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Role of δ-opioid receptors in mediating the aversive stimulus effects of morphine withdrawal in the rat

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Abstract

An unbiased place preference conditioning procedure was used to examine the role of δ-opioid receptors in mediating the aversive effects of opioid withdrawal. Rats were implanted s.c. with two pellets each containing placebo or 75 mg morphine. Single-trial conditioning sessions with saline and the opioid receptor antagonists naloxone (0.001–1.0 mg/kg, s.c.), naltrindole (0.01–3.0 mg/kg, s.c.) or naltriben (0.01–3.0 mg/kg, s.c.) commenced 4 days later. During these conditioning sessions, physical signs of withdrawal were also quantified. Tests of conditioning were conducted on day 5. Naloxone in doses of 0.01–1.0 mg/kg produced significant conditioned place aversions in morphine-implanted animals. A dose of 0.01 mg/kg produced few physical withdrawal signs whereas higher doses resulted in marked wet dog shakes, body weight loss, ptosis and diarrhea. No such effects were observed in control (placebo-implanted) animals. Administration of the selective δ-opioid receptor antagonists naltrindole and naltriben produced dose-related place aversions in morphine-implanted animals. The magnitude of these effects did not differ from that observed with naloxone. The minimum effective doses of naltrindole and naltriben were 0.1 mg/kg. Doses of 0.1–1.0 mg/kg produced few, if any, somatic signs of withdrawal whereas higher doses of these antagonists only produced diarrhea and wet-dog shakes. Other withdrawal signs were absent. In contrast to the opioid receptor antagonists tested, the dopamine D_1 receptor antagonist SCH23390 failed to produced conditioned place aversions or physical signs of withdrawal in morphine-pelleted animals. These data demonstrate that the selective blockade of either δ- or μ-opioid receptors is sufficient to induce conditioned aversive effects in morphine-dependent animals. They also indicate that physical symptoms associated with precipitated morphine withdrawal differ depending upon the opioid receptor antagonist employed.

Keywords: Naltrindole; Naltriben; δ-Opioid receptor; Opioid withdrawal

1. Introduction

In humans, withdrawal from chronic opioid use is associated with various physical symptoms as well as anxiety and dysphoria (Wikler et al., 1953; Henningfield et al., 1987). The avoidance of these aversive effects is thought to play an important role in the maintenance of opioid addiction. Several studies have also shown that the presentation of stimuli previously paired with the precipitation of opioid withdrawal can elicit physical as well as affective signs of withdrawal (Mucha, 1987; Higgins et al., 1991). An increasing body of evidence has suggested that such conditioned effects of opioid withdrawal may contribute, at least in part, to drug craving and the reinstatement of compulsive drug seeking behavior (Wikler et al., 1953; Lipkowitz et al., 1971; Henningfield et al., 1987).

One technique which can be used to characterize the conditioned reinforcing effects of drugs in experimental animals is that of place preference conditioning. Using this technique it has been shown that animals made physically dependent upon morphine exhibit a dose-related aversion for an environment previously associated with the administration of the opioid antagonists naloxone or naltrexone (Mucha, 1987; Higgins et al., 1991; Higgins and Sellers, 1994; Schulteis et al., 1994). This response, which occurs after only a single exposure to the antagonist, is not

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observed in opioid-naive animals. Such findings demonstrate that opioid withdrawal produces aversive states and that this effect, as observed in human subjects, can be conditioned to stimuli which previously signaled antagonist administration.

Fundamental questions however, remain as to whether such conditioned effects of opioid antagonists reflect the development of physical dependence or may, in fact, reflect changes in affective state which occur independently of quantifiable physical withdrawal signs. Development of an animal model which would permit assessment of withdrawal-induced changes in affect is of importance since recent clinical studies indicate that the affective component of opioid abstinence may be more relevant to drug craving and, relapse to compulsive drug use than somatic signs of withdrawal (Henningfield et al., 1987). Furthermore, although the existence of several opioid receptor types (μ, δ and κ) within the central nervous system is well-documented (Martin et al., 1976; Lord et al., 1977; Zukin and Zukin, 1984), naloxone binds with only slightly higher affinity to μ- as compared to δ- or κ-opioid receptors (James and Goldstein, 1984). Therefore, it remains unclear as to whether the aversive effects observed in response to this antagonist result from an interaction with μ- and/or other opioid receptor types. In this regard, a recent study has suggested an involvement of δ-opioid receptors in mediating the physical dependence which develops to morphine (Miyamoto et al., 1993). Thus, Miyamoto et al. (1993) reported that the blockade of δ-opioid receptors both prior to and throughout the duration of morphine treatment can attenuate physical signs of morphine withdrawal (e.g., jumping) produced by naloxone challenge. Furthermore, when the δ-opioid receptor antagonist naltriben was administered after a chronic morphine treatment regimen somatic signs of withdrawal were seen (Miyamoto et al., 1993). The role of this receptor type in mediating the conditioned aversive effects of opioid withdrawal has not, however, been investigated.

Recently, two selective non-peptide δ-opioid receptor antagonists, naltrindole and naltriben have been synthesized (Portoghese et al., 1988, Portoghese et al., 1991). By using these agents, it is possible to determine whether the selective blockade of δ-opioid receptors can induce conditioned aversive effects and/or physical signs of withdrawal in animals continuously exposed to morphine. Accordingly, in the present study, the place conditioning produced by the selective δ-opioid receptor antagonists, naltrindole and naltriben, were compared to that produced by the prototypic opioid receptor antagonist naloxone in rats implanted s.c. with pellets containing morphine or placebo. Physical signs of withdrawal were also quantified. The place conditioning produced by the dopamine D_1 receptor antagonist SCH23390 was also examined in both controls and morphine-pelleted animals to determine the specificity of the opioid receptor antagonist-induced effects.

2. Materials and methods

2.1. Animals

Male Sprague-Dawley rats (Charles River, Wilmington, MA, USA) weighing 250–300 g were housed three per cage in a temperature-controlled animal colony. The rats were maintained on a 12-h light-dark cycle (lights on: 7:00 a.m. to 7:00 p.m.) with laboratory rat chow and water available ad libitum. Animals were maintained in facilities accredited by the American Association for the Accreditation of Laboratory Animal Care and all experiments were conducted in accordance with the guidelines of the Institutional Care and Use Committee of the Division of Intramural Research/National Institute on Drug Abuse/National Institute of Health.

2.2. Morphine pellet implantation

Rats were implanted s.c. with two pellets containing morphine (75 mg base) under halothane anesthesia. This procedure has been shown to result in a naloxone-precipitated physical withdrawal syndrome within 72 h after pellet implantation (Yoburn et al., 1985). Control rats were implanted s.c. with two pellets containing placebo.

2.3. Place conditioning procedure

The apparatus consisted of $30 \times 60 \times 30$ cm wooden shuttle boxes with a clear Plexiglas front. For conditioning sessions, each box was divided into two equal-sized compartments by means of a sliding wall. One compartment was white with a textured floor, the other black with a smooth floor. For testing, the central wall was raised 12 cm above the floor, and a 5×2 cm 'neutral' steel mesh platform was inserted along the seam, separating the two compartments. Conditioning was conducted as previously described using an unbiased procedure (Shippenberg et al., 1993). Place conditioning commenced 4 days after pellet implantation. A total of two conditioning sessions (1 drug: 1 vehicle) were conducted with 6–8 h separating each. Conditioning drugs were the opioid receptor antagonist naloxone (0.001–1.0 mg/kg, s.c.), the δ-opioid receptor antagonists naltrindole (0.01–3.0 mg/kg, s.c.), naltriben (0.01–3.0 mg/kg, s.c.) and the dopamine D_1 receptor antagonist SCH23390 (0.01 mg/kg, s.c.; Iorio et al., 1983). For conditioning, rats were immediately confined to one compartment of the shuttle box after drug injection and to the other compartment after vehicle injection. Treatment compartment and the order of presentation of the drug and vehicle were counterbalanced for each drug dose. All conditioning sessions were 30 min in duration. Test sessions were carried out in pelleted animals one day after the last conditioning session. Uninjected rats were placed on the 'neutral' platform of the testbox and allowed free access to both sides of the shuttle box for 15 min. A

Panasonic WV-BL600 videocamera with integrated stopwatch was used for data recording. The time spent in a particular place (drug, vehicle or 'neutral' platform) was assessed by visual analysis of the recorded videotape in blind fashion.

2.4. Assessment of morphine withdrawal signs

Withdrawal signs were assessed, as previously described (Gellert and Holtzman, 1978; Funada et al., 1993), by visual inspection of video-recorded conditioning sessions and by direct observation of the rats in their home cages for 30 min following each session. The number of wet dog shakes was counted for 30 min after antagonist injection. The number of rats expressing ptosis, vocalization, teeth chattering, salivation and diarrhea within 60 min after antagonist injection was also recorded. Body weight was measured 30 and 60 min after antagonist injection.

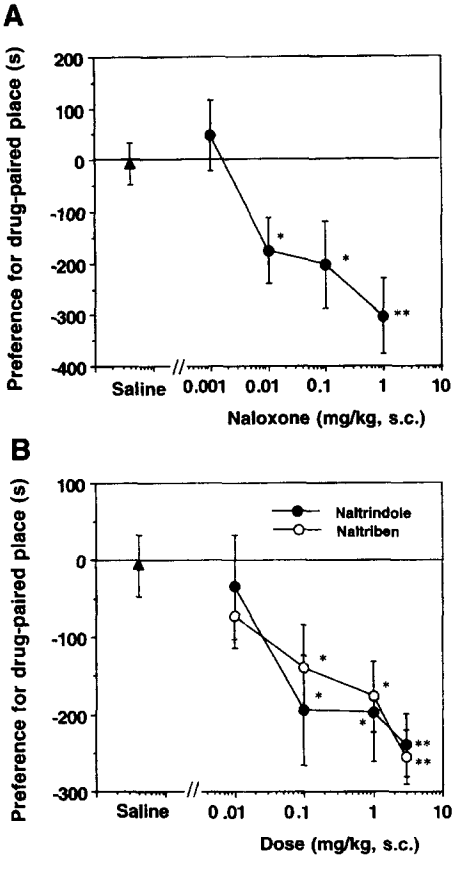

Fig. 1. Place conditioning produced by opioid receptor antagonists in morphine-dependent rats. Ordinate: mean difference (s) between the time spent in the drug- and saline-paired sides of the test box. (A) Place conditioning produced by naloxone (0.001–1.0 mg/kg, s.c.) in morphine-dependent rats. (B) Place conditioning produced by the δ-opioid receptor antagonists, naltrindole (0.01–3.0 mg/kg, s.c.) and naltriben (0.01–3.0 mg/kg, s.c.), in morphine-dependent rats. Each point represents the mean conditioning score ± S.E.M. of 7–12 animals. Morphine-implanted rats that received saline during each of the conditioning sessions exhibited no preference for either compartment of the test box; the mean conditioning score was −6.44 ± 37.9 s ($n = 9$). * $P < 0.05$, ** $P < 0.01$ vs. saline control.

2.5. Statistical analysis

Conditioning scores represent the time spent in the drug-paired place minus the time spent in the vehicle-paired place, and are expressed as means ± S.E.M. A negative conditioning score indicates that the time spent in the drug-paired place is less than that spent in the vehicle-paired place. Dose-response curves were analyzed using a one-way analysis of variance. The Dunnett's multiple comparison test was used to determine whether individual doses of each antagonist produced a conditioning score which was significantly different from that of pelleted-animals conditioned with saline. Changes in weight loss were analyzed using a repeated measures analysis of variance. The number and incidence of withdrawal signs were statistically evaluated using the Dunnett's multiple comparison test and the Fisher's probability test, respectively.

2.6. Drugs

The drugs used in the present study were naloxone hydrochloride (Research Biochemicals, Wayland, MA, USA), naltrindole hydrochloride (National Institute on Drug Abuse), naltriben hydrochloride (National Institute on Drug Abuse) and SCH23390 hydrochloride (*R*-3-methyl-7-chloro-8-hydroxy-1-phenyl-2,3,4,5,-tetrahydro-1*H*-3-benzazepine, Research Biochemicals). These drugs (salt) were dissolved in sterile 0.9% NaCl solution and administered s.c. Morphine (75 mg base) and placebo pellets were provided by the National Institute on Drug Abuse.

3. Results

3.1. Dose-response curves for place conditioning produced by naloxone in morphine-dependent rats

Morphine-implanted rats which received saline during each of the conditioning sessions exhibited no preference for either of the place cues. The mean time spent in the white and black compartments was 358 ± 31 and 365 ± 32 s ($n = 9$), respectively. In placebo pellet-implanted rats, naloxone (1.0 mg/kg) was ineffective as a conditioning stimulus (−51.6 ± 59.8 s, $n = 8$). Naloxone caused a dose-related ($F(4,41) = 5.07$; $P < 0.01$) aversion for the drug-associated place in morphine-implanted animals (Fig. 1A). The minimum dose of naloxone producing significant place aversions was 0.01 mg/kg (−176.2 ± 60.7 s, $F(1,19) = 4.77$; $P < 0.05$). At this dose of naloxone, 94.0% of the animals exhibited an aversion for the antagonist-associated place (e.g., a negative conditioning score).

3.2. Dose-response curves for place conditioning produced by δ-opioid receptor antagonists in morphine-dependent rats

In placebo-implanted rats, naltrindole (3.0 mg/kg) and naltriben (3.0 mg/kg) failed to produce significant place conditioning. The mean conditioning scores were -2.3 ± 36.0 s (naltrindole, $n = 6$) and -15.1 ± 59.2 s (naltriben, $n = 8$), respectively. The place conditioning produced by naltrindole or naltriben in morphine-dependent rats is shown in Fig. 1B. Naltrindole caused a dose-related ($F(4,45) = 3.36$; $P < 0.05$) aversion for the drug-associated place. The minimum effective dose of naltrindole was 0.1 mg/kg (-194.6 ± 68.0 s, $n = 11$, $F(1,18) = 5.15$; $P < 0.05$). At this dose, 94.0% of the animals exhibited an aversion for the drug-associated place. Similarly, administration of naltriben caused a dose-related ($F(3,36) = 5.35$; $P < 0.01$) aversion for the drug-associated place. The minimum effective dose was 0.1 mg/kg (-140.8 ± 53.2 s, $n = 11$, $F(1,18) = 5.12$; $P < 0.05$). At this dose, 90.0% of the animals exhibited an aversion for the drug-associated place.

3.3. Place conditioning produced by the dopamine D_1 receptor antagonist SCH23390 in morphine-dependent rats

The place conditioning produced by SCH23390 (0.01 mg/kg, s.c.) is shown in Table 1. In placebo-implanted rats, SCH23390 (0.01 mg/kg) failed to produce significant place conditioning. Similarly, SCH23390 was ineffective as a conditioning stimulus in morphine-pelleted animals. The mean conditioning scores were -23.1 ± 31.5 s (placebo-implanted animals, $n = 10$) and -28.0 ± 39.7 s (morphine-implanted animals, $n = 10$). Neither morphine- nor placebo-implanted animals exhibited any quantifiable physical signs of withdrawal following SCH23390 administration.

3.4. Naloxone-precipitated withdrawal signs in morphine-dependent rats

In morphine-implanted rats, administration of naloxone produced a dose-related incidence of diarrhea, salivation, vocalization, teeth chattering and ptosis (Table 2). As shown in Fig. 2, the amount of wet-dog shakes was significantly greater in morphine-implanted animals which received naloxone as compared to those conditioned with saline (0.1 mg/kg vs. saline, $F(1,15) = 14.57$; $P < 0.05$, 1.0 mg/kg vs. saline, $F(1,18) = 48.01$; $P < 0.01$). Administration of naloxone (0.1–1.0 mg/kg) also resulted in body weight loss and the magnitude of this effect was dose-related (Fig. 3, $F(4,27) = 34.61$; $P < 0.01$). This effect was significant both 30 min (0.1 mg/kg vs. saline, $F(1,20) = 6.83$; $P < 0.05$, 1.0 mg/kg vs. saline, $F(1,18) = 46.1$; $P < 0.01$) and 60 min (0.1 mg/kg vs. saline, $F(1,20) = 11.32$; $P < 0.01$; 1.0 mg/kg vs. saline, $F(1,18) = 72.52$; $P < 0.01$) after naloxone administration. Placebo-implanted animals failed to exhibit any physical signs of withdrawal following administration of 1.0 mg/kg naloxone.

Table 1
Place conditioning produced by SCH23390 in morphine-implanted rats

Pellet implantation	Time (s)		Conditioning score (mean ± S.E.M.)
	Drug-paired place	Saline-paired place	
Placebo	280.9 ± 30.1	304 ± 27.1	−23.1 ± 31.5
Morphine	304.1 ± 29.1	332.1 ± 43.1	−28.0 ± 39.7

Rats were implanted s.c. with two placebo or two 75 mg morphine pellets. Conditioning sessions with SCH23390 (0.01 mg/kg, s.c.) and saline (1.0 ml/kg, s.c.) were conducted on 4 days later. Each group represents the mean ± S.E.M. of 10 animals.

Table 2
Expression of somatic withdrawal signs after administration of naloxone in morphine-dependent rats

Withdrawal sign	Saline	Naloxone (mg/kg, s.c.)			
		0.001	0.01	0.1	1.0
Diarrhea	0	0	8.3	85.7 [a]	100 [a]
Salivation	0	0	0	42.9	70 [a]
Vocalization	10	10	27.3	100 [a]	100 [a]
Teeth chattering	0	0	8.3	57 [a]	100 [a]
Ptosis	0	12.5	16.7	100 [a]	100 [a]

Rats were implanted s.c. with two 75 mg morphine pellets. Conditioning sessions with the opioid receptor antagonist naloxone (0.001–1.0 mg/kg, s.c.) and saline (1.0 ml/kg, s.c.) were conducted on 4 days later. Physical signs of withdrawal were quantified for 60 min. Data represent the percentages of animals exhibiting each of the withdrawal signs. [a] $P < 0.05$ vs. saline-treated group.

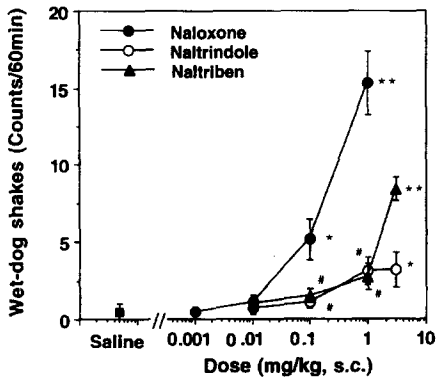

Fig. 2. The amount of opioid antagonist precipitated 'wet-dog' shakes in morphine-dependent rats. Withdrawal 'wet-dog' shakes was observed for 60 min after naloxone (0.001–1.0 mg/kg, s.c.), naltrindole (0.01–3.0 mg/kg, s.c.) or naltriben (0.01–3.0 mg/kg, s.c.) challenge in morphine-dependent rats. Each point represents the mean number of 'wet-dog' shakes ± S.E.M. of 7–12 animals. Saline administration resulted in 0.5 ± 0.5 counts/60 min ($n = 10$). * $P < 0.05$, ** $P < 0.01$ vs. saline control. # $P < 0.05$ vs. naloxone-treated groups.

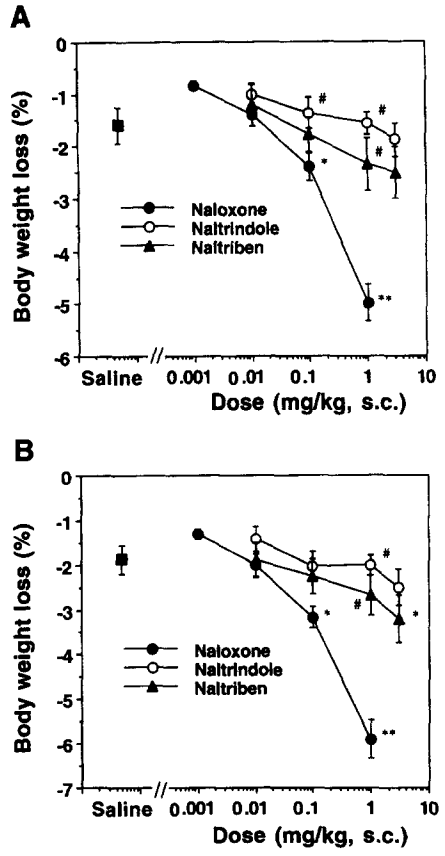

Fig. 3. Effect of opioid receptor antagonists on body weight in morphine-dependent rats. Body weight loss 30 min (A) and 60 min (B) after naloxone (0.001–1.0 mg/kg, s.c.), naltrindole (0.01–3.0 mg/kg, s.c.) or naltriben (0.01–3.0 mg/kg, s.c.) challenge. Each point represents mean body weight loss ± S.E.M. (%) of 7–12 animals. Administration of saline to morphine-dependent rats ($n = 10$) resulted in $-1.59 \pm 0.35\%$ (30 min) and $-1.88 \pm 0.33\%$ (60 min) body weight loss. * $P < 0.05$, ** $P < 0.01$ vs. saline control. # $P < 0.05$ vs. naloxone-treated groups.

3.5. δ-Opioid receptor antagonist-precipitated withdrawal signs in morphine-dependent rats

The incidence of withdrawal signs produced by the δ-opioid receptor antagonists, naltrindole and naltriben, are summarized in Tables 3 and 4, respectively. Naltrindole (0.01–3.0 mg/kg) failed to produce significant diarrhea, salivation, vocalization, teeth chattering and ptosis in morphine-implanted rats (Table 3). As shown in Fig. 2, the amounts of wet-dog shakes in morphine-implanted animals receiving either 0.1 or 1.0 mg/kg naltrindole did not differ from that of animals which had been conditioned with saline. Following administration of the 3.0 mg/kg dose of naltrindole, a significant incidence of wet-dog shakes was, however, observed (naltrindole vs. saline, $F(1,19) = 4.71$; $P < 0.05$). As shown in Fig. 3A,B, administration of naltrindole (0.01–3.0 mg/kg) did not induce significant body weight loss. Thus, body weight loss did not differ from rats which had received saline. As shown in Table 4, naltriben (0.01–1.0 mg/kg) did not produce diarrhea, salivation, vocalization, teeth chattering or ptosis in morphine-implanted rats. Administration of the 3.0 mg/kg dose, however, resulted in significant diarrhea, vocalization and ptosis, as compared to that seen in the saline-treated group. As shown in Fig. 2, naltriben (1.0 mg/kg) treatment slightly increased the incidence of wet-dog shakes (2.73 ± 0.82 counts/60 min, $n = 11$), but this effect was not statistically significant. A dose of 3 mg/kg naltriben resulted in 8.4 ± 0.75 wet-dog shakes/60 min ($n = 10$), and the incidence of this behavior was significantly higher than that in the saline-treated group (naltriben vs. saline: $F(1,18) = 77.0$; $P < 0.01$). As shown in Fig. 3B, administration of naltriben (3.0 mg/kg) also resulted in significant body weight loss as compared to saline-treated rats (naltriben vs. saline, $F(1,18) = 5.87$; $P < 0.05$). Neither naltrindole (3.0 mg/kg, s.c.) nor naltriben (3.0 mg/kg, s.c.) administration produced any of the above mentioned withdrawal signs in placebo-implanted rats.

Table 3
Expression of somatic withdrawal signs after administration of naltrindole in morphine-dependent rats

Withdrawal sign	Saline	Naltrindole (mg/kg, s.c.)			
		0.01	0.1	1.0	3.0
Diarrhea	0	0	0	0	9.1
Salivation	0	0	0	0	0
Vocalization	10	12.5	9.1	27.3	27.3
Teeth chattering	0	0	18.2	18.2	18.2
Ptosis	0	0	0	0	18.2

Rats were implanted s.c. with two 75 mg morphine pellets. Conditioning sessions with the opioid receptor antagonist naltrindole (0.01–3.0 mg/kg, s.c.) and saline (1.0 ml/kg, s.c.) were conducted on 4 days later. Physical signs of withdrawal were quantified for 60 min. Data represent the percentages of animals exhibiting each of the withdrawal signs.

Table 4
Expression of somatic withdrawal signs after administration of naltriben in morphine-dependent rats

Withdrawal sign	Saline	Naltriben (mg/kg, s.c.)			
		0.01	0.1	1.0	3.0
Diarrhea	0	0	0	0	90 [a]
Salivation	0	0	0	0	0
Vocalization	10	9.1	9.1	40	80
Teeth chattering	0	0	18.2	20	40
Ptosis	0	9.1	18.2	30	20

Rats were implanted s.c. with two 75 mg morphine pellets. Conditioning sessions with the opioid receptor antagonist naltriben (0.01–3.0 mg/kg, s.c.) and saline (1.0 ml/kg, s.c.) were conducted on 4 days later. Physical signs of withdrawal were quantified for 60 min. Data represent the percentages of animals exhibiting each of the withdrawal signs. [a] $P < 0.05$ vs. saline-treated group.

4. Discussion

The present study demonstrates that the administration of either naloxone or the selective δ-opioid receptor antag-

onists naltrindole and naltriben produce conditioned place aversions in animals continuously exposed to a high dose of morphine and that these effects occur in the presence of few physical withdrawal signs. Higher doses of these antagonists produced marked physical signs of withdrawal. However, both the magnitude and incidence of the withdrawal syndrome differed depending upon the opioid receptor antagonist employed.

Animals implanted with morphine pellets 4 days prior to the commencement of place conditioning sessions exhibited marked aversions for an environment previously associated with the administration of naloxone. This effect was observed in response to doses of 0.01–1.0 mg/kg naloxone. In contrast, naloxone failed to produce significant conditioning in animals implanted with placebo pellets. Such findings confirm those of previous studies (Mucha, 1987; Higgins et al., 1991; Kosten, 1994) and demonstrate that acute opioid receptor blockade produces aversive effects in animals chronically exposed to morphine. Furthermore, they confirm that these effects can be conditioned to environmental stimuli which previously signaled antagonist administration (Mucha, 1987).

Administration of naloxone to morphine-implanted animals also resulted in various physical signs of withdrawal. Thus, doses of 0.1 and 1.0 mg/kg produced marked diarrhea and body weight loss as well as ptosis, wet-dog shakes and vocalization upon handling. In contrast, none of these behaviors were observed in placebo-implanted animals. Such findings confirm that the morphine treatment regimen employed is sufficient to induce physical dependence (Gold et al., 1994; Schulteis et al., 1994). Furthermore, the present findings and others (Funada et al., 1993; Higgins and Sellers, 1994; Schulteis et al., 1994) indicate that the magnitude and incidence of naloxone-precipitated withdrawal vary depending upon the dose of naloxone administered. Indeed, a recent study has shown that somatic signs of withdrawal, such as a diarrhea, body weight loss, ptosis and wet-dog shakes, require doses of naloxone of 0.03 mg/kg or higher for their expression (Schulteis et al., 1994). As shown in the present study, low doses of naloxone precipitated few somatic signs of withdrawal. Thus, in animals receiving 0.001 and 0.01 mg/kg naloxone, somatic signs of withdrawal evaluated in this study did not differ from that of animals which received saline injections. In this regard, it is important to note that the 0.01 mg/kg dose was, however, effective in producing conditioned aversive effects. As such, these data add to growing body of evidence which indicate that the conditioning of the aversive effects of naloxone-precipitated withdrawal can occur in the presence of few physical withdrawal signs (Higgins and Sellers, 1994; Schulteis et al., 1994). Furthermore, the findings that the administration of the highly selective μ-opioid receptor antagonist Cys^2,Tyr^3,Orn^5,Pen^7-amide (CTOP) (Pelton et al., 1986) is also effective in producing physical signs of withdrawal (Baumeister et al., 1992) as well as conditioned place aversions (Funada, M., unpublished observation), strongly suggest that the blockade of μ-opioid receptors is sufficient for the expression of both the conditioned and unconditioned effects of morphine withdrawal.

The δ-opioid receptor antagonists naltrindole and naltriben produced dose-related place aversions in morphine-dependent rats. The minimum effective dose for both antagonists was 0.1 mg/kg. At this dose, the magnitude of place conditioning did not differ from that observed in response to naloxone. Under the experimental conditions employed, the effects of naloxone, naltrindole and naltriben on place conditioning were specific to morphine-implanted rats. Thus, whereas opioid antagonists such as naloxone produce significant place aversions in naive animals after several drug conditioning sessions (Mucha and Iversen, 1984; Vaccarino et al., 1992; Schulteis et al., 1994), none of the antagonists tested (Mucha, 1987; Higgins et al., 1991; Kosten, 1994) induced significant conditioning in naive or placebo-implanted rats after a single drug trial.

Like naloxone, administration of the dopamine D_1 receptor antagonist SCH23390 produces conditioned place aversions in previously drug-naive rats (Shippenberg and Herz, 1988). Typically, however, a minimum of three conditioning sessions is necessary for the establishment of this effect. In the present study, SCH23390 was ineffective as a conditioning stimulus in either control or morphine-pelleted animals after only one conditioning session. Thus, the conditioning score observed in response to SCH23390 did not differ from that produced by saline. Such findings are noteworthy in that they suggest that under the conditions used in the present study (e.g., single-trial conditioning), the aversive effects of opioid receptor antagonists appear to be selective. That is, a drug of a different pharmacological class is ineffective in producing place aversions in either morphine-treated or naive animals when a single trial place preference conditioning procedure is employed.

Both naltrindole and naltriben bind with high affinity to δ-opioid receptors and are presumed to be selective antagonists for this receptor type. Indeed, in vitro studies have shown that ligands selective for μ- or κ-opioid receptors only weakly inhibit the binding of naltrindole and naltriben to synaptosomal preparations from rodent brain (Contreras et al., 1993; Xu et al., 1993). Evidence that naltrindole in doses of 0.5–1.0 mg/kg selectivity antagonizes the antinociceptive effects of δ- but not μ-opioid receptor agonists has also been presented (Jackson and Kitchen, 1989; Kitchen and Pinker, 1990). In view of such findings, it is likely that the conditioned place aversions observed in response to the 0.1 and 1.0 mg/kg doses of naltrindole result from the selective blockade of δ-opioid receptors. As such, the results of the present conditioning studies suggest that the blockade of δ-opioid receptors can produce conditioned aversive effects in morphine-implanted animals. Furthermore, the findings that conditioned place

aversions can also be observed in response to the selective μ-opioid receptor antagonist CTOP (Funada, M., unpublished observation), indicating that the activation of either μ- and δ-opioid receptors is sufficient for the expression of this effect.

Administration of the 3.0 mg/kg doses of both naltrindole and naltriben resulted in conditioned place aversions as well as physical signs of withdrawal. Whether or not, however, these effects can be attributed to the selective blockade of δ-opioid receptors remains unclear. Thus, biphasic effects of naltrindole have been noted in several behavioral tests (Heidbreder et al., 1993; Shippenberg and Heidbreder, 1995). Furthermore, a recent study has shown that a dose of 2.0 mg/kg naltrindole prevents the stimulation of corticosterone release produced by the μ-opioid receptor agonist fentanyl (Kitchen and Kennedy, 1990). Therefore, the possibility arises that the effects of higher doses of both naltrindole and naltriben may result from interactions with δ- as well as μ-opioid receptor types. As such the physical withdrawal signs which were only observed in response to the 3.0 mg/kg doses of naltrindole and naltriben may reflect an antagonism of μ-opioid receptors. Indeed, evidence indicating a critical role for central μ-opioid receptors in the expression of wet-dog shakes has recently been presented (Maldonado et al., 1992).

A recent study showed that the systemic administration of naltriben can precipitate jumping and diarrhea in mice chronically treated with morphine (Miyamoto et al., 1993). Although such findings may indicate an involvement of δ-opioid receptors in the expression of morphine-induced physical dependence, the doses of naltriben producing this effect were those which attenuated the analgesic effects of μ- as well as δ-opioid receptor agonists (Miyamoto et al., 1993). Similarly, in the present study, somatic signs of withdrawal (e.g., diarrhea and wet-dog shakes) were only observed in response to a dose of naltrindole which was greater than that previously shown to antagonize the effects of the μ-opioid receptor agonist fentanyl (Kitchen and Kennedy, 1990). Therefore, it is likely that those physical withdrawal signs observed in response to both δ-opioid receptor antagonists may, in fact, reflect an interaction of these agents with multiple opioid receptor types. Interestingly, the administration of the non-equilibrium δ-opioid receptor antagonist naltrindole 5′-isothiocyanate to mice both prior to and during the course of chronic morphine treatment can attenuate physical withdrawal signs produced by naloxone (Miyamoto et al., 1993) suggesting a possible involvement of δ-opioid receptors in the development of morphine-induced physical dependence.

In summary, the present study demonstrates that selective δ-opioid receptor antagonists produce place aversions in rats chronically exposed to morphine and that these effects can occur in the presence of few, if any, physical signs of withdrawal. As such, an involvement of δ- as well as μ-opioid receptors in the expression of morphine dependence is suggested. It is further suggested that whereas μ- and δ-opioid receptors may play an important role in mediating those changes in affect which occur in response to withdrawal from morphine, alterations in μ-opioid receptor function may be critical for both the development and expression of morphine-induced physical dependence.

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