Possible involvement of μ_1-opioid receptors in the fentanyl- or morphine-induced antinociception at supraspinal and spinal sites

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Abstract

Fentanyl has been shown to be a potent analgesic with a lower propensity to produce tolerance and physical dependence in the clinical setting. The present study was designed to investigate the mechanisms of fentanyl- or morphine-induced antinociception at both supraspinal and spinal sites. In the mouse tail-flick test, the antinociceptive effects induced by both fentanyl and morphine were blocked by either the μ_1-opioid receptor antagonist naloxonazine or the μ_1/μ_2-opioid receptor antagonist β-funaltrexamine (β-FNA) after s.c., i.c.v. or i.t. injection. In contrast, both fentanyl and morphine given i.c.v. or i.t. failed to produce antinociception in μ_1-deficient CXBK mice. These findings indicate that like morphine, the antinociception induced by fentanyl may be mediated predominantly through μ_1-opioid receptors at both supraspinal and spinal sites in mice. We also determined the ED_{50} values for s.c.-, i.c.v.- and i.t.-administered fentanyl- or morphine-induced antinociception in mice. The ED_{50} values for s.c.-, i.c.v.- and i.t.-administered fentanyl-induced antinociception were 73.7, 18.5 and 1.2-fold lower than that of morphine, respectively. The present data clearly suggest the usefulness of peripheral treatment with fentanyl for the control of pain.   © 2002 Elsevier Science Inc. All rights reserved.

Keywords: Antinociception; Opioid; Fentanyl; μ-Opioid receptor; Intracerebroventricular injection; Intrathecal injection

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Introduction

μ-Opioid receptor acting drugs, such as morphine and methadone, have been used clinically as analgesics or anesthetics [1]. However, it is well known that the profound analgesia/antinociception induced by μ-opioid receptor agonists is accompanied by undesirable effects, such as respiratory depression and inhibition of gastrointestinal transit [2]. In 1965, fentanyl, the anilidopiperidine class of opioid, was reported as a potent synthetic analgesic. Fentanyl has a high affinity for μ-opioid receptors and exhibits 50–100 times more potent analgesic activity than that of morphine [3]. It is of interest to note that chronic administration of morphine to rats induced a tolerance to the antinociceptive effect produced by morphine, whereas chronic treatment with equieffective doses of fentanyl did not induce any tolerance to the fentanyl [4].

In the clinic, fentanyl is mainly used as a epidural anesthestic. It is likely that the reason for this limited clinical application is related to a poor understanding the functional mechanism of fentanyl to produce analgesic actions.

Opioid receptors have been classified into three types, μ-, δ- and κ-opioid receptors, based upon pharmacological, behavioral and biochemical studies. μ-Opioid receptor-mediated actions have been subdivided into μ_1- and μ_2-opioid receptor mechanisms, based upon the effect of the selective μ_1-opioid receptor antagonist naloxonazine [5]. Treatment with naloxonazine has been shown to reduce supraspinal antinociception induced by a selective μ-opioid receptor agonist [D-Ala^2,N-MePhe^4,Gly-ol^5]enkephalin (DAMGO), whereas the antinociceptive effect induced by intrathecally (i.t) administered DAMGO is not affected by naloxonazine [6]. The antinociception of i.t.-administered DAMGO is abolished by β-funaltrexamine (β-FNA) which selectively and irreversibly blocks μ-opioid receptors in binding assays, without distinguishing between μ_1- and μ_2-opioid receptor subtypes [7]. Therefore, it has long been considered that μ_1-opioid receptors mediate antinociception supraspinally, whereas μ_2-opioid receptors are responsible for spinal antinociception.

More recent studies show that the μ-opioid receptor agonists heroin and morphine-6β-glucuronide (M6G) induce antinociception mediated by a mechanism different from that of morphine. The CXBK mouse, which is μ_1-opioid receptor subtype deficient, does not exhibit antinociception with intracerebroventricular (i.c.v.) morphine injection, whereas i.c.v. heroin and M6G show potent antinociception in CXBK mice [8]. It is therefore worthwhile to further ascertain the possible involvement of μ-opioid receptor subtypes in the expression of antinociceptive effects induced by other μ-opioid receptor agonists with poorly understanding mechanisms, such as fentanyl. Thus, the goal of the present study was to investigate the role of the μ_1- and μ_2-opioid receptor subtypes in supraspinal and spinal antinociceptive effects induced by the potent opioid agonist fentanyl in mice.

Materials and methods

The present study was conducted in accordance with the Guiding Principles for the Care and Use of Laboratory Animals, Hoshi University, as adopted by the Committee on Animal

Research of Hoshi University, which is accredited by the Ministry of Education, Science, Sports and Culture of Japan.

Animals

Male ddY mice (20–30 g), male CXBK (20–30 g) and C57BL/6J (20–25 g) mice were obtained from Tokyo Animal Laboratories (Tokyo, Japan). The animals were housed at a room temperature of 22 ± 1 °C with a 12-h light-dark cycle (light on 8:30 a.m. to 8:30 p.m.), and were allowed to adapt to this environment for a period of 3 days before the experiments. Food and water were available *ad libitum*.

Antinociceptive assay

The tail-flick test was used to evaluate the antinociceptive effects of the drugs. The morphine- or fentanyl-induced antinociception was measured at 15 or 5 min after s.c. or i.c.v./
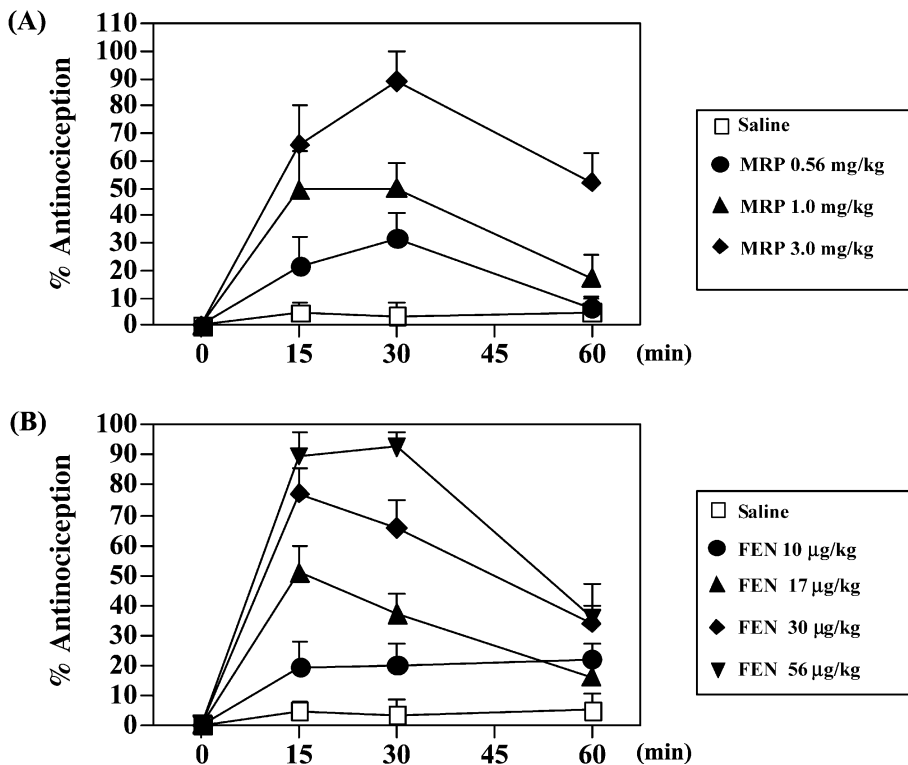

Fig. 1. Time-course changes in the antinociception after s.c. injection of morphine (MRP) (A) or fentanyl (FEN) (B) in mice. Antinociception was expressed as a percentage of maximum possible effect (% Antinociception). Each point represents the mean ± S.E.M. of 12 mice.

i.t. injection, respectively. The antinociception produced by i.c.v.-administered [D-Pen^2, Pen^5]enkephalin (DPDPE) and s.c.-administered U-50,488H was measured at 10 min and 15 min after each injection, respectively. A noxious beam of light was focussed on the tail
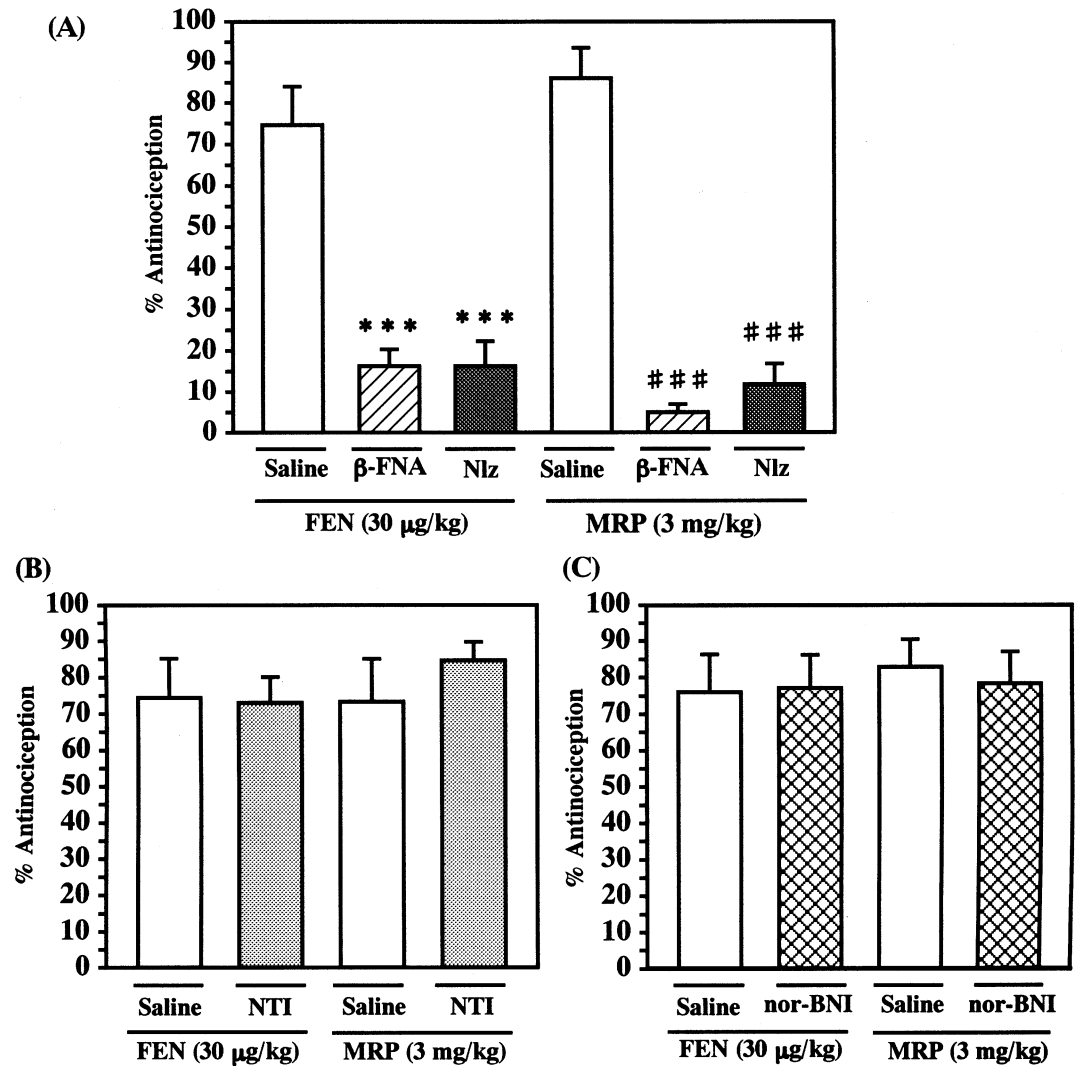

Fig. 2. (A) Effects of β-FNA or naloxonazine (Nlz) on s.c. morphine (MRP)- or fentanyl (FEN)-induced anti-nociception in the mouse tail-flick test. β-FNA (40 mg/kg) or Nlz (35 mg/kg) was administered s.c. 24 h before s.c. administration of agonists. ***: $p<0.001$ and ###: $p<0.001$ vs. respective agonist alone. (B) Effects of NTI on s.c. MRP- or FEN-induced antinociception in the mouse tail-flick test. NTI (3 mg/kg) was administered s.c. 30 min before s.c. administration of agonists. (C) Effects of nor-BNI on s.c. MRP- or FEN-induced antinociception in the mouse tail–flick test. Nor-BNI (3 mg/kg) was administered s.c. 4 h before s.c. administration of agonists. Each bar represents the mean ± S.E.M. of 10–15 mice.

about 4 cm from the tip, and the latency to removal was measured automatically. The intensity of the radiant heat source was adjusted to yield baseline latencies between 2 and 5 sec; this intensity was never changed and any animal in which baseline latency was outside the pre-established limits was excluded from the experiments. The cut-off time was 10 sec. Antinociception was calculated as a percentage of the maximum possible effect (% Anti-
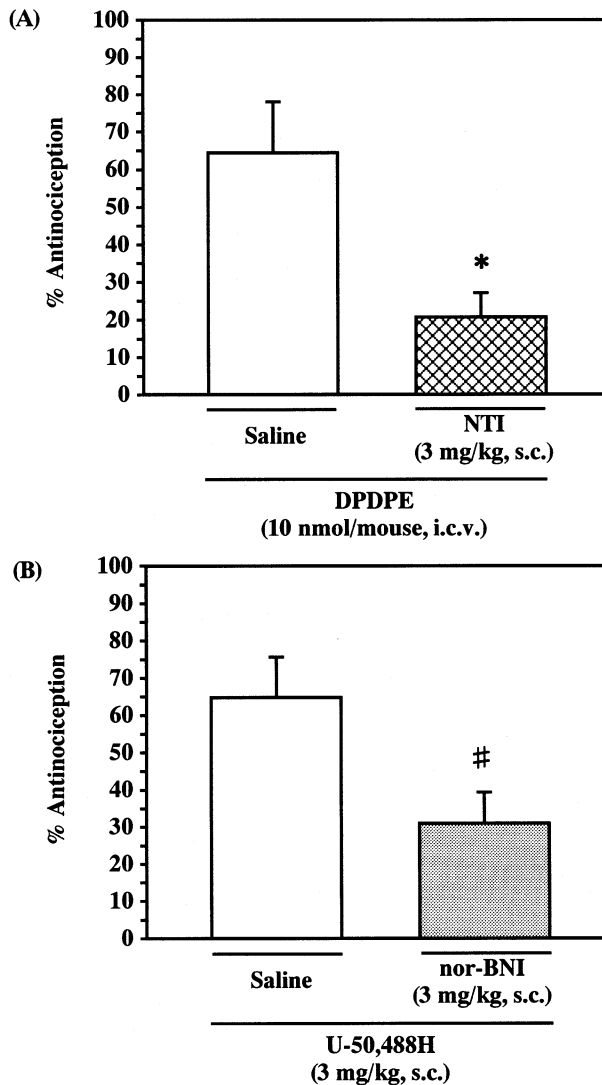

Fig. 3. (A) Effects of NTI on i.c.v. administration of [D-Pen^2, Pen^5]enkephalin (DPDPE)-induced antinociception in the tail-flick test. NTI (3 mg/kg) was administered s.c. 30 min before i.c.v. administration of DPDPE. (B) Effects of nor-BNI on s.c. administration of U-50,488H in the tail-flick test. Nor-BNI (3 mg/kg) was administered s.c. 4 h before s.c. administration of U-50,488H. Each bar represents the mean ± S.E.M. of 10–15 mice. *: p<0.05 and #: p<0.05 vs. respective agonist alone.

nociception) according to the following formula: % Antinociception = (test latency−predrug latency)×100/(cut-off time−predrug latency). Predrug latency was the mean of two values for each animal. Each group was consisted of 5–15 mice per drug or saline treatment and each animal was used for only one treatment.

Intracerebroventricular injection

Intracerebroventricular (i.c.v.) administration was performed following the method described previously [9]. The injection was made with a 2-mm double-needle (Natsume Seisakusho, Co., Ltd., Tokyo) attached to a 25-μl Hamilton microsyringe. Solution was injected in a volume of 4 μl per mouse.
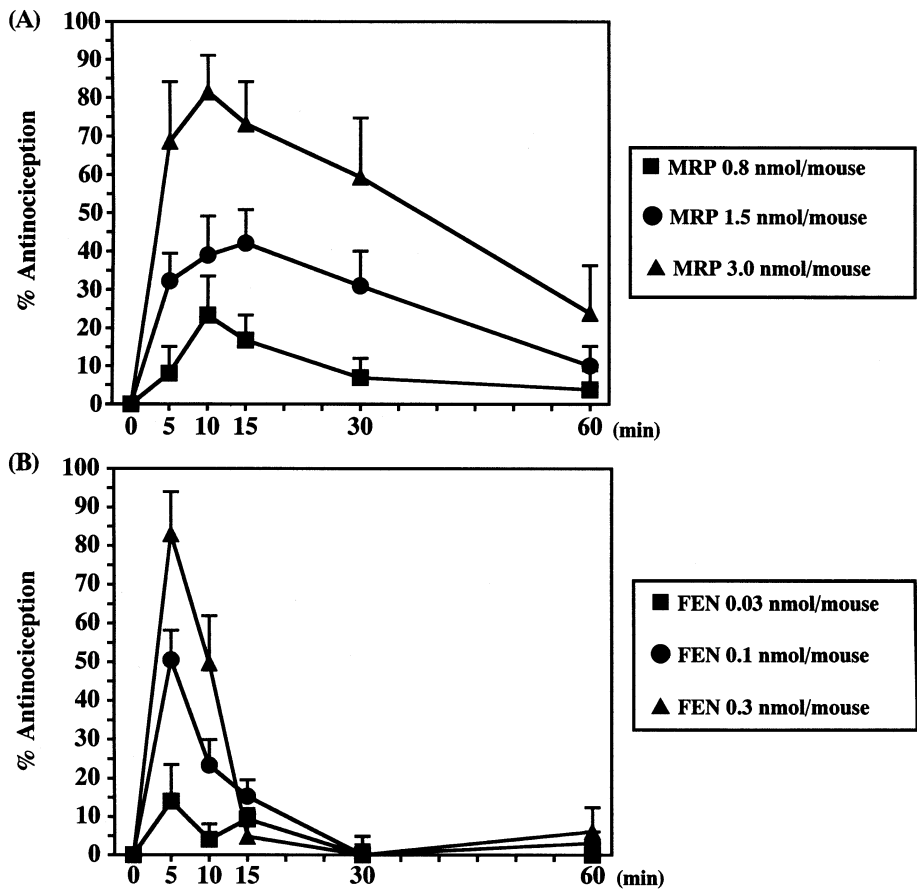

Fig. 4. Time-course changes in the antinociception after i.c.v. injection of morphine (MRP) (A) or fentanyl (FEN) (B) in mice. Antinociception was expressed as a percentage of maximum possible effect (% Antinociception). Each point represens the mean ± S.E.M. of 8–14 mice.

Intrathecal injection

Intrathecal (i.t.) administration was performed following the method described previously [10] using a 25-μl Hamilton syringe with a 30-gauge needle. Solution was injected in a volume of 4 μl per mouse.

Drugs

The drugs used in the present study were fentanyl citrate (Hisamitsu Pharmaceutical Co Inc, Tokyo, Japan), morphine hydrochloride (Sankyo Co, Tokyo, Japan), β-funaltrexamine hydrochloride (β-FNA), naloxonazine hydrochroride, naltrindole methanesulfonate (NTI), DPDPE, nor-binaltorphimine hydrochloride (nor-BNI) and U-50,488H (Toray Ind, Kamakura, Japan). β-FNA (40 mg/kg), naloxonazine (35 mg/kg), NTI (3 mg/kg) and nor-BNI
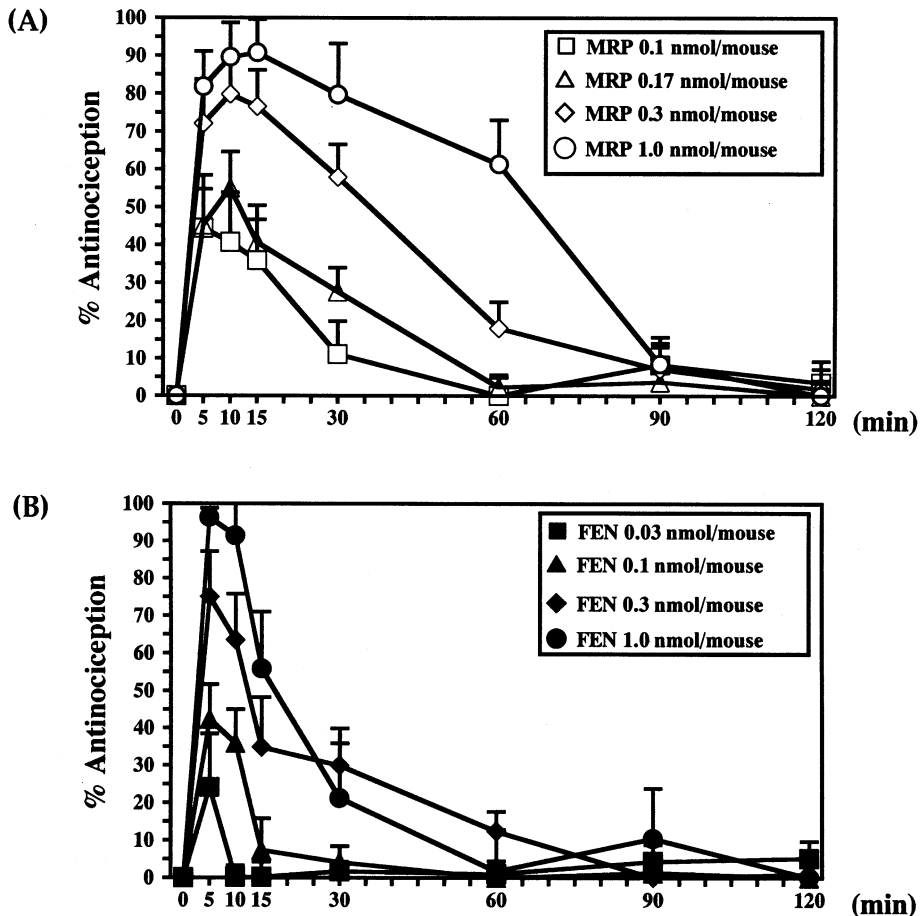

Fig. 5. Antinociceptive effects of morphine (MRP) (A) or fentanyl (FEN) (B) following i.t. administration in the mouse tail-flick test. Antinociception was expressed as a percentage of maximum possible effect (% Antinociception). Each point represents the mean ± S.E.M. of 8–12 mice.

(3 mg/kg) were adminstered s.c. 24 h, 24 h, 30 min and 4 h, respectively, before fentanyl or morphine injection. All drugs were dissolved in saline.

Statistical analysis

Antinociceptive effects were calculated using the general equation and were expressed as the mean with S.E.M. One-way repeated analysis of variance (ANOVA) followed by Dunnett's multiple comparison test was used for the statistical evaluation.

Results

Characterization of the antinociception induced by s.c. morphine or fentanyl

Morphine and fentanyl given s.c. produced antinociception in a dose-dependent manner, reaching maximal antinociceptive responses at 15 and 30 min, respectively, after the injection (Fig. 1A and 1B). The antinociception induced by s.c. morphine (3 mg/kg) or fentanyl (30 μg/kg) was significantly attenuated by pretreatment with both β-FNA (μ_1/μ_2-receptor antagonist) and naloxonazine (irreversible μ_1-receptor antagonist) (Fig. 2A). In contrast, NTI (selective δ-receptor antagonist) and nor-BNI (selective κ-receptor antagonist) had no effect on either morphine- or fentanyl-induced antinociception (Fig. 2B and 2C). The dose of NTI or nor-BNI used in the present study sufficiently anta-gonized the antinociception produced by i.c.v-administered DPDPE (selective δ-receptor agonist) or s.c.-administered U-50,488H (selective κ-receptor agonist), respectively (Fig. 3A and 3B). When opioid antagonists were administered i.c.v. at the doses used in the present study, these agonists did not produce any changes in the basal tail-flick latency (data not shown).

Spinal and supraspinal antinociceptive effects induced by morphine or fentanyl

As shown in Fig. 4, i.c.v. administration of either morphine or fentanyl produced significant antinociceptive effects in a dose-dependent manner, reaching maximal response

Table 1
Antinociceptive effects of fetanyl (FEN) or morphine (MRP)

| Injection site | ED$_{50}$ values | | MRP/FEN ratio |
| | Agonist | | |
	FEN	MRP	
s.c.	0.035 μmol/kg (0.025–0.049)	2.58 μmol/kg (1.37–4.85)	73.7
i.c.v.	0.10 nmol/mouse (0.04–0.23)	1.85 nmol/mouse (1.17–2.92)	18.5
i.t.	0.11 nmol/mouse (0.05–0.25)	0.13 nmol/mouse (0.06–0.28)	1.2

ED$_{50}$ values were determined using the analysis of variance and linear regression techniques. To calculate ED50 values, at least 3 drug doses were used and 8–15 mice were used for each dose. Values in parenthesis indicate the 95 % confidence range.
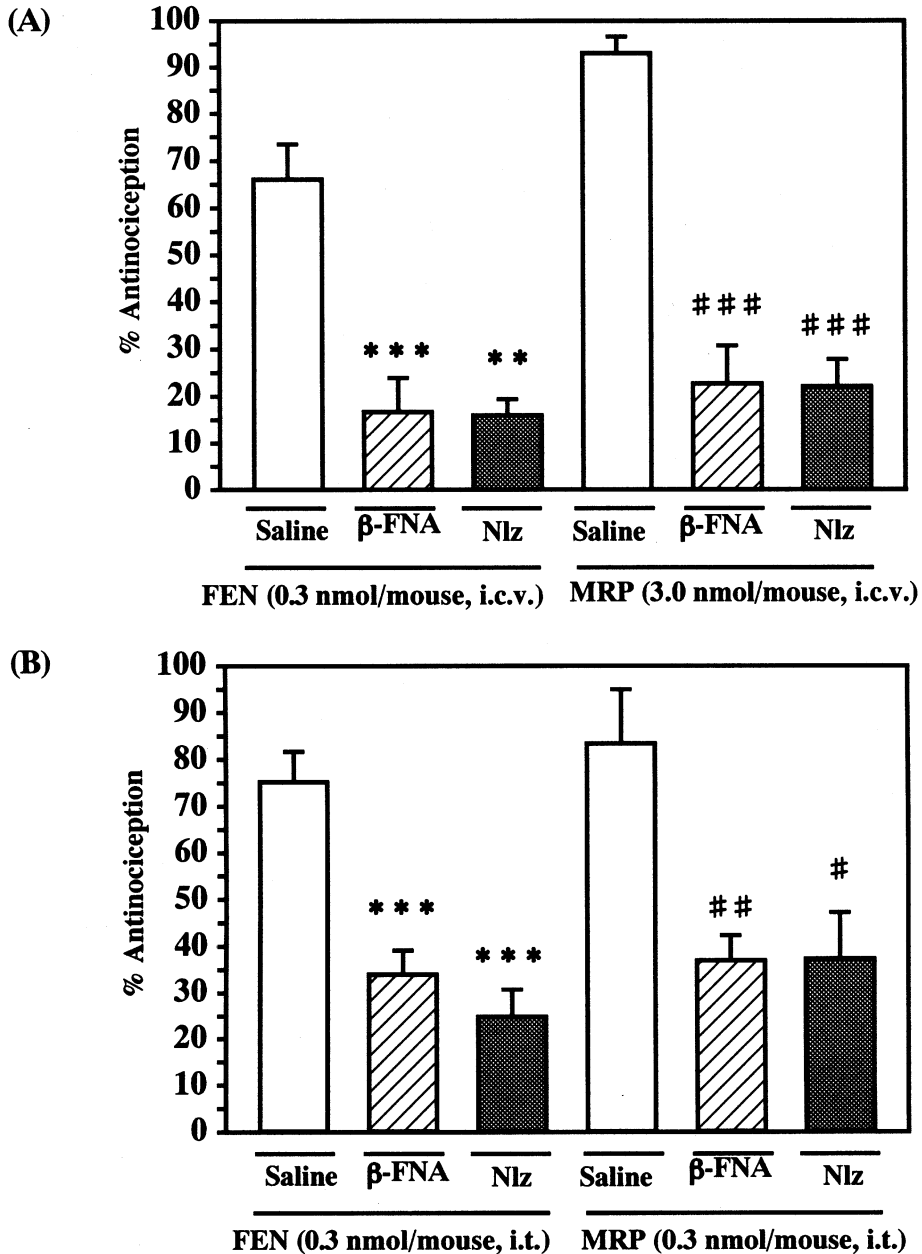
Fig. 6. (A) Effects of β-FNA or naloxonazine (Nlz) on i.c.v. morphine (MRP)- or fentanyl (FEN)-induced antinociception in the mouse tail-flick test. β-FNA (40 mg/kg) or Nlz (35 mg/kg) was administered s.c. 24 h before s.c. administration of agonists. **: p<0.01 and ***: p<0.001, ###: p<0.001 vs. respective agonist alone. (B) Effects of β-FNA or naloxonazine (Nlz) on i.t. morphine (MRP)- or fentanyl (FEN)-induced antinociception in the mouse tail-flick test. β-FNA (40 mg/kg) or Nlz (35 mg/kg) was administered s.c. 24 h before s.c. administration of agonists. ***: p<0.01, #: p<0.05 and ##: p<0.001 vs. respective agonist alone. Each bar represents the mean ± S.E.M. of 7−8 mice.

at 10–15 min or 5 min, respectively, following each injection. The dose-dependent antinociceptive effects were also elicited by i.t.-administered morphine and fentanyl (Fig. 5). Maximal antinociceptive effect of morphine or fentanyl was observed at 10–15 min or 5 min, respectively, following each injection.
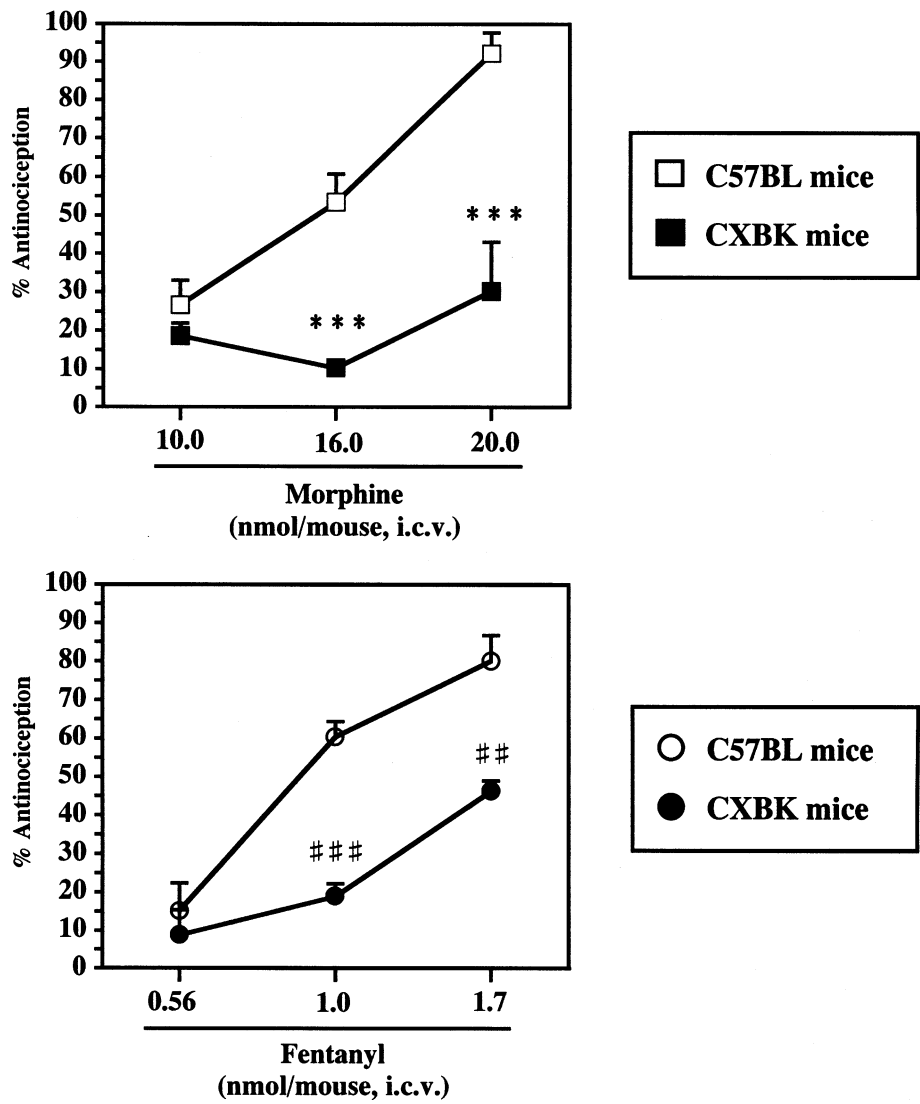

Fig. 7. The dose-response curve for antinociceptive effect induced by i.c.v. injection of morphine (A) or fentanyl (B) in CXBK and C57BL mice. Antinociception was expressed as a percentage of maximum possible effect (% Antinociception). Each point represents the mean ± S.E.M. of 5–6 mice. ***: p<0.001, ##: p<0.01 and ###: p<0.001 vs. C57BL mice.

Comparison of ED$_{50}$ values for antinociceptive effects induced by morphine or fentanyl when given s.c., i.c.v. or i.t.

In the present study, we determined the ED$_{50}$ values for s.c.-, i.c.v.- and i.t.-administered fentanyl- or morphine-induced antinociception (Table 1). In morphine-treated groups, the ED$_{50}$ values for s.c., i.c.v. and i.t. administration were 2.58 μmol/kg, 1.85 nmol/mouse and
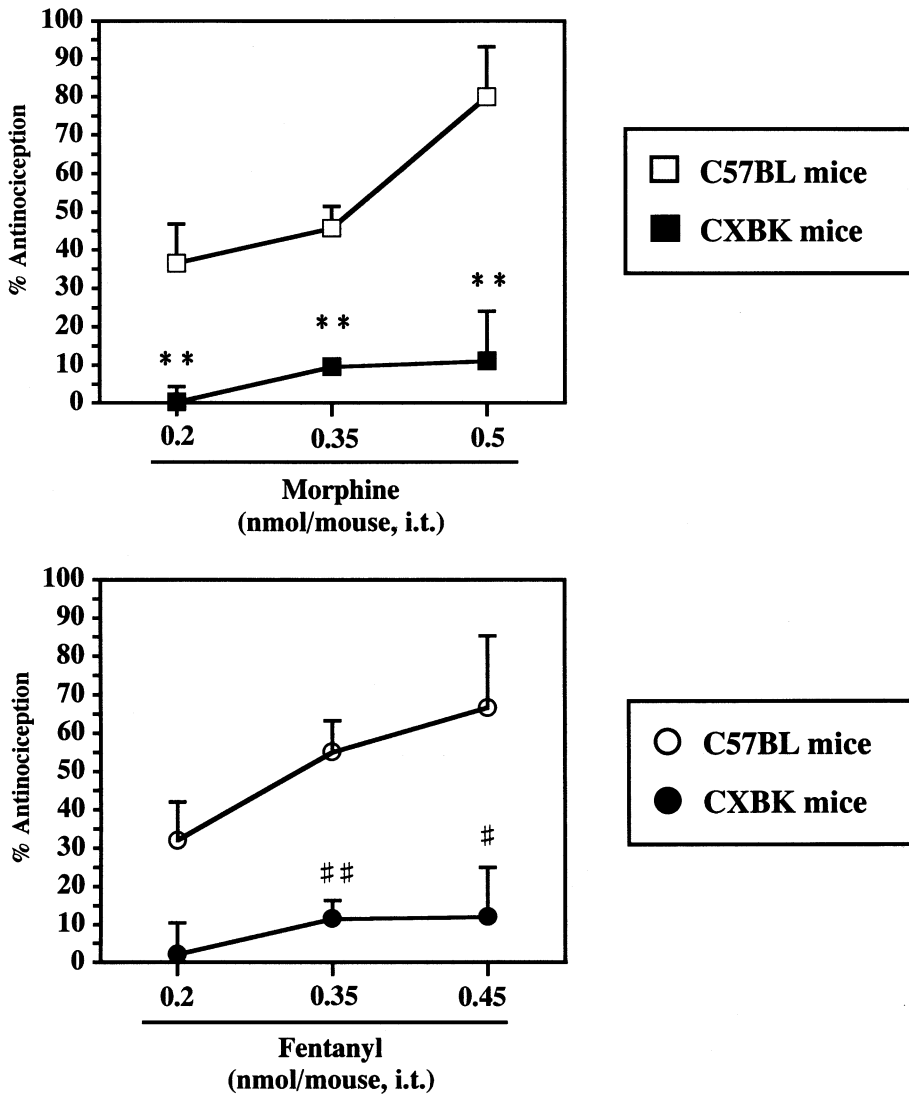

Fig. 8. The dose-response curve for antinociceptive effect induced by i.t. injection of morphine (A) or fentanyl (B) in CXBK and C57BL mice. Antinociception was expressed as a percentage of maximum possible effect (% Antinociception). Each point represents the mean ± S.E.M. of 5–6 mice. **: p<0.01, #: p<0.05 and ##: p<0.01 vs. C57BL mice.

0.13 nmol/mouse, respectively. In fentanyl-treated groups, the ED_{50} values for s.c., i.c.v. and i.t. administration were 0.035 μmol/kg, 0.10 nmol/mouse and 0.11 nmol/mouse, respectively. The ED_{50} values for fentanyl-induced antinociceptive effects following s.c., i.c.v. and i.t. injection were shown in Table 1. The morphine (MRP)/fentanyl (FEN) ratio for s.c., i.c.v. and i.t. routes were 73.7, 18.5 and 1.2, respectively.

Effects of β-FNA and naloxonazine on supraspinal and spinal antinociceptive effects produced by morphine or fentanyl when given i.c.v. or i.t.

The supraspinal antinociception induced by either i.c.v.-administered morphine or fentanyl was markedly suppressed by pretreatment with both β-FNA and naloxonazine (Fig. 6A). Furthermore, the morphine- or fentanyl-induced antinociception following i.t. injection was significantly attenuated by both β-FNA and naloxonazine (Fig. 6B).

Comparison of antinociceptive effects observed in CXBK and C57BL/6J mice at supraspinal and spinal sites

The supraspinal antinociceptive effects of morphine (i.c.v.) or fentanyl (i.c.v.) in CXBK and their parental strain C57BL/6J mice were investigated using the tail-flick test (Fig. 7). In C57BL/6J mice, either morphine or fentanyl produced a dose-dependent antinociceptive effect. In contrast, CXBK mice exhibited a weak antinociception induced by i.c.v. administration of both morphine and fentanyl as compared to that in C57BL/6J mice at the doses used in the present study.

We next examined the spinal antinociceptive effects of morphine (i.t.) or fentanyl (i.t.) in both strains. As shown in Fig. 8, the dose-dependent antinociceptive effects elicited by either morphine or fentanyl were observed in C57BL/6J mice. The antinociceptive effects induced by i.t. administration of both morphine and fentanyl in CXBK mice were reduced as compared to those in C57BL/6J at the doses used in the present study.

Discussion

In the present study using the mouse tail-flick test, both morphine and fentanyl produced dose-dependent antinociceptive effects after s.c. administration. These effects were significantly attenuated by pretreatment with both selective μ_1-opioid receptor antagonist naloxonazine and μ-opioid receptor antagonist β-FNA. The δ-opioid receptor antagonist NTI and κ-opioid receptor antagonist nor-BNI had no effects on antinociception induced by morphine or fentanyl, whereas the dose of NTI or nor-BNI was sufficiently effective to inhibit the antinociceptive effect produced by DPDPE or U-50,488H. These findings indicate that the μ_1-opioid receptor is involved in antinociception evoked by s.c. injection of morphine and fentanyl.

We determined the ED_{50} values for s.c., i.c.v. or i.t. injection of morphine- and fentanyl-induced antinociception in ddY mice. It is noteworthy that the MRP/FEN ratio for s.c.

administration was much higher than that for i.t. and i.c.v. injection. These differences may result from their pharmacokinetic profiles. This contention can be supported by the findings that unlike morphine, fentanyl has a lipid-soluble profile and thus easily diffuses into the brain across the blood-brain barrier. This may be the major reason that systemic (s.c.) injection of fentanyl can produce more potent antinociception than that of morphine.

The μ-opioid receptor has been further classified into μ_1- and μ_2-opioid receptors based upon the sensitivity to naloxonazine [5,11]. The CXBK mice, recombinant inbred mice of the C57BL/6J and BALB/J strains, are known to be deficient in μ_1-opioid receptor binding sites in whole-brain and spinal cord [12]. Whole-brain levels of endogenous opioid peptides do not appear to be altered in CXBK mice [13]. In the present study, both morphine and fentanyl induced antinociception in outbred ddY mice and inbred C57BL/6J mice when administered i.c.v. Conversely, antinociceptive activity induced by i.c.v. administration of both morphine and fentanyl was much lower in CXBK mice. Furthermore, i.c.v. injection of morphine- or fentanyl-induced antinociception was significantly attenuated by pretreatment with both β-FNA and naloxonazine. These findings indicate that antinociceptive effects of i.c.v.-administered morphine and fentanyl are mediated predominantly through μ_1-opioid receptors at the supra-spinal site. The results of our present study are consistent with previous reports that the μ_1-opioid receptors are implicated in supraspinal antinociception of μ-opioid receptor agonists [6,14].

In the present study, we found that both morphine and fentanyl when given i.t. failed to produce antinociceptive responses in CXBK mice. Furhtermore, i.t.-administered morphine- or fentanyl-induced antinociception was significantly attenuated by pretreatment with both β-FNA and naloxonazine. These data are inconsistent with the report that morphine has the potent and dose-dependent antinociceptive activity when given i.t. in CXBK mice and naloxonazine-treated mice using the tail-flick test [15]. Possible reason for this discrepancy may come from different experimental conditions and different doses of morphine used. Especially concerning antinociceptive assay using CXBK mice, the i.t. dose of morphine in the present study was 5-fold lower than that in their studies, indicating the possibility that lower doses of μ-opioid receptor agonists given i.t. may produce the antinociceptive effect mainly through the spinal μ_1-opioid receptors. In fact, μ_1-opioid receptors are concentrated in the lamina I and II of the spinal cord, which are key areas to modulate the nociceptive response [12]. CXBK mice have particularly low μ_1-opioid receptor levels in these areas [12]. Taken together, these findings suggest the substantial role for μ_1-opioid receptors in the antinoci-ception induced by morphine and fentanyl at the spinal level as well as supraspinal level.

In conclusion, the present study suggests that μ_1-opioid receptors at both spinal and supraspinal sites are the primary sites of antinociceptive action for fentanyl. Furthermore, the present data provide further evidence for the usefulness of peripheral treatment with fentanyl as therapeutic strategies for the control of pain.

Acknowledgments

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