole-Palmer, Chicago, IL) was applied to connect the minipump to a piece of L-shaped stainless steel injector tubing (32 gauge, 30 mm long) with one end having the same length as the guide cannula. All drug solutions were passed through a 0.2 mm sterile Acrodisk filter (Gelman Sciences, Ann Arbor, MI) before being introduced into the pumps, and the delivery apparatus was assembled under sterile conditions. Minipumps were primed overnight at room temperature in normal saline so that an optimal flow rate (1 μl/h) was obtained. Rats were injected i.c.v. with glutamate (5 or 50 nmol/5 μl, Sigma Chemical Corp., St. Louis, MO) or naloxone (48 nmol/5 μl, Sigma Chemical Corp., St. Louis, MO) 2 h after the termination of opioid infusion performed by cutting the tubing. The NMDA receptor antagonist, MK-801 {dizocilpine, (+)-5-methyl-10,11-dihydro-5*H*-dibenzo[*a*,*d*]cycloheptan-5,10-imine, Research Biomedicals International, Natick, MA}, 0.1 mg/kg, i.p., was injected 30 min prior to the glutamate or naloxone challenge.

Table 1
Withdrawal signs elicited in opioid non-dependent rats by i.c.v. injection of glutamate or naloxone

	Saline	GLU (nmol/5 μl)	NAL (nmol/5 μl)
Withdrawal signs		50	48
Escape behavior	0/14 *	0/7	0/7
Wet dog shakes	4/14	2/7	1/7
Teeth chattering	0/14	0/7	0/7
Rearing	0/14	0/7	1/7
Locomotion	1/14	1/7	0/7
Stretching	0/14	0/7	0/7
Scratching	0/14	0/7	0/7
Salivation	0/14	0/7	0/7
Penis-licking	0/14	0/7	0/7
Ptosis	0/14	0/7	0/7
Weight loss (> 3%)	0/14	0/7	0/7

Rats received i.c.v. infusion of saline (1 μl/h) for 3 days and were challenged with glutamate (GLU), naloxone (NAL), or saline 2 h after the termination of saline infusion. * Numbers denote the number of rats showing positive signs over the total number of rats tested.

2.4. Measurement of behavioral signs during morphine and butorphanol withdrawal

Ten distinct behaviors (escape behavior, wet dog shakes, teeth chattering, rearing, locomotion, stretching, scratching, salivation, penis-licking, and ptosis) were scored during a 30-min period following the glutamate or naloxone injection as behavioral signs of withdrawal. The reactions of each animal were evaluated by an independent observer who did not have prior knowledge of the nature of the treatment received. Loss of body weight (number of animals exhibiting > 3% body weight loss) was measured before and 1 h after the administration of naloxone or glutamate.

2.5. Statistics

Quantal (all or none) data from the behavioral studies on experimental groups and saline controls were compared by the chi-square test. A difference was considered significant at $P < 0.05$.

3. Results

3.1. Withdrawal signs elicited by i.c.v. injection of glutamate or naloxone

In animals continuously infused with saline (1 μl/h), glutamate (50 nmol/5 μl), and naloxone (48 nmol/5 μl) did not precipitate any withdrawal signs (Table 1).

Tables 2 and 3 demonstrate that the continuous i.c.v. infusion of morphine (26 nmol/μl per h) or butorphanol (26 nmol/μl per h) for 3 days induced physical dependence, manifested as withdrawal signs (escape behavior, wet dog shakes, teeth chattering, rearing, locomotion, stretching, scratching, salivation, penis-licking, ptosis, and body weight loss) when glutamate, 5 or 50 nmol/5 μl, or naloxone, 48 nmol/ 5 μl, was i.c.v. injected 2 h after the termination of opioid infusion. In animals treated with saline instead of glutamate or naloxone, no withdrawal response was observed in morphine- or butorphanol-dependent rats (Tables 2 and 3). On the other hand, the duration of the withdrawal signs from opioids was somewhat different between glutamate- and naloxone-injected animals. The withdrawal signs precipitated by glutamate disappeared within 10–15 min, while those precipitated by naloxone lasted for the entire observation period of 30 min (data not shown).

The i.c.v. injection of glutamate in morphine-depen-

Table 2
Withdrawal signs elicited in morphine-dependent rats by i.c.v. injection of glutamate or naloxone

	Saline	GLU (nmol/5 μl)		NAL (nmol/5 μl)
Withdrawal signs		5	50	48
Escape behavior	0/14 *	5/10 [a]	4/10 [a]	6/10 [b]
Wet dog shakes	3/14	7/10 [a]	9/10 [b]	9/10 [b]
Teeth chattering	0/14	3/10	4/10 [a]	8/10 [b]
Rearing	1/14	6/10 [a]	9/10 [b]	7/10 [b]
Locomotion	1/14	7/10 [b]	9/10 [b]	7/10 [b]
Stretching	0/14	1/10	5/10 [a]	7/10 [b]
Scratching	0/14	3/10	6/10 [b]	6/10 [b]
Salivation	0/14	0/10	0/10	6/10 [b]
Penis-licking	0/14	2/10	5/10 [a]	7/10 [b]
Ptosis	0/14	2/10	5/10 [a]	5/10 [a]
Weight loss (> 3%)	0/14	0/10	0/10	7/10 [b]

Rats received i.c.v. infusion of morphine (26 nmol/μl per h) for 3 days and were challenged with glutamate (GLU), naloxone (NAL), or saline 2 h after the termination of drug infusion. * Numbers denote the number of rats showing positive signs over the total number of rats tested. [a] $P < 0.05$, [b] $P < 0.01$, values are significantly higher than the control values as determined by the chi-square test.

Table 3
Withdrawal signs elicited in butorphanol-dependent rats by i.c.v. injection of glutamate or naloxone

Withdrawal signs	Saline	GLU (nmol/5 μl)		NAL (nmol/5 μl)
		5	50	48
Escape behavior	0/14 *	2/10	4/10 [a]	6/10 [b]
Wet dog shakes	4/14	6/10	9/10 [a]	8/10 [a]
Teeth chattering	0/14	3/10	5/10 [a]	9/10 [b]
Rearing	0/14	8/10 [b]	9/10 [b]	6/10 [b]
Locomotion	1/14	7/10 [b]	9/10 [b]	8/10 [b]
Stretching	0/14	1/10	5/10 [a]	9/10 [b]
Scratching	0/14	5/10 [a]	7/10 [b]	6/10 [b]
Salivation	0/14	0/10	0/10	5/10 [a]
Penis-licking	0/14	2/10	4/10 [a]	7/10 [b]
Ptosis	0/14	1/10	5/10 [a]	6/10 [b]
Weight loss (> 3%)	0/14	0/10	0/10	6/10 [b]

Rats received i.c.v. infusion of butorphanol (26 nmol/μl per h) for 3 days and were challenged with glutamate (GLU), naloxone (NAL), or saline 2 h after the termination of drug infusion. * Numbers denote the number of rats showing positive signs over the total number of rats tested. [a] $P < 0.05$, [b] $P < 0.01$, values are significantly higher than the control values as determined by the chi-square test.

Table 4
Effects of MK-801 on glutamate- or naloxone-precipitated withdrawal signs in morphine-dependent rats

Withdrawal signs	GLU (50 nmol/5 μl)		NAL (48 nmol/5 μl)	
	Vehicle	MK-801	Vehicle	MK-8-1
Escape behavior	2/7 *	0/7	4/7	0/7
Wet dog shakes	7/7	1/7 [b]	6/7	1/7 [a]
Teeth chattering	3/7	0/7	5/7	0/7 [a]
Rearing	6/7	0/7 [b]	5/7	0/7 [a]
Locomotion	7/7	1/7 [b]	5/7	0/7 [a]
Stretching	3/7	0/7	4/7	1/7
Scratching	4/7	1/7	4/7	1/7
Salivation	0/7	0/7	3/7	0/7
Penis-licking	2/7	0/7	4/7	1/7
Ptosis	4/7	0/7	3/7	1/7
Weight loss (> 3%)	1/7	0/7	6/7	0/7 [b]

Rats received i.c.v. infusion of morphine (26 nmol/μl per h) for 3 days and were challenged with glutamate (GLU), naloxone (NAL) 2 h after the termination of drug infusion. MK-801 (0.1 mg/kg) or saline vehicle was injected i.p. 30 min before glutamate or naloxone. * Numbers denote the number of rats showing positive signs over the total number of rats tested. [a] $P < 0.05$, [b] $P < 0.01$, values are significantly lower than the control values as determined by the chi-square test.

Table 5
Effects of MK-801 on glutamate- or naloxone-precipitated withdrawal signs in butorphanol-dependent rats

Withdrawal signs	GLU (50 nmol/5 μl)		NAL (48 nmol/5 μl)	
	Vehicle	MK-801	Vehicle	MK-801
Escape behavior	1/7 *	0/7	4/7	0/7
Wet dog shakes	6/7	1/7 [a]	6/7	0/7 [b]
Teeth chattering	2/7	0/7	6/7	0/7 [b]
Rearing	7/7	1/7 [b]	4/7	0/7
Locomotion	7/7	0/7 [b]	5/7	0/7 [a]
Stretching	3/7	0/7	6/7	0/7 [b]
Scratching	5/7	0/7 [a]	4/7	0/7
Salivation	0/7	0/7	3/7	1/7
Penis-licking	1/7	0/7	5/7	0/7 [a]
Ptosis	3/7	0/7	4/7	1/7
Weight loss (> 3%)	0/7	0/7	4/7	0/7

Rats received i.c.v. infusion of butorphanol (26 nmol/μl per h) for 3 days and were challenged with glutamate (GLU), naloxone (NAL) 2 h after the termination of drug infusion. MK-801 (0.1 mg/kg) or saline vehicle was injected i.p. 30 min before glutamate or naloxone. * Numbers denote the number of rats showing positive signs over the total number of rats tested. [a] $P < 0.05$, [b] $P < 0.01$, values are significantly lower than the control values as determined by the chi-square test.

dent animals significantly induced escape behavior (5 and 50 nmol/5 μl), wet dog shakes (5 and 50 nmol/5 μl), teeth chattering (50 nmol/5 μl), rearing (5 and 50 nmol/5 μl), locomotion (5 and 50 nmol/5 μl), stretching (50 nmol/5 μl), scratching (50 nmol/5 μl), penis-licking (50 nmol/5 μl), and ptosis (50 nmol/5 μl) as compared with the saline-challenged morphine-infused group. Salivation and body weight loss were not observed (Table 2). In butorphanol-infused animals, escape behavior (50 nmol/ 5 μl), wet dog shakes (50 nmol/5 μl), teeth chattering (50 nmol/5 μl), rearing (5 and 50 nmol/5 μl), locomotion (5 and 50 nmol/5 μl), stretching (50 nmol/5 μl), scratching (5 and 50 nmol/5 μl), penis-licking (50 nmol/5 μl), and ptosis (50 nmol/5 μl) were produced by an injection of glutamate, but other signs did not appear (Table 3). Following the naloxone challenge, all withdrawal signs from morphine and butorphanol were precipitated (Tables 2 and 3).

3.2. Effects of MK-801 on the withdrawal signs elicited by i.c.v. injection of glutamate or naloxone

As shown in Tables 4 and 5, pretreatment with MK-801, 0.1 mg/kg, i.p., 30 min prior to the glutamate or naloxone challenge completely blocked glutamate- or naloxone-precipitated withdrawal signs from morphine- and butorphanol-dependent animals.

4. Discussion

It has been reported that naloxone-precipitated withdrawal from morphine is associated with increased extracellular levels of glutamate within the pontine locus coeruleus, suggesting that glutamate may be involved in the expression of opioid withdrawal signs (Aghajanian et al., 1994; Feng et al., 1995; Zhang et al., 1994). However, there was no report on the direct application of glutamate to the brain in opioid-dependent animals. In the present study, direct i.c.v. injection of this excitatory amino acid to the brains of morphine- or butorphanol-dependent rats precipitated withdrawal signs in a dose-dependent manner, while prominent behavioral changes were not observed when animals continuously infused with saline were challenged with glutamate. Hence, these results provide direct evidence that glutamate is essential for the expression of opioid withdrawal signs.

The study also revealed that withdrawal signs elicited by glutamate are similar to those produced by naloxone except that the duration of withdrawal from morphine and butorphanol by i.c.v. injection of glutamate was shorter (15 min at the most) than that precipitated by naloxone (30 min). In addition, salivation and body weight loss were not observed in the glutamate-challenged groups, while i.c.v. injection of naloxone precipitated all behavioral signs observed. The discrepancy between glutamate- and naloxone-precipitated withdrawal is mainly due to the difference in the time of action of both agents; that is, naloxone acts longer than glutamate. Indeed, naloxone has been shown to increase the glutamate release from the locus coeruleus in morphine- or butorphanol-dependent animals for 30 min (Feng et al., 1995; Zhang et al., 1994). These results suggest that in order for severe withdrawal signs such as salivation and body weight loss to appear, the withdrawal state shoud be lasted for a longer period of time.

At the present time, it is still unclear as to which area in the brain is mainly involved in the expression of opioid withdrawal signs precipitated by glutamate. Recently, some interesting reports have been published that extracellular fluid levels of glutamate within the locus coeruleus are increased during withdrawal from morphine (Aghajanian et al., 1994; Zhang et al., 1994) and butorphanol (Feng et al., 1995). In fact, this region has been most widely studied as an important anatomical site for mediating opioid withdrawal signs (Aghajanian, 1978; Ennis and Aston-Jones, 1988; Rasmussen and Aghajanian, 1989; Redmond et al., 1976; Redmond and Krystal, 1984). These findings, together with the present study, support our hypothesis that the rapid release of glutamate from locus coeruleus under an opioid dependent-state might be a trigger or key element for the expression of opioid withdrawal signs. However, other regions in the brain are not ruled out since it is well known that glutamate modulates dopamine release in the striatum, substantia nigra, and ventral tegmental area (Imperato et al., 1990; Krebs et al., 1991; Mount et al., 1989). Dopaminergic neurons have also been reported to be involved with the expression of opioid withdrawal signs (Ahtee et al., 1987; De la Baume et al., 1979).

Presently, opioid receptors can be classified into at least three different types, i.e. μ, δ, and κ receptors (Goldstein and Naidu, 1989; Martin, 1984), and butorphanol is known to act on μ-, δ-, and κ-opioid receptors (Horan and Ho, 1989; Lahti et al., 1985; Pircio et al., 1976). Previous studies from our laboratory have demonstrated that β-funaltrexamine (a μ-opioid receptor selective antagonist) failed to precipitate withdrawal in butorphanol-dependent rats (Jaw et al., 1993b), while both naltrindole (a δ-opioid receptor selective antagonsit) and nor-binaltorphimine (a κ-opioid receptor selective antagonsit) have been shown to precipitate withdrawal signs similar to those precipitated by naloxone (Jaw et al., 1993a, c). These data have indicated that κ- and/or δ-opioid receptors are more involved than μ-receptors in the expression of behavioral signs of withdrawal from butorphanol. In the present study, glutamate precipitated morphine or butorphanol withdrawal to a similar degree, suggesting

that the expression of withdrawal signs from both opioids is mediated through a mutual mechanism which activates glutamate receptors. The increased release of glutamate in our previous studies (Feng et al., 1994, 1995; Zhang et al., 1994) may be due to the effects of antagonists acting at different opioid receptors.

Interest in a possible role for the NMDA receptor in the expression of opioid withdrawal has been stimulated by evidence that the behavioral symptoms of morphine withdrawal by naloxone precipitation are blocked by a non-competitive NMDA receptor antagonist, MK-801 (Tanganelli et al., 1991; Trujillo and Akil, 1991). The present results substantiate this contention by demonstrating that MK-801 completely prevented the withdrawal signs elicited by glutamate or naloxone challenge in opioid-dependent animals. Furthermore, recent studies indicate that the potency of MK-801 in displacing [^{3}H]naloxone from its binding sites in the brain membrane was low with an IC_{50} value of 34 μM. In comparison, the IC_{50} values of naloxone and morphine were 1.5 and 28.4 nM, respectively (Lutfy et al., 1993). Therefore, MK-801 does not appear to act on opioid receptors. These indicate that the NMDA receptor-mediated system is important in the expression of opioid withdrawal.

In conclusion, the present study is the first report to show that direct i.c.v. injection of glutamate is able to precipitate the expression of opioid withdrawal signs. The data indicate that rapid central release of glutamate under an opioid-dependent state may be a trigger or key factor for the expression of opioid withdrawal signs. It is further suggested that the NMDA subtype of glutamate receptor-mediated system plays a substantial role in the appearance of these behavioral changes caused by opioid withdrawal.

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References

Abdelhamid, E.E., M. Sultana, P.S. Portoghese and A.E. Takemori, 1991, Selective blockade of delta-opioid receptors prevents the development of morphine tolerance and dependence in mice, J. Pharmacol. Exp. Ther. 258, 299.

Aghajanian, G.K., 1978, Tolerance of locus coeruleus neurons to morphine and suppression of withdrawal response by clonidine, Nature 276, 186.

Aghajanian, G.K., J.H. Kogan and B. Moghaddam, 1994, Opiate withdrawal increases glutamate and aspartate efflux in the locus coeruleus: an in vivo microdialysis study, Brain Res. 636, 126.

Ahtee, L., P. Attila, V. Lauhakangas, A. Solkinen and J. Sipila, 1987, The fall of homovanillic acid and 5-hydroxyindoleacetic acid concentrations in brain of mice withdrawn from repeated morphine treatment and their restoration by acute morphine administration, J. Neural Transm. 68, 63.

De la Baume, S., G. Patey, H. Marcais, P. Protais, J. Costentin and J.-C. Schwartz, 1979, Changes in dopamine receptors in mouse striatum following morphine treatments, Life Sci. 24, 2333.

Ennis, M. and G. Aston-Jones, 1988, Activation of locus coeruleus from nucleus paragigantocellularis: a new excitatory amino acid pathway in brain, J. Neurosci. 8, 3644.

Feng, Y.Z., T. Zhang., R.W. Rockhold and I.K. Ho, 1994, Opioid receptor subtypes modurate pontine excitatory amino acid (EAA) levels differently in butorphanol and morphine dependence, Soc. Neurosci. Abstr. 20, 1232.

Feng, Y.Z., T. Zhang., R.W. Rockhold and I.K. Ho, 1995, Increased locus coeruleus glutamate levels are associated with naloxone-precipitated withdrawal from butorphanol in the rat, Neurochem. Res. 20, 675.

Goldstein, A. and A. Naidu, 1989, Multiple opioid receptors: ligand selectivity profiles and binding site signatures, Mol. Pharmacol. 36, 265.

Gulya, K., M. Krivan, N. Nyolczas, Z. Sarnyai and G.L. Kovács, 1988, Central effects of the potent and highly selective μ opioid antagonist D-Phe-Cys-Tyr-D-Trp-Orn-Thr-Pen-Thr-NH_2 (CTOP) in mice, Eur. J. Pharmacol. 150, 355.

Honore, T., S.N. Davies, J. Drejer, E.J. Fletcher, P. Jacobsen, D. Lodge and F.E. Nielsen, 1988, Quinoxalinediones: potent, competitive non-NMDA glutamate receptor antagonist, Science 241, 701.

Horan, P.J. and I.K. Ho, 1989, Comparative pharmacological and biochemical studies between butorphanol and morphine, Pharmacol. Biochem. Behav. 34, 847.

Horan, P.J. and I.K. Ho, 1991, The physical dependence liability of butorphanol: a comparative study with morphine, Eur. J. Pharmacol. 203, 387.

Imperato, A., M.G. Scrocco, S. Bacchi and L. Angelucci, 1990, NMDA receptors and in vivo dopamine release in the nucleus accumbens and caudatus, Eur. J. Pharmacol. 187, 555.

Jaw, S.P., B. Hoskins and I.K. Ho, 1993a, Involvement of δ-opioid receptors in physical dependence on butorphanol, Eur. J. Pharmacol. 240, 67.

Jaw, S.P., B. Hoskins and I.K. Ho, 1993b, Opioid antagonists and butorphanol dependence, Pharmacol. Biochem. Behav. 44, 497.

Jaw, S.P., M. Makimura, B. Hoskins and I.K. Ho, 1993c, Effect of nor-binaltorphimine on butorphanol dependence, Eur. J. Pharmacol. 239, 133.

Johnson, J.W. and P. Ascher, 1987, Glycine potentiates the NMDA response in cultured mouse brain neurons, Nature 325, 529.

Krebs, M.O., J.M. Desce, M.L. Kemel, C. Gauchy, G. Godeheu, A. Cheramy and J. Glowinski, 1991, Glutamate control of dopamine release in the rat striatum: evidence for presynaptic *N*-metyl-D-aspartate receptors on dopaminergic nerve terminals, J. Neurochem. 56, 81.

Lahti, R.A., M.M. Mickelson, J.M. McCall and P.F. Von Voigtlander, 1985, [^{3}H]U-69,593 a highly selective ligand for the opioid kappa receptor, Eur. J. Pharmacol. 109, 281.

Lodge, D. and G. Collingridge, 1991, The pharmacology of excitatory amino acids: a TIPS special report, Trends Pharmacol. Sci. (Elsevier Trends Journals, Cambridge, UK).

Lutfy, K., D.E. Hurlbut and E. Weber, 1993, Blockade of morphine induced analgesia and tolerance in mice by MK-801, Brain Res. 616, 83.

Martin, W.R., 1984, Pharmacology of opioids, Pharmacol. Rev. 35, 283.

Miyamoto, Y., P.S. Portoghese and A.E. Takemori, 1993, Involvement of $delta_2$ opioid receptors in the development of morphine dependence in mice, J. Pharmacol. Exp. Ther. 264, 1141.

Mount, H.S., S. Welner, R. Quirion and P. Boska, 1989, Glutamate stimulation of [^{3}H]dopamine release from dissociated cell cultures of rat ventral mesencephalon, J. Neurochem. 52, 1300.

Oh, K.W., M. Makimura, S.P. Jaw, B. Hoskins and I.K. Ho, 1992, Effects of β-funaltrexamine on butorphanol dependence, Pharmacol. Biochem. Behav. 42, 29.

Paxinos, G. and C. Watson, 1986, The Rat Brain in Stereotaxic Coordinates, 2nd edn. (Academic Press, Orlando, FL).

Pircio, A.W., J.A. Gylys, R.L. Cavanagh, J.P. Buyniski and M.E. Bierwagen, 1976, The pharmacology of butorphanol, a 3,14-dihydroxymorphinan narcotic antagonist analgesic, Arch. Int. Pharmacodyn. Ther. 220, 231.

Rasmussen, K. and G.K. Aghajanian, 1989, Withdrawal-induced activation of locus coeruleus neurons in opiate-dependent rats: attenuation by lesions of the nucleus paragigantocellularis, Brain Res. 505, 346.

Redmond, Jr., D.E. and J.H. Krystal, 1984, Multiple mechanisms of withdrawal from opioid drugs, Annu. Rev. Neurosci. 7, 443.

Redmond, Jr., D.E., Y.H. Huang, D.R. Snyder and J.W. Maas, 1976, Behavioral effects of stimulation of the nucleus locus coeruleus in the stump-tailed monkey (*Macaca arctoides*), Brain Res. 116, 502.

Schoepp, D.D. and P.J. Conn, 1993, Metabolic glutamate receptors in brain function and pathology, Trends Pharmacol. Sci. 14,13.

Tanganelli, S., T. Antonelli, M. Morari, C. Bianchi and L. Beani, 1991, Glutamate antagonists prevent morphine withdrawal in mice and guinea pigs, Neurosci. Lett. 122, 270.

Tokuyama, S., Y.Z. Feng, H. Wakabayashi and I.K. Ho, 1995a, Ca^{2+} channel blocker, diltiazem, prevents physical dependence and the enhancement of protein kinase C activity by opioid infusion in rats, Eur. J. Pharmacol. 279, 93.

Tokuyama, S., Y.Z. Feng, H. Wakabayashi and I.K. Ho, 1995b, Possible involvement of protein kinases in physical dependence on opioids: studies using protein kinase inhibitors, H-7 and H-8, Eur. J. Pharmacol. 284, 101.

Trujillo, K. and H. Akil, 1991, Inhibition of morphine tolerance and dependence by the NMDA receptor antagonist MK-801, Science 251, 85.

Watkins, J.C., P. Krogsgaard-Larsen and T. Honore, 1990, Structure-activity relationships in the development of excitatory amino acid receptor agonists and competitive antagonists. in: The Pharmacology of Excitatory Amino Acids. Tips Special Report, eds. D. Lodge and G. Collingridge (Elsevier, Cambridge) p. 4.

Zhang, T., Y.Z. Feng, R.W. Rockhold and I.K. Ho, 1994, Naloxone-precipitated morphine withdrawal increases pontaine glutamate levels in the rat, Life Sci. 55, 25.