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Involvement of δ_1 and δ_2 Opioid Receptor Subtypes in the Development of Physical Dependence on Morphine in Mice

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SUZUKI, T., M. TSUJI, T. MORI, M. MISAWA AND H. NAGASE. *Involvement of δ_1 and δ_2 opioid receptor subtypes in the development of physical dependence on morphine in mice.* PHARMACOL BIOCHEM BEHAV **57**(1/2) 293–299, 1997.—The effects of the highly selective δ opioid receptor antagonists naltrindole (NTI) for δ_1 and δ_2 naltriben (NTB) and naltrindole 5′-isothiocyanate (5′-NTII) for δ_2 and 7-benzylidenenaltrexone (BNTX) for δ_1 on the development of physical dependence on morphine were investigated in mice. Neither NTI (3 mg/kg, sc), NTB (0.5 mg/kg, sc), 5′-NTII (0.5 mg/kg, sc) nor BNTX (0.5 mg/kg, sc) suppressed the antinociception induced by morphine (10 mg/kg, sc). Pretreatment with NTI (3 mg/kg, sc), NTB (0.5, 1.0 mg/kg, sc) or 5′-NTII (0.5, 1.0 mg/kg, sc) during chronic treatment with morphine for 5 days significantly suppressed naloxone-induced body-weight loss in morphine-dependent mice. The incidence of jumping and body shakes in morphine-dependent mice that were pretreated with NTI, NTB or 5′-NTII were significantly lower than with morphine alone. Pretreatment with BNTX (0.5, 1.0 mg/kg, sc) during chronic treatment with morphine also significantly suppressed naloxone-induced body-weight loss in morphine-dependent mice, but this suppression was weaker than that by the antagonists. In contrast to mice that had been pretreated with NTI, NTB or 5′-NTII, the incidence of several withdrawal signs, such as jumping and body shakes, was not significantly affected in morphine-dependent mice that were pretreated with BNTX. These findings suggest that both δ_2 and δ_1 opioid receptors may play important roles in modulating the development of physical dependence on morphine. © 1997 Elsevier Science Inc.

δ Opioid receptors Morphine Physical dependence NTI BNTX NTB

IT is well known that various types of opioid receptors are involved in the development of physical dependence on morphine. For example μ opioid receptors play a particularly important role in the development of physical dependence on morphine (3,5,7). However, morphine also interacts with both δ and κ opioid receptors *in vivo* and *in vitro* (44), and δ and κ opioid receptors are reported involved in the development of tolerance to and dependence on morphine (1,17, 18,38,50). For example, Abdelhamid et al. (1) recently demonstrated that selective blockade of δ opioid receptors inhibits the development of physical dependence on morphine. More recently, it has been shown that there are multiple δ opioid receptor subtypes, i.e., δ_1 and δ_2 opioid receptors (12,16,34). In addition, highly selective δ opioid receptor antagonists, such as naltriben (NTB) and naltrindole 5′-isothiocyanate (5′-NTII) for δ_2 opioid receptors and 7-benzylidenenaltrexone (BNTX) for δ_1 opioid receptors, have recently been synthesized (25–29). Miyamoto et al. (17) demonstrated that δ_2 opioid receptors are involved in the development of acute physical dependence on morphine, and that continuous blockade, rather than intermittent blockade, of δ_2 opioid receptors is necessary to inhibit the development of physical dependence on morphine. In addition, they also demonstrated that δ_1, as opposed to δ_2, opioid receptors are not involved in the development of physical dependence on morphine, since signs of opiate withdrawal were not affected by chronic treatment of animals with [D-Ala^2, Leu^5, Cys^6]enkephalin during implantation of a morphine pellet (19). However, jumping and diarrhea were the only naloxone-precipitated withdrawal signs they studied; other withdrawal signs, such as body-weight loss, body

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shakes, ptosis, forepaw tremor and rearing, were not investigated.

In the present study, we investigated the roles of the δ opioid receptor subtypes in the development of physical dependence on morphine using highly selective δ opioid receptor antagonists and seven naloxone-precipitated withdrawal signs.

METHODS

Animals

Male ddY mice (25–30 g) were obtained from Tokyo Animal Laboratories Inc. (Tokyo, Japan). The animals were housed at a room temperature of 22 ± 1°C with a 12 h light-dark cycle (light on 0800-2000). Food and water were available ad lib.

Antinociceptive Assay

The antinociceptive test in the present study used a 51°C warm-plate as the nociceptive stimulus with the latency to paw-tap, paw-lick or an attempt to escape by jumping taken as the endpoint. Control latencies were determined prior to drug administration. To prevent tissue damage, mice that showed no response within 60 s (cut-off time) were removed from the warm-plate. The percent of antinociception was calculated as % antinociception = 100 × (test latency–control latency)/(60 s–control latency). Morphine was given sc in a dose of 10 mg/kg. Antinociception was tested 30 min (peak time) after sc morphine treatment. NTI (3 mg/kg, sc), NTB (1.0 mg/kg, sc), BNTX (1.0 mg/kg, sc) and 5′-NTII (1.0 mg/kg, sc) were injected 30 min, 30 min, 20 min and 24 h prior to the morphine injection, respectively, according to previous reports (17,22,39).

Chronic Morphine Treatment

Morphine was injected sc daily at 0900 and 1900. According to the schedule described by Maldonado et al. (15), the morphine dose was increased progressively from 8 to 45 mg/kg over a period of 5 days, i.e., 1st day (8, 15 mg/kg at 0900 and 1900, respectively), 2nd day (20, 25), 3rd day (30, 35), 4th day (40, 45) and 5th day (45 mg/kg at 0900 only).

Chronic Treatment with δ Opioid Receptor Antagonists

NTI (3 mg/kg, sc) and NTB (0.5, 1 mg/kg, sc) were injected chronically 30 min before morphine injection. BNTX (0.5, 1 mg/kg, sc) was injected chronically 20 min before morphine injection. Injection of 5′-NTII (0.5, 1 mg/kg, sc) was begun 24 h prior to beginning chronic morphine treatment. Thereafter, 5′-NTII was injected at 900 during chronic morphine treatment.

Morphine Withdrawal

Withdrawal signs were precipitated by injecting naloxone (3 mg/kg, sc) 2 h after the final morphine administration. After the naloxone challenge, mice were immediately placed on a circular platform (30 cm diameter × 70 cm height). Naloxone-precipitated withdrawal signs were recorded for 60 min according to our previous reports (36–38). Body weight was measured initially and at 15, 30, 45 and 60 min after the naloxone injection.

Drugs

The drugs used in the present study were morphine hydrochloride (Sankyo Co., Tokyo, Japan), naltrindole hydrochloride (NTI), naltriben methanesulfonate hydrate (NTB), 7-benzylidenenaltrexone methanesulfonate hydrate (BNTX), naltrindole 5′-isothiocyanate methanesulfonate hydrate (5′-NTII) and naloxone hydrochloride (Research Biochemicals Inc., Wayland, MA, U.S.A.). NTI, NTB, BNTX and 5′-NTII were synthesized by us. All doses refer to the salt forms of the drugs, and all drugs were dissolved in 0.9% NaCl.

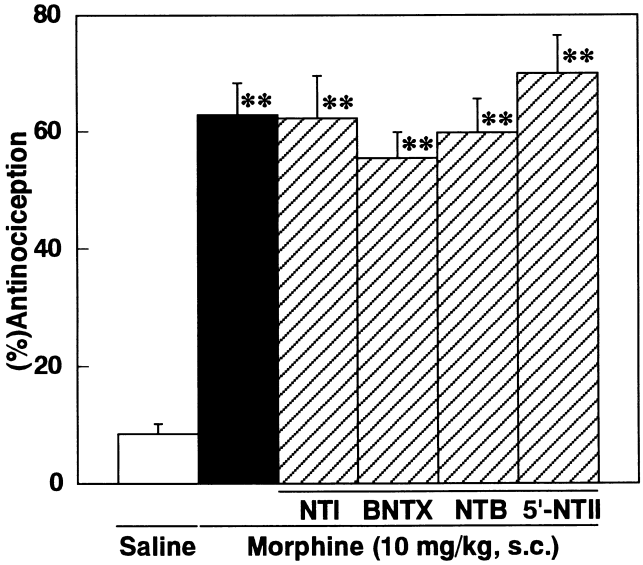

FIG. 1. Effect of pretreatment with NTI, NTB, 5′-NTII or BNTX on morphine-induced antinociception. NTI (3 mg/kg, sc), NTB (1 mg/kg, sc), 5′-NTII (1 mg/kg, sc) and BNTX (1 mg/kg, sc) were given to mice 30 min, 30 min, 24 h and 20 min prior to morphine treatment, respectively. Each column represents the mean % antinociceptive effect in 10 mice with SEM using a 51°C warm-plate as the nociceptive stimulus. $^{**}p < 0.01$ vs. saline control.

Statistical Analysis

Changes in weight loss were evaluated using a repeated measures analysis of variance (ANOVA). The factors of variation were treatment and time. If a significant effect of treatment or interaction with treatment was observed, a one-way analysis of variance was used to determine the significance at each time point. Individual group comparisons were made using Dunnett's multiple comparison test. The incidence of withdrawal signs was statistically evaluated using Fisher's probability test.

RESULTS

Effect of δ Opioid Receptor Subtype Antagonists on Morphine-Induced Antinociception

The effects of δ opioid receptor antagonists on morphine-induced antinociception are summarized in Fig. 1. Morphine (10 mg/kg, sc) produced a significant antinociception in mice. Pretreatment with the δ opioid receptor antagonists NTI (3 mg/kg, sc), NTB (1 mg/kg, sc), 5′-NTII (1 mg/kg, sc) or BNTX (1 mg/kg, sc) had no effect on the antinociceptive effect of acute morphine treatment (10 mg/kg, sc).

Effect of NTI on the Development of Physical Dependence on Morphine in Mice

The effects of NTI on the development of physical dependence on morphine in mice are summarized in Fig. 2A and

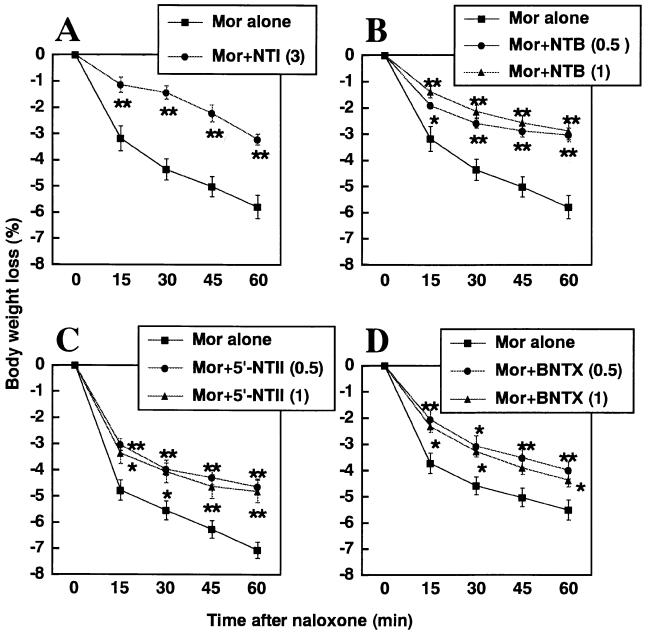

FIG. 2. Effect of pretreatment with NTI, NTB, 5′-NTII or BNTX on naloxone-precipitated body-weight loss in morphine-dependent mice. Mice were chronically pretreated with NTI (3 mg/kg, sc), NTB (0.5 and 1 mg/kg, sc) or BNTX (0.5 mg/kg, sc) 30, 30 and 20 min before treatment with morphine (8–45 mg/kg, sc), respectively. Treatment with 5′-NTII (0.5 mg/kg, sc) was begun 24 h prior to beginning chronic morphine treatment. Thereafter, 5′-NTII was administered daily at 0900 h during chronic morphine treatment. Withdrawal was precipitated by naloxone (3 mg/kg, sc) and withdrawal body-weight loss was observed for 60 min. Each plot represents the mean body weight loss of 10 animals with SEM $^{**}p < 0.01$ and $^{*}p < 0.05$ vs. morphine alone group (Mor alone).

Table 1. There was no significant difference between the pre-withdrawal body weight in the morphine-alone groups and the morphine-plus-δ opioid receptor antagonist groups. Naloxone-precipitated body-weight loss was significantly less with NTI than with morphine alone (Fig. 2A). As shown in Table 1, the incidence of jumping and body shakes in morphine-dependent mice treated with NTI was significantly less than with morphine alone. In addition, the incidence of forepaw tremor, rearing and diarrhea in morphine-dependent mice treated with NTI tended to be less than with morphine alone.

Effect of NTB on the Development of Physical Dependence on Morphine in Mice

The effects of NTB on the development of physical dependence on morphine in mice are summarized in Fig. 2B and Table 1. Naloxone-precipitated body-weight loss was dose-dependently and significantly suppressed by treatment with NTB, as compared with morphine alone (Fig. 2B). As shown in Table 1, the incidence of jumping in morphine-dependent mice treated with NTB (0.5, 1.0 mg/kg, sc) was significantly less than that with morphine alone. Moreover, the incidence of body shakes in morphine-dependent mice treated with NTB (1.0 mg/kg, sc) was also significantly less than with morphine alone. In addition, the incidence of forepaw tremor, rearing and diarrhea tended to be less than with morphine alone.

Effect of 5′-NTII on the Development of Physical Dependence on Morphine in Mice

The effects of 5′-NTII on the development of physical dependence on morphine in mice are summarized in Fig. 2C and Table 1. Naloxone-precipitated body-weight loss was significantly suppressed by 5′-NTII treatment, as compared with morphine alone. The incidence of jumping and body shakes in morphine-dependent mice treated with 5′-NTII (0.5, 1 mg/kg, sc) was significantly less than with morphine alone. Moreover, the incidence of diarrhea in morphine-dependent mice treated with 5′-NTII (1.0 mg/kg, sc) was also significantly less than with morphine alone. In addition, the incidence of rearing tended to be less than with morphine alone (Table 1).

Effect of BNTX on the Development of Physical Dependence on Morphine in Mice

The effects of BNTX on the development of physical dependence on morphine in mice are summarized in Fig. 2D and Table 1. Although the naloxone-precipitated body-weight loss was significantly suppressed by BNTX treatment, as compared with morphine alone, the suppression by BNTX was weaker than that by NTI, NTB or 5′-NTII (Fig. 2D). The incidence of jumping and body shakes in mice treated with BNTX tended to be less than with morphine alone. However, BNTX failed to affect the incidence of other withdrawal signs (Table 1).

DISCUSSION

μ-Opioid receptors have been reported to play a role in several of morphine's pharmacological effects. For example, the selective and irreversible μ opioid receptor antagonist β-funaltrexamine (β-FNA) (24,43,49) inhibits the development of physical dependence on morphine in rats (4,6). Moreover, we previously found that intracerebroventricular (ICV) injection of β-FNA drastically antagonized morphine-induced antinociception and dopamine (DA)-dependent hyperlocomotion, and enhanced DA turnover in the limbic forebrain of mice (20,21). Thus, the activation of μ opioid receptors plays an important role in the expression of several of morphine's pharmacological effects. However, there have also been reports that β-FNA is not selective for μ-opioid receptors. For example, β-FNA can interfere with the binding of both μ- and δ-ligands (14,33). Moreover, the possibility that β-FNA is selective for μ-opioid receptors that are in a functional complex with δ opioid receptors has been reported by Rothman et al. (32). Thus, β-FNA may have a partial δ-opioid antagonistic effect. Recently, several investigators have reported an interaction between morphine and Leu-enkephalin *in vivo* and in opioid receptor binding assays. *In vivo* treatment with Leu-enkephalin either before or after morphine administration enhanced morphine-induced antinociception, tolerance and physical dependence (13,48). In opioid receptor binding assays, [^{3}H]Leu-enkephalin was displaced by morphine (30,31), and δ opioid binding sties were up-regulated in the striatum of morphine-dependent and -tolerant mice (2). Thus, some interaction between μ and δ opioid binding sites may exist in the antinociception produced by, the tolerance to, and the physical dependence on morphine. Moreover, in rats chronically infused with δ opioid receptor agonist into the cerebral aqueduct, development of dependence on this agonist was observed (5). These reports confirm that activation of not only μ opioid receptors but also δ opioid receptors may play a significant a role in the development of physical dependence

TABLE 1

EFFECT OF δ OPIOID RECEPTOR ANTAGONISTS ON NALOXONE-PRECIPITATED WITHDRAWAL SIGNS IN MORPHINE-DEPENDENT MICE

	Positive Mice/Total Mice								
	Saline		Chronic Treatment with Morphine (8–45 mg/kg/sc)						
			Pretreatment						
				NTB		5′-NTII		BNTX	
Withdrawal Signs	Non	Non	NTI 3	0.5	1	0.5	1	0.5	1
Jumping	0/10*	9/10	3/10*	3/10*	2/10*	3/10*	2/10*	6/10	6/10
Body shakes	1/10*	10/10	3/10*	6/10	3/10*	4/10*	4/10*	6/10	6/10
Ptosis	0/10*	10/10	10/10	10/10	8/10	10/10	9/10	9/10	8/10
Forepaw tremor	1/10*	10/10	5/10*	6/10	6/10	10/10	10/10	10/10	10/10
Rearing	1/10*	10/10	7/10	7/10	8/10	8/10	7/10	10/10	8/10
Diarrhea	0/10*	10/10	4/10*	6/10	6/10	6/10	5/10*	10/10	10/10

The morphine dose was increased progressively from 8 to 45 mg/kg (sc) over a period of 5 days. NTI (3 mg/kg, sc) or NTB (0.5, 1 mg/kg, sc) was injected chronically 30 min before the morphine injection. BNTX (0.5, 1 mg/kg, sc) was injected chronically 20 min before the morphine injection. Injection of 5′-NTII (0.5, 1 mg/kg, sc) was started 24 h prior to the beginning of chronic morphine treatment, thereafter, 5′-NTII was injected at 0900 h during the chronic morphine treatment. Withdrawal was precipitated by injecting naloxone (3 mg/kg, sc) 2 h after the final morphine injection. Withdrawal signs were observed for 60 min.

*$p < 0.05$ vs. chronic morphine-treated group (non-pretreated).

on morphine. In addition, recent studies have shown that chronic morphine treatment produced a change in endogenous enkephalin systems such as enkephalin gene expression or enkephalin immunoreactivity (23,47). Therefore, it is possible that these changes may also be involved in the mechanisms of morphine dependence. Based on these previous reports, we expected that blockade of δ opioid receptors during chronic morphine treatment may prevent the development of morphine dependence. In fact, in the present study, we could demonstrate the involvement of δ opioid receptors in the development of physical dependence on morphine.

Recently, evidence for the presence of δ opioid receptor subtypes (δ_1 and δ_2 opioid receptors) has been reported (12,16,34). Thus, it is necessary to investigate the roles that these δ opioid receptor subtypes play in the expression of several pharmacological effects of morphine. A series of highly selective, non-peptide δ opioid receptor antagonists, including NTI (δ_1 and δ_2 opioid receptor antagonist), 5′-NTII (δ_2 opioid receptor antagonist), NTB (δ_2 opioid receptor antagonist) and BNTX (δ_1 opioid receptor antagonist), have recently been synthesized (25–29). We used these antagonists to investigate the involvement of δ opioid receptor subtypes in the development of physical dependence on morphine.

In the present study, although systemic morphine produced a potent antinociceptive effect, neither NTI (3 mg/kg, sc), 5′-NTII (0.5, 1 mg/kg, sc), NTB (0.5 1 mg/kg, sc) nor BNTX (0.5, 1 mg/kg, sc) alone produced antinociceptive effects. Furthermore, pretreatment with NTI, 5′-NTII, NTB or BNTX had no effect on the antinociceptive effect of morphine. These results demonstrate that δ opioid receptor antagonists did not affect the pain threshold or the antinociceptive effect of morphine, and suggest that pretreatment with the δ opioid receptor antagonists used in the present study doe s not block μ opioid receptors.

Pretreatment with NTI (3 mg/kg, sc) during chronic morphine treatment significantly suppressed the incidence of jumping and diarrhea, as compared with morphine alone. These results suggest that not only μ opioid receptors, but also NTI-sensitive δ opioid receptors may play a significant role in the development of physical dependence on morphine. Our finding is consistent with the results reported by Abdelhamid et al. (1). Although they observed jumping and diarrhea after the injection of naloxone in mice treated with a morphine pellet, other naloxone-precipitated withdrawal signs were not investigated. In the present study, pretreatment with NTI during chronic morphine injection also suppressed the naloxone-precipitated body-weight loss and the incidence of not only jumping and diarrhea, but also of body shakes and forepaw tremor, as compared with morphine alone. Thus, we have newly demonstrated that blockade of δ opioid receptors during chronic morphine treatment inhibits not only naloxone-precipitated jumping and diarrhea but also other withdrawal signs. Pretreatment with 5′-NTII (0.5 mg/kg, sc), a long-acting δ_2 opioid receptor antagonist, during chronic morphine treatment also suppressed the incidence of jumping and diarrhea, as compared with morphine alone. Although these results are consistent with the results of Miyamoto et al. (17), they also observed only jumping and diarrhea. In the present study, chronic treatment with 5′-NTII and morphine suppressed naloxone-precipitated body-weight loss and the incidence of body shakes to the same degree as in the NTI-pretreated group. These results demonstrate that the blockade of δ_2 opioid receptors during chronic morphine treatment suppresses naloxone-precipitated body-weight loss and the incidence of body shakes, as well as of jumping and diarrhea, and suggest that δ_2 opioid receptors may play a significant role in the development of physical dependence on morphine. Moreover, pretreatment with NTB (0.5, 1.0 mg/kg, sc), a short-acting δ_2 opioid receptor antagonist, during chronic morphine treatment also significantly suppressed the naloxone-precipitated body-weight loss, jumping and body shakes. However, Miyamoto et al. (17) reported that chronic treatment with NTB did not affect the ED_{50} values of naloxone for jumping and diarrhea in mice that were treated with NTB 30 min before, and 24 and 48 h after the implantation of a morphine pellet. These results contradict our findings. Miyamoto et al. (17)

reported that continuous blockade, rather than intermittent blockade, of δ_2 opioid receptors is necessary to inhibit the development of physical dependence on morphine. In the present study, when NTB was administered 30 min before each morphine injection, the development of physical dependence on morphine was inhibited. Therefore, these results confirm that δ_2 opioid receptors may play a significant role in the development of physical dependence on morphine.

It is previously reported that high doses of NTI and NTB have κ opioid receptor agonist-like activity (35,45). In addition, we and other investigators suggest that activation of κ opioid receptors prevents the development of physical dependence on morphine (38) and the naloxone-precipitated withdrawal signs (3,8). It is therefore possible that the present results are due to the κ opioid receptor agonist-like activities of NTI and NTB. However, the selectivity of the doses of antagonists used in the present study has been confirmed by our and other studies which rule out the possible non-selective actions of these compounds (19,40,41,45). Therefore, we conclude that the present inhibitory effects of NTI and NTB may be solely the results of the selective action to δ opioid receptor subtypes.

On the other hand, the role of δ_1 opioid receptors in the development of physical dependence on morphine is still unclear. Recently, a highly selective and non-peptide δ_1 opioid receptor antagonist, 7-benzylidenenaltrexone (BNTX), was synthesized by Portoghese et al. (29), which makes it possible to investigate the involvement of δ_1 opioid receptors in the development of dependence on morphine. In the present study, we found that pretreatment with BNTX (0.5, 1.0 mg/kg, sc) during chronic morphine treatment inhibited naloxone-precipitated body-weight loss, but this inhibition was weaker than that caused by NTI, 5′NTII or NTB. Moreover, the incidence of naloxone-precipitated jumping and body shakes in morphine-dependence mice treated with BNTX also tended to be less than with morphine alone. These results suggest that not only δ_2 but also δ_1 opioid receptors may play a significant role in the development of physical dependence on morphine. Contrast to the present results, Miyamoto et al. (19) recently demonstrated that treatment with [D-Ala^2, Leu^5, Cys^6]enkephalin (DALCE), a δ_1 opioid receptor antagonist, during chronic morphine treatment did not suppress naloxone-precipitated jumping and diarrhea, and concluded that δ_1 opioid receptors are not involved in the development of physical dependence on morphine. There may be several reasons for the difference between our present results and their results (19). In their study, the experimental design is completely different from our design. The degree of morphine dependence was evaluated by naloxone ED_{50} values in naloxone-precipitated withdrawal jumping and diarrhea. They observed only withdrawal jumping and diarrhea, and did not observe effects of δ opioid receptor antagonists on other naloxone-precipitated withdrawal behaviors, such as body-weight loss, body shakes, ptosis, forepaw tremor and rearing. Therefore, it is unclear whether other withdrawal signs are inhibited by the blockade of δ_1 opioid receptors. Moreover, the methods used to develop dependence and the antagonist used to antagonize δ_1 opioid receptor subtypes are also different from the present study. Miyamoto et al. (19) implanted s.c. with morphine pellet to develop dependence, in contrast, chronic injection method was used in the present study. Moreover, although we used BNTX as δ_1 opioid receptor antagonist, they used non-equilibrium antagonist DALCE. All these factors may contribute to the different findings. Recently several studies indicate the existence of μ–δ_1 opioid receptor interaction in the expression of morphine's pharmacological effects. In an antinociceptive assay, Porreca and colleagues demonstrated that a subeffective dose of DPDPE, a selective δ_1 opioid receptor agonist, potentiates morphine antinociception (9–11). We also reported the similar results that a subeffective dose of TAN-67, nonpeptide δ opioid receptor agonist, potentiates morphine antinociception, and the effects of TAN-67 are selectively antagonized by δ_1 opioid receptor antagonist BNTX (41). In receptor binding assay, change in μ opioid receptor binding site which was determined by using [^{3}H]Tyr-D-Ala-Gly-MePhe-Gly-ol (DAMGO) was observed after chronic DPDPE treatment (46), suggesting the existence of the interaction between μ and δ_1 binding site. Moreover, we previously found that pretreatment with BNTX abolished the place preference conditioning produced by morphine (39). In contrast, a subeffective dose of TAN-67 potentiates the morphine-induced place preference and increase in dopamine turnover in the limbic forebrain, and the effects of TAN-67 were antagonized by BNTX (42). Therefore, it is possible that the δ_1 opioid receptor subtypes are involved in the development of morphine dependence. Cowan et al. (5) demonstrated that chronic DPDPE infusions directly into the cerebral aqueduct of rats developed a low- to moderate-dependence on this agonist, suggesting that activation of DPDPE-sensitive δ opioid receptors, i.e., δ_1 opioid receptor subtypes, may be associated with the development of opioid physical dependence. In view of these previous reports, including the present work, we concluded that not only δ_2 opioid receptors but also δ_1 opioid receptors may be involved in the development of physical dependence on morphine.

In conclusion, we found that pretreatment with not only δ_2 but also δ_1 opioid receptor antagonist during chronic morphine treatment reduces several naloxone-precipitated withdrawal signs. These findings suggest that both δ_2 and δ_1 opioid receptors may play an important role in modulating the development of physical dependence on morphine. However, δ_2 opioid receptors may play a more vital role than δ_1 receptors.

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REFERENCES

1. Abdelhamide, E. E.; Sultana, M.; Portoghese, P. S.; Takemori, A. E. Selective blockage of delta opioid receptors prevents the development of morphine tolerance and dependence in mice. J. Pharmacol. Exp. Ther. 258:299–303; 1991a.
2. Abdehamide, E. E.; Takemori, A. E. Characteristics of μ and δ opioid binding sites in striatal slices of morphine-tolerant and -dependent mice. Eur. J. Pharmacol. 198:157–163; 1991b.
3. Aceto, M. D.; Dewey, W. L.; Chang, J.-K.; Lee, N. M. Dynorphin-(1-13): Effect in nontolerant and morphine-dependent rhesus monkeys. Eur. J. Pharmacol. 83:139–142; 1982.
4. Aceto, M. D.; Dewey, W. L.; Portoghese, P. S.; Takemori, A. E. Effect of β-funaltrexamine (β-FNA) on morphine dependence in rats and monkeys. Eur. J. Pharmacol. 123:387–393; 1986.
5. Cowan, A.; Zhu, X. Z.; Mosberg, H. I.; Omnaas, R. J.; Porreca,

F. Direct dependence studies in rats with agents selective for different types of opioid receptor. J. Pharmacol. Exp. Ther. 246: 950–955; 1988.
6. Delander, G. E.; Portoghese, P. S.; Takemori, A. E. Role of spinal mu opioid receptors in the development of morphine tolerance and dependence. J. Pharmacol. Exp. Ther. 231:91–96; 1984.
7. Gmerek, D.; Woods, J. H. Effect of β-funaltrexamine in normal and morphine dependent rhesus monkeys: Observation studies. J. Pharmacol. Exp. Ther. 235:296–301; 1985.
8. Green, P. G.; Lee, N. M. Dynorphin A-(1-13) attenuates withdrawal in morphine-dependent rats: effect of route of administration. Eur. J. Pharmacol. 145:267–272; 1988.
9. Heyman, J. S.; Vaught, J. L.; Mosberg, H. I.; Haaseth, R. C.; Porreca, F. Modulation of μ-mediated antinociception by δ agonists in the mouse: selective potentiation of morphine and normorphine by [D-Pen^2, D-Pen^5]enkephalin. Eur. J. Pharmacol. 165: 1–10; 1989.
10. Horan, P.; Tallarida, R. J.; Haaseth, R. C.; Matsunaga, T. O.; Hruby, V. J.; Porreca, F. Antinociceptive interactions of opioid delta receptor agonists with morphine in mice: Supra- and subadditivity. Life Sic. 50:1535–1541; 1992.
11. Jiang, Q. I.; Mosberg, H. I.; Porreca, F. Modulation of the potency and efficacy of mu-mediated antinociception by delta agonists in the mouse. J. Pharmacol. Exp. Ther. 254:683–689; 1990.
12. Jiang, Q.; Takemori, A. E.; Sultana, M.; Portoghese, P. S.; Bowen, W. D.; Mosberg, H. I.; Porreca, F. Differential antagonism of opioid delta antinociception by [D-Ala^2, Leu^5, Cys^6]enkephalin and naltrindole 5′-isothiocyanate: Evidence for delta receptor subtypes. J. Pharmacol. Exp. Ther. 257:1069–1075; 1991.
13. Lee, N. M.; Leybin, L.; Chang, J. K.; Loh, H. H. Opiate and peptide interaction: Effect of enkephalin on morphine analgesia. Eur. J. Pharmacol. 68:181–185; 1980.
14. Liu-Chen, L-Y.; Li, S.; Wheeler-Aceto, H.; Cowan, A. (1991) Effects of intracerebroventricular β-funaltrexamine on μ and δ opioid receptors in the rat: dichotomy between binding and antinociception. Eur. J. Pharmacol. 203:195–202; 1991.
15. Maldonado, R.; Dauge, V.; Callebert, J.; Villette, J. M.; Fourine-Zaluski, M.-C.; Feger, J.; Roques, B. P. Comparison of selective and complete inhibitors of enkephalin-degrading enzymes on morphine withdrawal syndrome. Eur. J. Pharmacol. 165:199–207; 1989.
16. Mattia, A.; Vanderah, T.; Mosberg, H. I.; Porreca, F. Lack of antinociceptive cross-tolerance between [D-Pen^2, D-Pen^5]enkephalin and [D-Ala^2]deltorphine II in mice: Evidence for delta receptor subtypes. J. Pharmacol. Exp. Ther. 258:583–587; 1991.
17. Miyamoto, Y.; Portoghese, P. S.; Takemori, A. E. Involvement of delta$_2$ opioid receptors in the development of morphine dependence in mice. J. Pharmacol. Exp. Ther. 264:1141–1145; 1993a.
18. Miyamoto, Y.; Portoghese, P.S.; Takemori, A. E. Involvement of δ_2 opioid receptors in acute dependence on morphine in mice. J. Pharmacol. Exp. Ther. 265:1325–1327; 1993b.
19. Miyamoto, Y.; Bowen, W. D.; Portoghese, P. S.; Takemori, A. E. Lack of involvement of delta-1 opioid receptors in the development of physical dependence on morphine in mice. J. Pharmacol. Exp. Ther. 270:37–39; 1994.
20. Narita, M.; Suzuki, T.; Misawa, M.; Nagase, H. Effects of kappa opioids on the morphine-induced pharmacological actions. Eur. J. Pharmacol. 183:2328; 1990.
21. Narita, M.; Suzuki, T.; Funada, M.; Misawa, M.; Nagase, H. Blockade of the morphine-induced increase in turnover of dopamine on the mesolimbic dopaminergic system by κ-opioid receptor activation in mice. Life Sic. 52:397–404; 1993a.
22. Narita, M.; Suzuki, T.; Funada, M.; Misawa, M.; Nagase, H. Involvement of δ-opioid receptors in the effects of morphine on locomotor activity and the mesolimbic dopaminergic system in mice. Psychopharmacology 111:423–426; 1993b.
23. Nylander, I.; Vlaskovska, M.; Terenius, L. The effects of morphine treatment and morphine withdrawal on the dynorphin and enkephalin systems in Sprague–Dawley rats. Psychopharmacology 118:391–400; 1995.
24. Portoghese, P. S.; Larson, D. L.; Sayre, L. E.; Fries, D. S.; Takemori, A. E. A novel opioid receptor site directed alkylating agent with irreversible narcotic antagonistic and reversible agonistic activity. J. Med. Chem. 23:233–234; 1980.
25. Portoghese, P. S.; Sultana, M.; Takemori, A. E. Naltrindole, a highly selective and potent non-peptide δ opioid receptor antagonist. Eur. J. Pharmacol. 146:185–186; 1988.
26. Portoghese, P. S.; Sultana, M.; Takemori, A E. Naltrindole 5′-isothiocyanate: A nonequilibrium, highly selective δ opioid receptor antagonist. J. Med. Chem. 33:1547–1548; 1990a.
27. Portoghese, P. S.; Sultana, M.; Takemori, A. E. Design of peptidomimetic δ opioid antagonists using the message-address concept. J. Med. Chem. 33:1714–1720; 1990b.
28. Portoghese, P. S.; Nagase H.; Maloney-Huss, K. E.; Lin, C. E.; Takemori, A. E. Role of spacer and address components in peptidomimetic δ opioid receptor antagonists related to naltrindole. J. Med. Chem. 34:1715–1720; 1991.
29. Portoghese, P. S.; Sultana, M.; Nagase, H.; Takemori, A. E. A highly selective δ_1-opioid receptor antagonist: 7-benzylidenenaltrexone. Eur. J. Pharmacol. 218:195–196; 1992.
30. Rothman, R. B.; Westfall, T. C. Morphine allosterically modulates the binding of [^{3}H]-leucine enkephalin to a particulate fraction of rat brain. Mol. Pharmacol. 21:538–547; 1982a.
31. Rothman, R. B.; Westfall, T. C. Allosteric coupling between morphine and enkephalin receptors *in vivo*. Mol. Pharmacol. 21:548–557; 1982b.
32. Rothman, R. B.; Long, J. B.; Bykov, V.; Jacobson, A. E.; Rice, K. C.; Holaday, J. W. β-FNA binds irreversibly to opiate receptor complex: *in vivo* and *vitro* evidence. J. Pharmacol. Exp. Ther. 247:405–416; 1988.
33. Sanchez-Blazquez, P.; Garzon, J. Evaluation of δ receptor mediation of supraspinal opioid analgesia by *in vivo* protection against the β-funaltrexamine antagonist effect. Eur. J. Pharmacol. 159: 9–23; 1989.
34. Sofuoglu, M.; Portoghese, P. S.; Takemori, A. E. Differential antagonism of delta opioid agonists by naltrindole and its benzofuran analog (NTB) in mice: evidence for delta opioid receptor subtypes. J. Pharmacol. Exp. Ther. 257:676–680; 1991.
35. Stewart, P. E.; Holper, E. M.; Hammond, D. L. δ Antagonist and κ agonist activity of naltriben: evidence for differential κ interaction with the δ_1 and δ_2 opioid receptor subtypes. Life Sci. 55:PL79–84; 1994.
36. Suzuki, T.; Yoshii, T.; Yanaura, S. Induction of physical dependence on morphine in mice by the drug-admixed food method. Jpn. J. Pharmacol. 34:319–325; 1984.
37. Suzuki, T.; Hayashi, Y.; Misawa M. The role of mu$_1$ receptor in physical dependence on morphine using the mu receptor deficient CXBK mouse. Life Sci. 50:849–856; 1992a.
38. Suzuki, T.; Narita, M.; Takahashi, Y.; Misawa, M.; Nagase, H. Effects of norbinaltrophimine on the development of analgesic tolerance to and physical dependence on morphine. Eur. J. Pharmacol. 213:91–97; 1992b.
39. Suzuki, T.; Yoshiike, M.; Mizoguchi, H.; Kamei, J.; Misawa, M.; Nagase, H. Blockade of δ-opioid receptors prevents morphine-induced place preference in mice. Jpn. J. Pharmacol. 66:131–137; 1994.
40. Suzuki, T.; Mori, T.; Tsuji, M.; Misawa, M.; Nagase, H. The role of δ-opioid receptor subtypes in cocaine- and methamphetamine-induced place preferences. Life Sci. 55:PL339–344; 1994.
41. Suzuki, T.; Tsuji, M.; Mori, T.; Misawa, M.; Endoh, T.; Nagase, H. Effects of a highly selective nonpeptide δ opioid receptor agonist, TAN-67, on morphine-induced antinociception in mice. Life Sci. 57:155–168; 1995.
42. Suzuki, T.; Tsuji, M.; Mori, T.; Misawa, M.; Endoh, T.; Nagase, H. Effect of the highly selective and nonpeptide δ opioid receptor agonist TAN-67 on the morphine-induced place preference in mice. J. Pharmacol. Exp. Ther. 279:177–185; 1996.
43. Takemori, A. E.; Larson, D. L.; Portoghese, P. S. The irreversible narcotic antagonistic and reversible agonistic properties of the fumaramate methyl ester derivative of naltrexone. Eur. J. Pharmacol. 70:445–451; 1981.
44. Takemori, A. E.; Portoghese, P. S. Evidence for the interaction of morphine with kappa and delta opioid receptors to induced

analgesia in β-funaltrexamine-treated mice. J. Pharmacol. Exp. Ther. 243:91–94; 1987.
45. Takemori, A. E.; Sultana, M.; Nagase, H.; Portoghese, P. S. Agonist and antagonist activities of ligands derived from naltrexone and oxymorphone. Life Sci. 50:1491–1495; 1992.
46. Tao, P.-L.; Tsai, C.-L.; Chang, L.-R.; Loh, H. H. Chronic effect of [D-Pen^2, D-Pen^5]enkephalin on rat brain opioid receptors. Eur. J. Pharmacol. 201:209–214; 1991.
47. Uhl, G. R.; Ryan, J. P.; Schwartz, J. P. Morphine alters preproenkephalin gene expression. Brain Res. 459:391–397; 1988.
48. Vaught, J. L.; Takemori, A. E. Differential effects of leucine and methionine enkephalin on morphine-induced analgesia, acute tolerance, and dependence. J. Pharmacol. Exp. Ther. 208: 86–90; 1979.
49. Ward, S. J.; Portoghese, P. S.; Takemori, A. E. Pharmacological characterization *in vivo* of the novel opiate, β-funaltrexamine. J. Pharmacol. Exp. Ther. 220:494–498; 1982.
50. Yamamoto, T.; Ohno, M.; Ueki, S. A selective κ-opioid agonist, U-50, 588H, blocks the development of tolerance to morphine analgasis in rats. Eur. J. Pharmacol. 156:173–176; 1988.