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# Attenuation of morphine withdrawal signs by intracerebral administration of 18-methoxycoronaridine

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**Abstract**

18-Methoxyroconaridine (18-MC), a synthetic derivative of ibogaine, reduces morphine self-administration and alleviates several signs of acute opioid withdrawal in rats. Although there is already well documented evidence of the mechanism mediating 18-MC's action to reduce the rewarding effects of morphine, nothing is known about the mechanism responsible for 18-MC's attenuation of opioid withdrawal. In vitro studies have demonstrated that 18-MC is a potent antagonist of $\alpha_3\beta_4$ nicotinic receptors ($IC_{50}$=0.75 μM), which are predominantly located in the medial habenula and interpeduncular nuclei. Previous work indicating that $\alpha_3\beta_4$ nicotinic receptors mediate 18-MC's effects on drug self-administration prompted us to assess whether brain areas having high or moderate densities of $\alpha_3\beta_4$ receptors might be involved in 18-MC's modulation of opioid withdrawal. To test this possibility, 18-MC was locally administered into the medial habenula, interpeduncular nucleus and locus coeruleus of morphine-dependent rats; this treatment was followed by naltrexone to precipitate a withdrawal syndrome. Pretreatment with various doses of 18-MC into the locus coeruleus significantly reduced wet-dog shakes, teeth chattering, burying and diarrhea, while pretreatment into the medial habenula attenuated teeth chattering, burying, and weight loss. Some doses of 18-MC administered into the interpeduncular nucleus significantly ameliorated rearing, teeth chattering, and burying, while other doses exacerbated diarrhea and teeth chattering. The present findings suggest that 18-MC may act in all three nuclei to suppress various signs of opioid withdrawal.


*Keywords:* Morphine; Naltrexone; Medial habenula; Interpeduncular nucleus; Locus coeruleus; Physical dependence

## 1. Introduction

18-Methoxycoronaridine, a synthetic derivative of ibogaine, reduces the intravenous self-administration of morphine and other drugs (Glick et al., 1996, 2000) and alleviates several signs of acute opioid withdrawal in rats (Rho and Glick, 1998). Using patch clamp methodology, Glick et al. (2002a) demonstrated that 18-MC is a potent antagonist of $\alpha_3\beta_4$ nicotinic receptors ($IC_{50}$=0.75 μM). In the brain, $\alpha_3\beta_4$ nicotinic receptors are predominantly located in the medial habenula and interpeduncular nucleus (Wonnacott, 1997; Quick et al., 1999). The habenulo-interpeduncular pathway is primarily cholinergic, and is one of the largest cholinergic pathways in the brain (Morley, 1986). Several drugs of abuse, such as amphetamine and nicotine, have been shown to alter the morphology and function of this neuronal pathway (Ellison, 1992, 2002; Carlson et al., 2000, 2001). Thus, the habenulo-interpeduncular pathway may play an important role in modulating the effects of addictive drugs.

Effects of morphine on central cholinergic neurotransmission during opioid withdrawal are well documented (Antonelli et al., 1986; Rada et al., 1991, 1996; Crossland and Ahmed, 1984; Jhamandas and Sutak, 1974; Bhargava and Way, 1976). For example, an increase of cortical acetylcholine release was demonstrated during naloxone-precipitated opioid withdrawal in guinea pigs (Antonelli et al., 1986). Similarly, extracellular acetylcholine in the nucleus accumbens and prefrontal cortex was shown to be increased during naloxone-precipitated morphine withdrawal in rats; the increase was correlated with the intensity of withdrawal signs (Rada et al., 1991, 1996). Similar changes in extracellular acetylcholine release during naloxone-precipitated withdrawal were observed in other brain areas

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(Jhamandas and Sutak, 1974; Crossland and Ahmed, 1984; Cheney et al., 1974). Actions of acetylcholine at both muscarinic and nicotinic receptors appear to be important for the modulation of opioid withdrawal signs (Holland et al., 1993; Buccafusco, 1991; Taraschenko et al., 2005).

18-MC's action at $\alpha_3\beta_4$ nicotinic receptors originally led to the hypothesis that antagonism of these receptors may be a mechanism mediating 18-MC's attenuation of opioid reward and physical dependence. Consistent with this hypothesis, in a previous study, the non-selective $\alpha_3\beta_4$ nicotinic receptor antagonists dextromethorphan, mecamylamine and bupropion all reduced the self-administration of morphine and methamphetamine in rats (Glick et al., 2002a). Dextromethorphan (also an *N*-methyl-D-aspartate receptor antagonist), mecamylamine (an antagonist at all nicotinic receptors), and bupropion (a dopamine transporter blocker) are unrelated agents with unique actions. However, all three drugs share the common action of blocking $\alpha_3\beta_4$ receptors (Hernandez et al., 2000; Papke et al., 2001; Fryer and Lukas, 1999). Based on the rationale that low-dose combinations of these drugs should produce additive effects at the $\alpha_3\beta_4$ site, previous work in this laboratory showed that all combinations of low doses of any two of these $\alpha_3\beta_4$ antagonists were equally effective in reducing morphine, methamphetamine, and nicotine self-administration in rats (Glick et al., 2002b; Maisonneuve and Glick, 2003). Dextromethorphan, mecamylamine and bupropion have also been tested alone and in combination in a rat model of acute morphine withdrawal (Taraschenko et al., 2005). The latter study demonstrated that although each drug alone produced variable effects on withdrawal, a combination of low doses of these agents consistently reduced at least two of seven signs of withdrawal. Thus, it was concluded that an additive action of the antagonists at $\alpha_3\beta_4$ receptors may be responsible for the alleviation of part of the morphine withdrawal syndrome.

High densities of nicotinic $\alpha_3\beta_4$ receptors are located in the medial habenula and interpeduncular nucleus, while moderate densities are located in the locus coeruleus (Quick et al., 1999; Perry et al., 2002). In the present study, to investigate the role of brain areas mediating 18-MC's effects on signs of opioid withdrawal, 18-MC was locally administered into the medial habenula, interpeduncular nucleus or locus coeruleus. Morphine withdrawal has been shown to increase glucose utilization in the medial habenula (Kimes et al., 1990) and interpeduncular nucleus in rats (Wooten et al., 1982). Injection of the opioid antagonist methylnaloxonium into the locus coeruleus precipitated withdrawal signs in morphine-dependent rats (Koob et al., 1992) while lesions of the locus coeruleus attenuated withdrawal signs (Maldonado et al., 1992). Thus all three of these areas have been implicated in the actions of chronic morphine administration.

## 2. Materials and methods

### 2.1. Animals

Naïve female Sprague-Dawley rats (230–300 g; Taconic, Germantown, NY) were housed individually and maintained on a normal 12 h light cycle (light on/off at 7 a.m./7 p.m.). Food and water were provided ad libidum. The experiments were conducted in accordance with the "Guide for the Care and Use of Laboratory Animals" (1996).

### 2.2. Drugs

Morphine sulfate and naltrexone hydrochloride (Research Biochemicals Inc., Natick, MA) were dissolved in saline and administered subcutaneously and intraperitonealy, respectively. 18-Methoxycoronaridine hydrochloride (Albany Molecular Research, Albany, NY) was dissolved in 50% dimethyl sulfoxide (DMSO, vehicle), pH = 5.0.

### 2.3. Stereotaxic brain cannulation surgery

Under sodium pentobarbital anesthesia (50 mg/kg i.p.) rats were secured in a stereotaxic instrument. An incision was made, the bone was exposed and holes for the microinjection guide cannulae were drilled bilaterally. Microinjection guide cannulae (22-gauge) (Plastics One, Roanoke, VA, USA) were lowered into place such that when injectors were inserted the tips were located in the medial habenula, interpeduncular nucleus or locus coeruleus. The coordinates for those placements were, respectively, as follows: (AP −4.2 mm, ML ±2.9 mm, DV −5.0 mm, using a 24° angle; AP −6.3, ML ±2.6 mm, DV −7.7 mm, using a 15° angle; AP −9.8 mm, ML ±2.6 mm, DV −6.2 mm, using a 12° angle) (Paxinos and Watson, 1986). The microinjection guide cannulae were permanently secured with stainless steel screws (Small Parts Inc., Miami Lakes, FL) and cranioplastic cement (Plastics One). The incision was closed with staples. The rats were then allowed to recover for at least 24 h before the beginning of morphine treatment.

### 2.4. Induction of dependence

Morphine was administered subcutaneously at 9:30 a.m. and 3:30 p.m. for 7 days. The following schedule was used: 10 and 20 mg/kg on the first day, 40 and 60 mg/kg on the second day, and 60 and 80 mg/kg on the subsequent days. This schedule was previously used to induce morphine dependence in rats (Rho and Glick, 1998; Taraschenko et al., 2005).

### 2.5. Intracerebral drug administration

On day 8, rats were first weighted and allocated to treatment groups; the groups were balanced such that the average body weights were similar in all groups (overall average weight ± SEM was 232.2 ± 1.98 g). Subsequently, using a microsyringe (Hamilton, Reno, Nevada), 18-MC (5 μg, 10 μg or 20 μg) or vehicle was infused into the medial habenula, interpeduncular nucleus, or locus coeruleus in a volume of 1 μl over 1 min (Fig. 1). Tzo prevent backflow through the microinjection guide, the injection cannula (26 gauge) was kept in place for an additional minute after the solution was administered. The number of animals per study group, ranging from 5 to 8, was previously shown to have sufficient power for data analysis

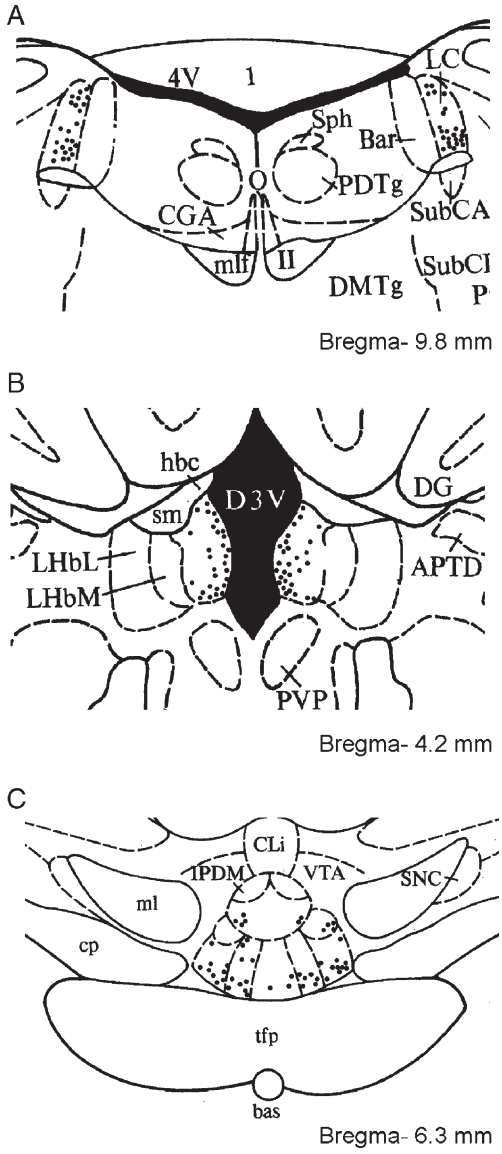


Fig. 1. Localization of microinjection cannulae placements in the locus coeruleus (A), medial habenula (B) and interpeduncular nucleus (C).

(Taraschenko et al., 2005). All drug injections, including daily morphine injections, were made by the same experimenter; thus animals were well habituated to the experimenter by the time withdrawal signs were assessed.

### 2.6. Naltrexone-precipitated withdrawal and behavioral scoring

Intracerebral drug administration into the medial habenula, interpeduncular nucleus, or locus coeruleus was followed immediately by the systemic injection of naltrexone hydrochloride (1 mg/kg i.p.). The signs of withdrawal were monitored and recorded continuously every 15 min for 2 h. The rats were scored in their home cages (transparent Plexiglass, 10″×18″, with wood chip bedding; Sani-Chips, Montville, NJ). The illumination of the experimental room was similar to that in the colony room where the animals were maintained. The behaviors assessed included wet-dog shakes, rearing, grooming, teeth chattering, burying, and diarrhea. The frequencies of incidents were recorded for each 15-min interval and each behavior was regarded as one incident regardless of its duration. Wet-dog shakes were defined as whole body shaking, while the rearing was defined as standing on the hind paws. Grooming was defined as licking, scratching or cleaning any part of the body. Teeth chattering was defined as audible gnawing of teeth, while burying was defined as any incident of digging into the bedding. Body weights were obtained twice, before intracerebral injection and 2 h later. Two rats (drug and vehicle-pretreated) were observed simultaneously; observations were made blindly, without knowledge of whether rats received 18-MC or vehicle.

### 2.7. Verification of the injector sites

Following the completion of the experiment, rats were euthanized and decapitated. Their brains were frozen at −80 °C and then sectioned in a cryostat. The intracerebral injection placements were mapped without knowledge of the behavioral data.

### 2.8. Statistical analysis

All of the withdrawal scores were expressed as the percentage of respective control means. The data were analyzed with two-way analysis of variance (ANOVA) with doses and sign as factors. *Post-hoc* comparison tests (Fisher Least Squares Difference, LSD) were performed when appropriate.

## 3. Results

Injection of naltrexone precipitated an acute withdrawal syndrome in morphine-dependent rats. The following signs were observed: wet-dog shakes, rearing, grooming, teeth chattering, burying, diarrhea and weight loss. The time-courses of the average scores of all vehicle-pretreated rats ($n=24$) for each sign are shown in Fig. 2(A–F). The mean scores for wet-dog shakes and diarrhea decreased during the entire time of observation. On the other hand, the mean scores for rearing and grooming decreased during the first hour and remained unaltered for the rest of the experiment. The mean scores for teeth chattering and burying diminished for 90 and 45 min after naltrexone, respectively, and remained unchanged thereafter. The effects were similar to those previously described in morphine-dependent rats without brain cannulae (Taraschenko et al., 2005).

### 3.1. Pretreatment with 18-MC into the locus coeruleus

Mean withdrawal scores in control rats ($n=6$) for Fig. 3A were as follows (mean incidents per 2 h±S.E.M., except for weight loss in g): wet-dog shakes, 94.3±18.9; rearing, 17.5±3.5; grooming, 12.8±2.8; teeth chattering, 17.0±2.2; burying, 7.5±1.7; diarrhea, 3.5±0.3; weight loss, 11.5±1.4. Two-way ANOVA with dose and sign as two factors revealed a significant

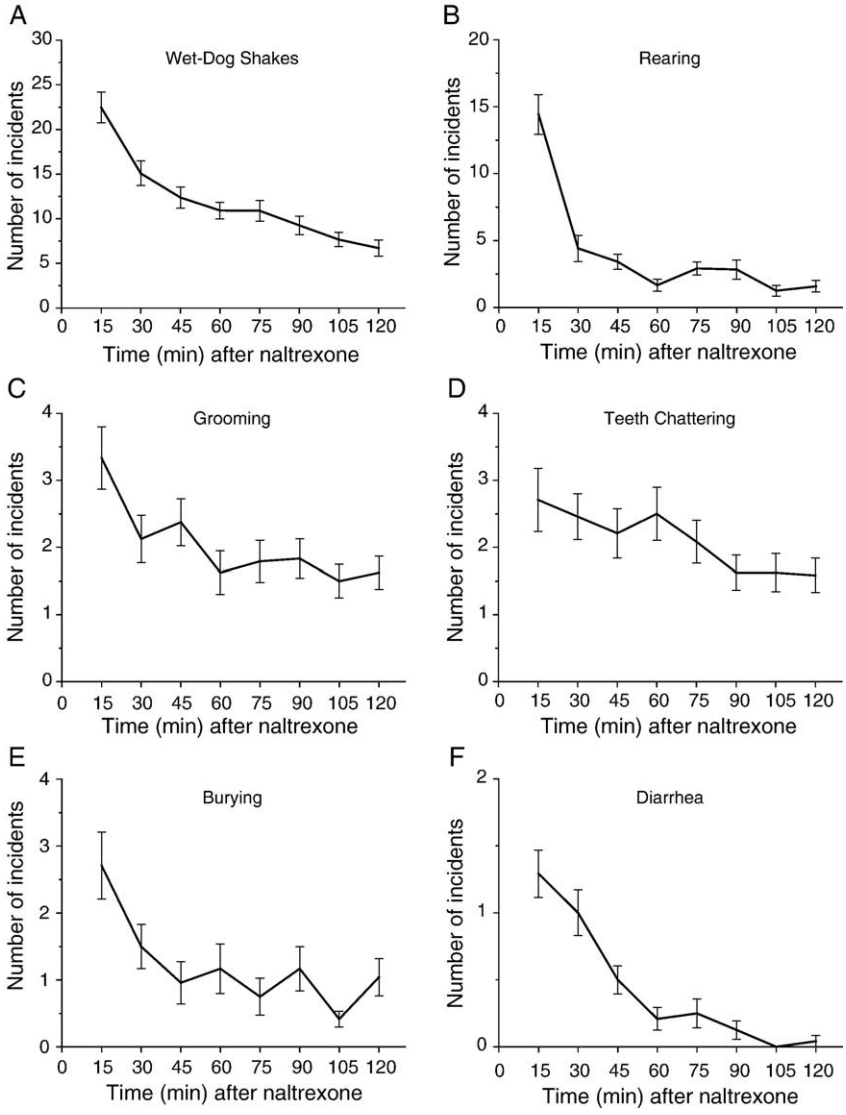


Fig. 2. Time-course of naltrexone-precipitated withdrawal signs in morphine-dependent rats. Vehicle (50% DMSO) was administered into the medial habenula, interpeduncular nucleus or locus coeruleus immediately before naltrexone (1 mg/kg i.p.), and withdrawal signs (mean±S.E.M., $n=24$) were assessed for 2 h.

dose and a significant dose×sign interaction [$F(3,20)=4.49$, $P=0.02$ and $F(18,120)=2.14$, $P=0.01$, respectively]. Post-hoc LSD tests showed that all three dosages of 18-MC significantly attenuated burying, while two dosages (i.e., 5 μg and 20 μg) attenuated teeth chattering. At 10 μg, 18-MC significantly reduced wet-dog shaking and diarrhea.

### 3.2. Pretreatment with 18-MC into the medial habenula

Mean withdrawal scores in vehicle-pretreated rats ($n=10$) for Fig. 3B were as follows (mean incidents per 2 h±S.E.M., except for weight loss in g): wet-dog shakes, 101.3±12.7; rearing, 34.9±3.4; grooming, 18.1±2.5; teeth chattering, 14.6±2.7; burying, 9.4±2.0; diarrhea, 3.5±0.3; weight loss, 12.5±0.8. Two-way ANOVA with dose and sign as the two factors revealed a significant dose×sign interaction [$F(18,150)=1.94$, $P=0.017$]. Further post-hoc LSD tests showed that at 5 μg 18-MC significantly attenuated teeth chattering and weight loss. At 10 μg, 18-MC significantly increased and attenuated teeth chattering and burying, respectively. The highest dose of 18-MC (i.e., 20 μg) failed to alter signs of morphine withdrawal.

### 3.3. Pretreatment with 18-MC into the interpeduncular nucleus

Mean withdrawal scores in vehicle-pretreated rats ($n=8$) for Fig. 3C were as follows (mean incidents per 2 h±S.E.M., except for weight loss in g): wet-dog shakes, 88.6±7.0; rearing, 40.5±7.2; grooming, 16.4±1.8; teeth chattering, 15.1±3.0; burying, 11.6±3.3; diarrhea, 3.1±0.4; weight loss, 12.8±0.9. Two-way ANOVA with dose and sign as two factors revealed a significant dose×sign interaction [$F(18,120)=3.21$, $P=0.00007$]. Further post-hoc LSD tests showed the lowest dose of 18-MC (i.e., 5 μg) significantly attenuated teeth chattering and burying in morphine-dependent rats. Interestingly, the same dose of 18-MC significantly increased diarrhea while the highest dose (i.e., 20

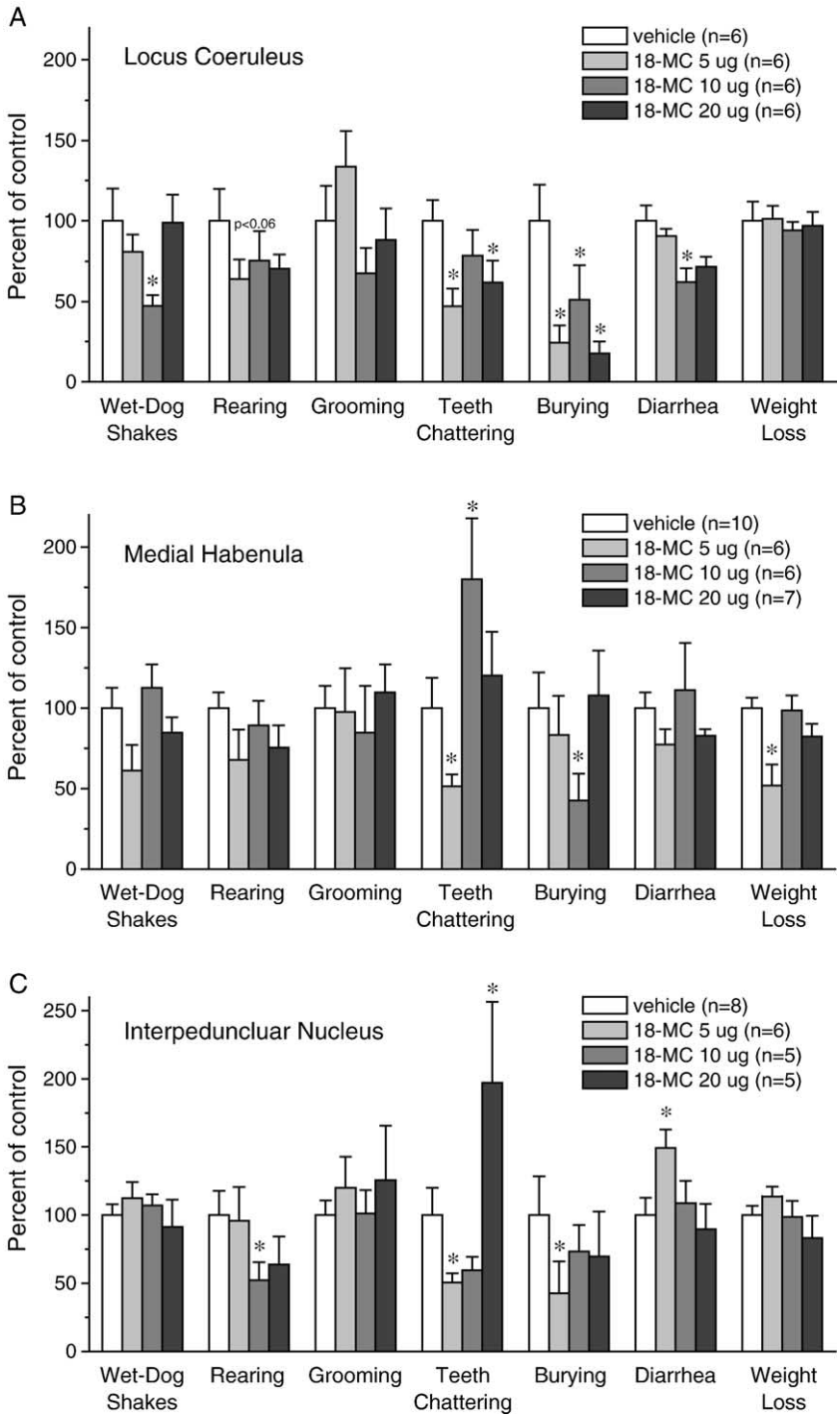


Fig. 3. Effects of 18-MC locally administered into the locus coeruleus (A), medial habenula (B) and interpeduncular nucleus (C) on morphine withdrawal signs. 18-MC (or vehicle) were administered immediately before naltrexone (1 mg/kg, i.p.), and withdrawal signs (percent of respective control±S.E.M.) were assessed for 2 h (*$P<0.05$, LSD test).

μg) increased teeth chattering. At 10 μg, 18-MC attenuated rearing.

## 4. Discussion

18-MC, a potent antagonist at $\alpha_3\beta_4$ nicotinic receptors, has previously been shown to reduce the expression of opioid withdrawal in rats (Rho and Glick, 1998); however, the sites of action of 18-MC in the brain were not previously identified. Systemic administration of 18-MC decreased weight loss, teeth chattering, burying, and diarrhea in a dose-dependent manner (Rho and Glick, 1998). The present study describes the effects of intracerebral administration of 18-MC on signs of naltrexone-precipitated opioid withdrawal in rats. We investigated the medial habenula, interpeduncular nucleus, and locus coeruleus in this study because these regions are enriched in

$\alpha_3\beta_4$ nicotinic receptors (Quick et al., 1999; Perry et al., 2002). Significant attenuation of four of seven tested signs of withdrawal was produced by 18-MC locally injected into the locus coeruleus. Similar pretreatment into the medial habenula or interpeduncular nucleus attenuated only three of seven signs of withdrawal.

Some comments about the microinjection technique should be noted. That is, although some diffusion inevitably occurred to neighboring brain areas, the diameter of the cannulae used was appropriate for the size of the studied brain structures. The same cannulae have previously been used in this laboratory for injections into the interpeduncular nucleus. In one study, 18-MC microinjected into the interpeduncular nucleus decreased morphine self-administration (Maisonneuve and Glick, 2003) while similar injections of 18-MC into the neighboring ventral tegmental area had no effect (unpublished data).

In the present study 5 μg and 20 μg of 18-MC administered into the locus coeruleus reduced the expression of teeth chattering, while at 10 μg, it reduced wet-dog shakes (Fig. 3A). Mediated by several brain areas (i.e., locus coeruleus, substantia nigra and hypothalamus), both teeth chattering and wet dog shakes are thought to be an adaptation of the body to an increase in set-point temperature in the central nervous system during withdrawal (Maldonado et al., 1992; Koob et al., 1992; Baumeister et al., 1992). The demonstrated effect of 18-MC on wet-dog shakes and teeth chattering could therefore be mediated either directly by locus coeruleus or indirectly via its connections with the hypothalamus (Almeida et al., 2004; Aston-Jones et al., 1991; Grzanna and Fritschy, 1991).

At 10 μg, 18-MC locally administered into the locus coeruleus attenuated diarrhea (Fig. 3A). Neurons originating in the locus coeruleus are known to be involved in the expression of diarrhea in physically dependent rats, presumably via an enhancement of noradrenergic output and an increase of corticotropin-releasing hormone levels in the brain mediating stress-induced colonic overactivity (Taylor et al., 1988; Kimes et al., 1990; Funada et al., 2001; Milanes et al., 1998; Tache et al., 2004). Therefore, the demonstrated effect of 18-MC on diarrhea was likely to be mediated directly by locus coeruleus neurons.

All doses of 18-MC locally administered into the locus coeruleus attenuated naltrexone-precipitated burying. While burying is thought to be a withdrawal-induced defensive response (Mucha, 1991), no specific brain sites have been linked to this behavior. It is unclear at the present time whether the attenuation of burying by 18-MC was mediated by the locus coeruleus itself or by its connections with other brain areas.

When administered into the medial habenula, 18-MC (5 μg and 10 μg) significantly attenuated and enhanced teeth chattering, respectively (Fig. 3B). The effect of 18-MC on teeth chattering could be directly mediated by the medial habenula or indirectly via the habenula's connections with other brain areas such as locus coeruleus and substantia nigra (Sutherland, 1982). Both locus coeruleus and substantia nigra are among the brain areas known to be responsible for the expression of teeth chattering (Maldonado et al., 1992; Baumeister et al., 1992). At the lowest dose (5 μg), 18-MC locally administered into the medial habenula attenuated weight loss; the two higher dosages had no effect. The attenuation of weight loss could be due to an indirect alteration of the hypothalamo-hypophyseal axis known to be activated during opioid withdrawal (Gonzalvez et al., 1994; Ignar and Kuhn, 1990; Milanes et al., 1998). At 10 μg, 18-MC locally administered into the medial habenula also attenuated burying; the neural basis for this interaction remains elusive at the present time.

18-MC, at 5 μg, administered into the interpeduncular nucleus, ameliorated teeth chattering and burying (Fig. 3C). The effect on teeth chattering could be mediated by connections of the interpeduncular nucleus with the mammilary nuclei which are known to participate in the regulation of body temperature (Smaha and Kaelber, 1973; Dean and Boulant, 1989). It is uncertain whether the interpeduncular nucleus itself is important for the regulation of body temperature during opioid withdrawal.

In summary, 18-MC was more effective in attenuating signs of morphine withdrawal when locally administered into the locus coeruleus than into the medial habenula and interpeduncular nucleus. However, all these effects of 18-MC were not as pronounced as they were when the drug was administered systemically (Rho and Glick, 1998). This disparity may be due to the fact that no single brain structure is responsible for the expression of all signs of morphine withdrawal (Koob et al., 1992; Maldonado et al., 1992). Other brain areas implicated in the modulation of the opioid withdrawal syndrome, e.g., the periaqueductal gray matter, nucleus raphe magnus, and anterior preoptic area, remain to be investigated. Although antagonism of $\alpha_3\beta_4$ nicotinic receptors by 18-MC in the locus coeruleus, medial habenula and interpeduncular nucleus is a possible mechanism for the described effects, there is no conclusive evidence of this; other mechanisms may also be involved (cf. Maisonneuve and Glick, 2003). Irrespective of the mechanisms involved, systemic 18-MC may be useful in ameliorating opioid withdrawal in humans.

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