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Precipitated κ-opioid receptor agonist withdrawal increases glutamate in rat locus coeruleus

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Abstract

Extracellular fluid levels of excitatory amino acids (glutamate, Glu; and aspartate, Asp) in the locus coeruleus and the behavioral signs during naloxone-precipitated withdrawal from κ-opioid receptor agonists, butorphanol and (5α,7α,8β)-(+)-*N*-methyl-*N*-[7-(1-pyrrolidinyl)-1-oxaspiro[4,5]dec-8-yl]-benzeneacetamide (U-69,593), were investigated by in vivo microdialysis. Increases in levels of Glu, but not of Asp, were noted after naloxone (12 or 48 nmol/5 μl, locus coeruleus)-precipitated withdrawal in the rats which had been intracerebroventricularly infused with butorphanol (26 nmol/1 μl/h) or U-69,593 (26 nmol/10 μl/h) for 3 days. The Glu levels in the locus coeruleus increased following administration of naloxone before and during the first 15-min sample after the precipitation of withdrawal in the butorphanol- or U-69,593-dependent rats. Furthermore, behavioral evidence of withdrawal (teeth-chattering, wet-dog shakes, etc.) was detected following the naloxone challenge in the butorphanol- and U-69,593-infused rats, but not in saline-infused controls. These results provide direct evidence to support the role of excitatory amino acids within the locus coeruleus in butorphanol or U-69,593 withdrawal.

Keywords: Butorphanol; Glutamate; κ-Opioid receptor agonist; Locus coeruleus; Microdialysis; U-69,593

1. Introduction

Butorphanol is a potent analgesic but has been found to have antagonistic effects in morphine-dependent subjects (Preston et al., 1988). It is generally classified as an opioid agonist/antagonist and exerts effects through actions at μ-, δ- and κ-opioid receptors (Butelman et al., 1995). On the other hand, U-69,593, a selective κ-opioid receptor agonist, is an effective antinociceptive agent at both supraspinal and spinal sites in a variety of models (Barro et al., 1995). With respect to ligands, U-69,593 binds with high affinity and specificity to κ-opioid receptors, especially κ_1-opioid receptors, rather than the other subtypes, κ_2 and κ_3 (Mosaddeghi et al., 1995; Pasternak, 1993; Yasuda et al., 1993).

The locus coeruleus possesses a high density of μ- and κ-opioid receptors and is the most sensitive site for inducing withdrawal signs by intracerebral injection of opioid antagonists (Maldonado et al., 1992; Rasmussen and Aghajanian, 1989). It has been demonstrated that an increase in the release of excitatory amino acids occurs in the locus coeruleus as a result of opioid withdrawal (Aghajanian et al., 1994). Recent results from our laboratory also showed that i.c.v. injection of naloxone precipitated morphine withdrawal and increased pontine Glu levels (Zhang et al., 1994). These results provide direct evidence to support the role of locus coeruleus excitatory amino acids in withdrawal. A study examining increases in Glu levels in rats during naloxone precipitated (both i.c.v. and s.c.) withdrawal from butorphanol also substantiates the role of excitatory amino acids within the locus coeruleus in butorphanol withdrawal (Feng et al., 1995).

The purpose of the present studies was to directly substantiate a role for increased levels of excitatory amino acids within the locus coeruleus in butorphanol withdrawal precipitated by direct injection of naloxone into the locus coeruleus. To more clearly establish that κ-opioid receptors play a key role in the locus coeruleus in mediating withdrawal from butorphanol-dependent animals, a κ-specific opioid receptor agonist, U-69,593, was included for comparison with butorphanol.

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2. Materials and methods

2.1. Animals

Male Sprague-Dawley rats (270–300 g; Harlan, Prattville, AL, USA) were purchased and maintained under conditions of 22 ± 2°C with a 12–12 h light-dark cycle for 1 week prior to surgery. All procedures involving the rats were performed using protocols approved by the Animal Care and Use Committee of our institution.

2.2. Drugs

Butorphanol tartrate was the gift from Bristol Myers Squibb (Evansville, IN, USA). U-69,593, (5α,7α,8β)-(+)-*N*-methyl-*N*-[7-(1-pyrrolidinyl)-1-oxaspiro[4,5]dec-8-yl]-benzeneacetamide, was purchased from Research Biochemicals International (Natick, MA, USA). Amino acids were purchased from Sigma (St. Louis, MO, USA). Other reagents were of analytical grade.

2.3. Surgical procedures

Rats were anesthetized with equithensin (4.14 g chloral hydrate, 2.22 g $MgSO_4 \cdot 7\ H_2O$, 0.993 g pentobarbital Na, 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 apparatus. A midline skin incision was made and the skull leveled between bregma and lambda. A stainless steel guide cannula (26-gauge, 10 mm long) was implanted into the right lateral cerebral ventricle for i.c.v. infusion or injection. A stylet (32-gauge sealed stainless steel tubing) was inserted into the guide cannula to keep the cannula patent. The presence of cerebrospinal fluid in the guide cannula was noted as verification of proper placement. The coordinates for implantation were, relative to bregma: posterior 0.5 mm, lateral 1.3 mm and 4.5 mm below the dorsal skull surface (Paxinos and Watson, 1986). A CMA/11 guide cannula (Bioanalytical Systems, West Lafayette, IN, USA) was implanted with the tip directed toward the locus coeruleus. The coordinates for the locus coeruleus implantation were posterior −0.8 mm, lateral 1.1 mm and 6.8 mm below the skull surface (Paxinos and Watson, 1986). Dental acrylic (Lang Dental, Wheeling, IL, USA) was applied to the surface of the skull; two protective aluminum caps were placed around the guide cannula and anchored by five screws. After surgery, rats were given 300 000 U of procaine penicillin G s.c. and were allowed 1 week to recover before implantation of osmotic minipumps for inducing butorphanol or U-69,593 dependence. Upon completion of the experimental sequence, each brain was removed and fixed at least 2 days in formalin. The i.c.v. cannula track was verified visually by cutting vertically through the cannula marks on the cortex surface. The remaining brain fragment was frozen and 14-μm sections were cut through the locus coeruleus using a microtome-cryostat (Ames Lab-Tek, Westmont, IL, USA). The probes were located within the locus coeruleus area and part of the tips extended into the area of the subcoeruleus nucleus.

2.4. Induction of butorphanol or U-69,593 dependence

Animals were rendered dependent on butorphanol or U-69,593 by i.c.v. infusion of butorphanol (26 nmol/1 μl/h) or U-69,593 (26 nmol/10 μl/h) for 3 days via osmotic minipumps (Alzet 2001 and 2 ML; Alza, Palo Alto, CA, USA) as previously described (Horan and Ho, 1991; Zhang et al., 1994). Under ether anesthesia, each animal had a minipump implanted s.c. between the scapulae. A 4-cm piece of Tygon tubing (0.38 mm i.d.; Cole-Palmer, Chicago, IL, USA) was used to connect the outlet of the minipump to a piece of 'L'-shaped stainless steel injector tubing (32-gauge, 30 mm long), which was then placed into the i.c.v. guide cannula. Both normal saline and butorphanol solution were passed through a 0.2-μm sterile Acrodisk filter (Gelman Sciences, Ann Arbor, MI, USA) before they were introduced into the pumps. Minipumps were primed overnight at room temperature in normal saline so that the nominal flow rate (1 μl/h) was attained. The control rats received an i.c.v. infusion of normal saline (1 μl/h) for the same period of time. U-69,593 was diluted in ethanol (9.5%) and normal saline (90.5%) and passed through a Millipore filter as described above. The U-69,593 solution was then used to fill osmotic minipumps (Alzet 2 ML; Alza). As a control for this experimental group, osmotic minipumps were filled with the vehicle which contained ethanol (9.5% in normal saline). A 6-cm piece of Tygon tube was directly attached to the guide cannula.

2.5. Measurement of behavioral signs during butorphanol and U-69,593 withdrawals

After 3 days of i.c.v. infusion of butorphanol or U-69,593, the locus coeruleus injection of naloxone (12 or 48 nmol/5 μl) precipitated withdrawal signs (teeth-chattering, wet-dog shakes, penis-licking, locomotion, stretching, scratching, salivation, piloerection, rearing and ptosis) were scored during a 30-min period following the injection. For statistical purposes, a simple scoring system (1 point for each sign and summation of the 10 signs to give a total score for each rat) was used to compare responses between the control group which received the vehicle and the naloxone-precipitated withdrawal group.

2.6. Samplings for microdialysis

The microdialysis probes (CMA/11, 2 mm tip) and guide cannula were purchased from Bioanalytical Systems (BAS, Lafayette, IN, USA) and used within 3 months. The

dialysis membrane tip of the probe has an o.d. of 240 μm and i.d. of 210 μm, a dead volume of 1 μl and a molecular mass cut-off of 20 000 Da. The in vitro recoveries of Glu and Asp were determined by immersing probes in Ringer's solution containing 100 μM each of Glu and Asp at room temperature. Probes were dialyzed with Ringer's solution and dialysate samples were analyzed by high-performance liquid chromatography (HPLC). Recoveries of Glu and Asp were 9.6 ± 0.4 and 9.6 ± 0.7% of the external concentrations, respectively. Due to the variability of probe recovery, the extracellular fluid levels of amino acids were individually calculated for each animal.

2.7. Analysis of amino acids

Analysis of amino acids was conducted by the method of Ellison et al. (1987) with minor modification. Briefly, the measurements were performed on an HPLC (BAS LC-44 Detector) with electrochemical detection using a Rainin 4.6 × 15 cm (5 μm, 100A) column. The mobile phase consisted of 50 mM sodium phosphate (mono and dibasic)/methanol buffer (70/30, v/v; pH 5.38). The derivatizing agent consisted of *o*-phthaldialdehyde (50 mg), 2-mercaptoethanol (40 μl), absolute ethanol (0.9 ml) and 2 mM borate buffer to make a final volume of 10 ml. The peaks of Glu and Asp were verified by retention time, peak shape and comparison of samples with a standard consisting of eight amino acids (aspartate, glutamate, glutamine, histidine, arginine, glycine, taurine and alanine, each at 5 μM concentration).

2.8. General experimental procedures

1 week following stereotaxic surgery and the day before the beginning of the experiment, a freshly calibrated microdialysis probe was placed into the locus coeruleus guide cannula. The probe was then perfused with filtered Ringer's solution at a low rate (0.2 μl/min) overnight. On the morning of the following day, the flow rate was increased to 2 μl/min. Collection of consecutive 15-min samples for determination of basal values was begun after 2–3 h of equilibration. Following collection of 3 consecutive stable (not more than 20% of intersample variation) basal samples, a single i.c.v. injection of butorphanol (26 nmol/5 μl), U-69,593 (26 nmol/50 μl) or saline (5 μl) was given over a 5-min period (over a 10-min for U-69,593) and consecutive 15-min samples were collected for an hour. The microdialysis probe was left in place following completion of the acute study. The inlet and outlet of the probe were protected by connecting the ports with a short PE-50 tube filled with Ringer's solution. Each animal was then anesthetized, a minipump was implanted and the i.c.v. infusion of butorphanol or U-69,593 was initiated. The infusion of both drugs was continued by means of the implanted osmotic minipump for 3 days. On the 4th day after the initiation of drug infusion, the connecting tube between the i.c.v. cannula and the outlet of the osmotic minipump was disconnected (2 h before the naloxone challenge was given) and perfusion of the microdialysis probe was reinitiated at 2 μl/min. After 2 h of equilibration, 3 or 4 samples were collected for determination of basal values. Naloxone (12 or 48 nmol/5 μl, locus coeruleus) was then given over a 5-min period using a hand-held microliter syringe and samples were collected for an additional hour.

2.9. Data analysis

One-way analysis of variance (ANOVA) and the Newman-Keuls test were used. Calculated values of $P < 0.05$ were considered as statistically significant. Selected values are expressed as a percentage change from basal values. Mean values ± S.E.M. are reported.

3. Results

3.1. Effects of acute administration of butorphanol and U-69,593 on excitatory amino-acid levels in the locus coeruleus

No significant changes were noted in Glu levels following a single acute i.c.v. injection of butorphanol, U-69,593, or vehicles (Fig. 1). Basal values of Glu averaged 5.1 ± 0.6, 4.6 ± 0.2 and 4.7 ± 0.5 μM, respectively, in the butorphanol, U-69,593 and control groups. Basal values of Asp were also the same (butorphanol, 4.6 ± 0.6 μM; U-69,593, 4.6 ± 0.9 μM; and control, 4.4 ± 0.7 μM) (data not shown).

3.2. Effects of naloxone-precipitated butorphanol or U-69,593 withdrawal on excitatory amino-acid levels in the locus coeruleus

Increases in Glu levels within the locus coeruleus during naloxone-precipitated withdrawal from butorphanol were observed. Following a low-dose (12 nmol/5 μl, locus coeruleus) naloxone injection, Glu levels were increased ($P < 0.05$) to 138% of the basal value in the first 15-min sample (from 6.1 ± 0.1 to 8.2 ± 0.2 μM). Increases in Glu ($P < 0.05$) to 156% of the basal value (from 5.7 ± 0.1 to 9.1 ± 0.4 μM) were noted following treatment with a high dose of naloxone (48 nmol/5 μl, locus coeruleus) (Fig. 2). However, no significant changes were noted in Asp levels following naloxone (12 and 48 nmol/5 μl, locus coeruleus) injection. The Glu levels in the control group receiving normal saline were not altered following naloxone (48 nmol/5 μl, locus coeruleus) treatment (from 5.4 ± 0.3 to 5.9 ± 0.4 μM before and in the first 15-min sample following naloxone, respectively) (Fig.

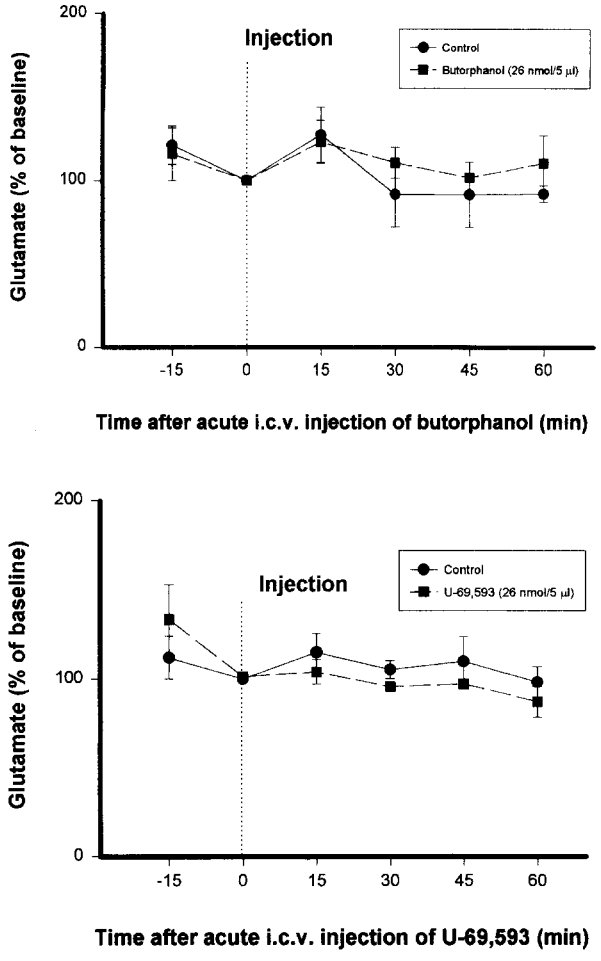

Fig. 1. Effects of acute i.c.v. administration of butorphanol (26 nmol/5 μl; $n = 3$: top panel), U-69,593 (26 nmol/50 μl; $n = 3$: bottom panel) or normal saline (5 μl; $n = 3$) on the extracellular fluid levels of glutamate within the locus coeruleus. Solutions were injected i.c.v. over a 5- or 10-min period beginning at the time marked, 'injection' (see Section 2 for details). Values are expressed as percentage change (mean ± S.E.M.) from basal values. Basal values were calculated as the mean of the three individual basal samples.

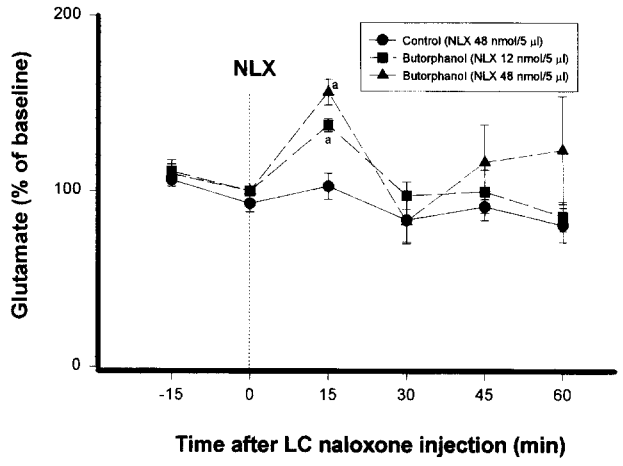

Fig. 2. Increases in extracellular fluid levels of Glu within the locus coeruleus during naloxone (NLX)-precipitated butorphanol withdrawal. Animals received 3 days of i.c.v. infusion of either butorphanol (26 nmol/1 μl/h; $n = 10$) or normal saline (1 μl/h; $n = 11$). Naloxone was given by locus coeruleus injection in low (12 nmol/5 μl; $n = 5$) or high dose (48 nmol/5 μl; $n = 5$) to butorphanol-dependent rats over a 5-min period. Values are expressed as percentage change (mean ± S.E.M.) from basal values. [a] $P < 0.05$ vs. control.

3.3. Behavior signs during naloxone-precipitated butorphanol or U-69,593 withdrawal

We observed no significant differences between the low and high doses of naloxone in the butorphanol- and U-69,593-treated groups. Therefore, withdrawal scores from both naloxone-treated opioid-infused groups were combined. As shown in Table 1, the incidence of appearance of selected behavioral signs of withdrawal in the butor-

2). On the other hand, the Glu levels within the locus coeruleus during the low-dose naloxone-precipitated U-69,593 withdrawal were increased ($P < 0.05$) to 193% of the basal value in the first 15-min sample (from 5.6 ± 0.3 to 10.2 ± 1.3 μM). Increases in Glu levels ($P < 0.01$) during the high-dose naloxone-precipitated U-69,593 withdrawal were the same value (193%) (from 5.4 ± 0.4 to 10.0 ± 0.5 μM) as those of the low-dose naloxone treatment (Fig. 3). However, there was no significant increase in Asp levels after either a low or high dose of naloxone in the U-69,593-treated group. Significant decreases were noted in the Glu levels ($P < 0.01$) in the control group (from 5.0 ± 1.0 to 3.5 ± 0.5 μM) between 0- and 30-min samples following the high dose of naloxone (Fig. 3). There was no difference in Asp levels before and after the naloxone injection in the control group which received vehicle (data not shown).

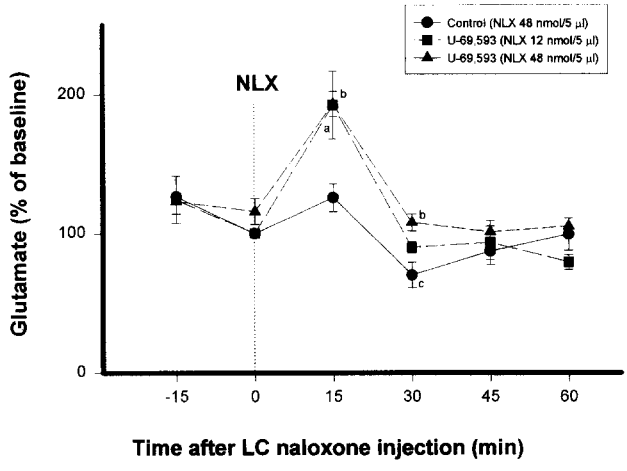

Fig. 3. Increases in extracellular fluid levels of Glu within the locus coeruleus during naloxone (NLX)-precipitated U-69,593 withdrawal. Animals received 3 days of i.c.v. infusion of either U-69,593 (26 nmol/10 μl/h; $n = 12$) or normal saline containing 9.5% ethanol (10 μl/h; $n = 12$). Naloxone was given by locus coeruleus injection in low (12 nmol/5 μl; $n = 5$) or high dose (48 nmol/5 μl; $n = 7$) to U-69,593-dependent rats over a 5-min period. Values are expressed as percentage change (mean ± S.E.M.) from basal values. [a] $P < 0.05$ vs. control; [b] $P < 0.01$ vs. control; [c] $P < 0.01$, 0-min vs. 30-min sample.

Table 1
Withdrawal signs elicited in butorphanol- and U-69,593-dependent or control rats by locus coeruleus naloxone administration

Withdrawal signs	Control	Butorphanol	Control	U-69,593
Teeth chattering	2/11 [a]	9/11 [c]	3/11	10/11 [d]
Wet-dog shakes	3/11	9/11 [c]	4/11	10/11 [c]
Locomotion	N.D. [b]	8/11 [d]	N.D.	10/11 [d]
Stretching	N.D.	5/11 [c]	N.D.	7/11 [d]
Scratching	N.D.	5/11 [c]	N.D.	7/11 [d]
Penis-licking	N.D.	3/11	N.D.	3/11
Piloerection	N.D.	2/11	N.D.	2/11
Rearing	N.D.	2/11	N.D.	3/11
Salivation	N.D.	N.D.	N.D.	N.D.
Ptosis	N.D.	2/11	N.D.	2/11

Rats received 3 days i.c.v. infusion of butorphanol (26 nmol/1 μl/h), U-69,593 (26 nmol/10 μl/h) or vehicle control (1 μl/h), then they were challenged by locus coeruleus injection of naloxone (12 and 48 nmol/5 μl).

[a] Numbers denote the number of rats showing positive signs over the total number of rats tested.

[b] N.D., none detected.

[c] $P < 0.05$, [d] $P < 0.01$ (values are significantly higher than the control values as determined by the χ^2 test).

phanol-infused group varied from a low of 2/11 for piloerection, rearing and ptosis, to a high of 9/11 for teeth-chattering and wet-dog shakes. The incidence of these in the U-69,593 group varied from a low of 2/11 for piloerection and ptosis, to a high of 10/11 for wet-dog shakes, teeth-chattering and locomotion. A significant enhancement of withdrawal signs was not observed following naloxone injection in the control groups. The composite withdrawal score was significantly ($P < 0.05$) higher in the butorphanol-infused rats (5.4 ± 0.9; $n = 11$) as compared to that of the control group (1.7 ± 0.3; $n = 11$). The withdrawal score was also significantly ($P < 0.01$) higher in the U-69,593-infused rats (5.4 ± 0.7; $n = 11$) as compared to that of the control group (1.8 ± 0.2; $n = 11$). In addition, no significant differences were noted between butorphanol and U-69,593 groups.

4. Discussion

Our results indicate that naloxone-precipitated butorphanol or U-69,593 withdrawal resulted in increased Glu levels in the locus coeruleus of butorphanol- and U-69,593-dependent rats. This study provides further evidence to indicate that excitatory amino acids within the locus coeruleus are involved in butorphanol and U-69,593 withdrawal.

Previous electrophysiological studies have provided evidence for increased excitatory amino-acid release in the locus coeruleus during opioid withdrawal (Rasmussen and Aghajanian, 1989; Rasmussen, 1991). Recently, it has been shown that naltrexone- or naloxone-precipitated opioid withdrawal produces an increased efflux of Glu and Asp in the locus coeruleus of morphine-dependent rats (Aghajanian et al., 1994; Zhang et al., 1994). The locus coeruleus possesses a high density of opioid receptors, particularly of the μ- and κ-subtype (Atweh and Kuhar, 1977), and administration of an opioid antagonist was found to increase firing of locus coeruleus neurons in morphine-dependent rats (Rasmussen, 1991). The hyperactivity of locus coeruleus neurons seen after opioid receptor antagonist-induced withdrawal is believed to be mediated by an excitatory amino-acid pathway from the nucleus paragigantocellularis to the locus coeruleus (Akaoka and Aston-Jones, 1991; Aston-Jones et al., 1986; Rasmussen, 1991; Rasmussen and Aghajanian, 1989).

We noted increases in the extracellular fluid levels of Glu but not of Asp after the naloxone (both 12 and 48 nmol/5 μl, locus coeruleus)-precipitated withdrawal in rats which had been i.c.v. infused with butorphanol or U-69,593 for 3 days. This may indicate that the increased Glu levels within the locus coeruleus during naloxone-precipitated withdrawal is due to increase in the firing rate of glutamatergic neurons in the paragigantocellularis-locus coeruleus pathway (Aghajanian et al., 1994). However, no increases in Asp were seen during the 1-h collection period, despite the fact that there were withdrawal signs observed after naloxone injection in κ-opioid receptor agonist-infused animals. Aghajanian et al. (1994) have shown that there was a significant but less pronounced increase in the efflux of Asp in the locus coeruleus of morphine-dependent rats. Therefore, our results may indicate that the amount of Asp released per nerve impulse in the locus coeruleus was less than that of Glu released per impulse. This may also be due to the fact that the withdrawal-induced activation of locus coeruleus in butorphanol- or U-69,593-infused animals is relatively weaker than that of morphine. In addition, the time course of levels of Glu following naloxone injection in the locus coeruleus indicated a very short half-life as reported previously for electrophysiological experiments in vivo (Olsson et al., 1995; Rasmussen, 1991; Rasmussen et al., 1990).

As shown in Fig. 3, in saline-treated rats, a significant decrease in Glu levels within the locus coeruleus was temporarily observed at 30 min following a naloxone injection, implying that naloxone temporarily depleted neurotransmitters contained in the neuronal terminals in the locus coeruleus. This may probably be due to the relatively large volume of vehicle injected. On the other hand, alcohol which was present in the vehicle could also contribute to the effect. This possibility is remained to be investigated.

Acute i.c.v. administration of both butorphanol and U-69,593 did not cause a change in the release of either Glu or Asp in the region of the locus coeruleus. Opioids have the capacity to block directly the release of neurotransmitters contained in the neuronal terminals (Olsson et al., 1995; Crowder et al., 1986). The discrepancy between these results can probably be explained by the differences

in experimental protocols, e.g., in vitro preparations in brain slices or in vivo microdialysis performed in conscious animals.

Excitatory amino-acid neurotransmission in the locus coeruleus neurons is closely related to the morphine-withdrawal behavior (Aghajanian, 1978; Aghajanian et al., 1994; Rasmussen et al., 1991; Zhang et al., 1994). In our experiment, increases in Glu efflux were observed only when the microdialysis probe was properly located in the locus coeruleus; no increase was seen in adjacent regions. In addition, the elevation of Glu levels and behavioral signs of butorphanol or U-69,593 withdrawal showed virtually identical time courses. Both measures rose markedly by 15 min after the naloxone injection and returned to control levels by 30 min. These data are direct biochemical evidence that the increased Glu is due to the hyperactivity of locus coeruleus neurons observed after naloxone-precipitated opioid withdrawal (Rasmussen et al., 1990). Therefore, release of excitatory amino acids in the locus coeruleus may play an important role in mediating the opioid-withdrawal syndrome (Aghajanian et al., 1994; Feng et al., 1995; Zhang et al., 1994). In fact, i.c.v. administration of kynurenic acid, a non-selective excitatory amino-acid antagonist, can block increases in the locus coeruleus neuronal firing and behavioral changes noted during morphine withdrawal (Rasmussen et al., 1991). In this study, the parallel between withdrawal behavior and the increased Glu levels within the locus coeruleus suggests that activation of locus coeruleus neurons plays a role in mediating aspects of butorphanol- or U-69,593-induced withdrawal. This may be, at least in part, ascribed to the relationship between excitatory amino-acid transmitters and a κ-opioid receptor in the locus coeruleus which mediates butorphanol- or U-69,593-induced withdrawal. The increased levels of Glu observed in this study may prove to be indicative of excitatory amino-acid release from nerve terminals within the locus coeruleus.

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References

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.

Akaoka, H. and G. Aston-Jones, 1991, Opiate withdrawal-induced hyperactivity of locus coeruleus neurons is substantially mediated by augmented excitatory amino acid input, J. Neurosci. 11, 3830.

Aston-Jones, G., M. Ennis, V.A. Pieribone, W.T. Nickell and M.T. Shipley, 1986, The brain nucleus locus coeruleus: restricted afferent control of a broad efferent network, Science 234, 734.

Atweh, S.F. and M.J. Kuhar, 1977, Autoradiographic localization of opioid receptors in rat brain. II. The brain stem, Brain Res. 129, 1.

Barro, M., F. Ruiz and M.A. Hurle, 1995, κ-Opioid receptor mediated antinociception in rats is dependent on the functional state of dihydropyridine-sensitive calcium channels, Brain Res. 672, 148.

Butelman, E.R., G. Winger, G. Zernig and J.H. Woods, 1995, Butorphanol: characterization of agonist and antagonist effects in rhesus monkeys, J. Pharmacol. Exp. Ther. 272, 845.

Crowder, J.M., D.K. Norris and H.F. Bradford, 1986, Morphine inhibition of calcium fluxes, neurotransmitter release and protein and lipid phosphorylation in brain slices and synaptosomes, Biochem. Pharmacol. 35, 2501.

Ellison, D.W., M.F. Beal and J.B. Martin, 1987, Amino acid neurotransmitters in postmortem human brain analyzed by high performance liquid chromatography with electrochemical detection, J. Neurosci. Methods 19, 305.

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, 745.

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

Maldonado, R., L. Stinus, L.H. Gold and G.F. Koob, 1992, Role of different brain structures in the expression of the physical morphine withdrawal syndrome, J. Pharmacol. Exp. Ther. 261, 669.

Mosaddeghi, M., D.R. Kapusta, L.D. Minor, N. Duan and D. Paul, 1995, Effects of κ-opioid receptor agonists on stimulated phosphoinositide hydrolysis in rat kidney, Eur. J. Pharmacol. 289, 411.

Olsson, Y., H.S. Sharma, F. Nyberg and J. Westman, 1995, The opioid receptor antagonist naloxone influences the pathophysiology of spinal cord injury, in: Progress in Brain Research, Vol. 104, eds. F. Nyberg, H.S. Sharma and Z. Wiesenfeld-Hallin (Elsevier, Amsterdam) p. 381.

Pasternak, G.W., 1993, Review: pharmacological mechanisms of opioid analgesics, Clin. Neuropharmacol. 16, 1.

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

Preston, K.L., G.E. Bigelow and I.A. Liebson, 1988, Butorphanol-precipitated withdrawal in opioid-dependent human volunteers, J. Pharmacol. Exp. Ther. 246, 441.

Rasmussen, K., 1991, Afferent effects on locus coeruleus in opiate withdrawal, in: Progress in Brain Research, Vol. 88, eds. C.D. Barnes and O. Pompeiano (Elsevier, Amsterdam) p. 207.

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.

Rasmussen, K., D.B. Beitner-Johnson, J.H. Krystal, G.K. Aghajanian and E.J. Nestler, 1990, Opiate withdrawal and the rat locus coeruleus: behavioral, electrophysiological, and biochemical correlates, J. Neurosci. 10, 2308.

Rasmussen, K., J.H. Krystal and G.K. Aghajanian, 1991, Excitatory amino acids and morphine withdrawal: differential effects of central and peripheral kynurenic acid administration, Psychopharmacology 105, 508.

Yasuda, K., K. Raynor, H. Kong, C.D. Breder, J. Takeda, T. Reisine and G.I. Bell, 1993, Cloning and functional comparison of κ and δ opioid receptors from mouse brain, Proc. Natl. Acad. Sci. USA 90, 6736.

Zhang, T., Y. Feng, R.W. Rockhold and I.K. Ho, 1994, Naloxone-precipitated morphine withdrawal increases pontine glutamate levels in the rat, Life Sci. 55, PL25.