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Antinociceptive properties of FR140423 mediated through spinal δ-, but not μ- and κ-, opioid receptors

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Abstract

We investigated the antinociceptive effect of FR140423, 3-(difluoromethyl)-1-(4-methoxyphenyl)-5-[4-(methylsulfinyl)phenyl] pyrazole, in the tail-pinch test in mice, and evaluated the mechanism of action using various opioid receptor antagonists. P.o. and i.t. injection of FR140423 exerted dose-dependent antinociceptive activities with ED_{50} values of 21 mg/kg and 3.1 μg/mouse, respectively. However, i.c.v. injection of FR140423 did not show an antinociceptive effect. The antinociceptive effects of FR140423 were completely abolished by naloxone and naltrindole but not by naloxonazine, β-funaltrexamine and nor-binaltorphimine. FR140423 did not affect any opioid receptor binding in mouse spinal membranes at concentrations up to 100 μM in vitro. Naloxone-induced jumping and diarrhea tests for morphine-like physical dependence of FR140423 gave negative results. These results suggest that FR140423 can induce antinociception by acting on the spinal but not the supraspinal site, and that spinal δ-opioid systems indirectly play a role in the antinociception produced by FR140423 in mice.

Keywords: FR140423; Antinociception; Opioid; Spinal cord; (Mice)

1. Introduction

In pain therapy, there are two main classes of analgesic drugs (Rang and Urban, 1995), namely non-steroidal anti-inflammatory drugs (NSAIDs) which prevent the sensitization of pain receptors in peripheral sites by inhibiting cyclooxygenase (Cashman, 1996) and opiates which directly abolish the promotion of nociceptive transmission in the central nervous system (CNS) by binding to opioid receptors (Hoskin and Hanks, 1991; Zadina et al., 1997). There are, however, drugs in the second group which may act as antinociceptives, i.e., by blocking ongoing hyperalgesia through the release of endogenous opioid-like substances (Ferreira et al., 1995).

FR140423 is a novel pyrazole derivative discovered by the screening of compounds to find new anti-inflammatory drugs without side effects (Tsuji et al., 1997). We reported that FR140423 showed potent anti-inflammatory and analgesic effects with selective cyclooxygenase-2 inhibition (Ochi et al., 1999b). Furthermore, FR140423 had antinociceptive effects in the tail-flick test which has been used for testing the central analgesic action of morphine, and its effect was antagonized by naloxone, a opioid receptor antagonist (Ochi et al., 1999b). Thus, we consider that FR140423 is an analgesic drug having two different types of action mechanisms, cyclooxygenase-2 inhibition in inflamed tissues and morphine like activities (Ochi et al., 1999a).

The present study was undertaken to characterize the antinociceptive effect caused by FR140423 on the regulation of pain pathways in the CNS. To assess the antinociceptive activity of FR140423, FR140423-induced antinociception was measured in the tail-pinch test in mice. We investigated the possible mechanism and site of action of systemically, spinally and supraspinally administered FR140423 using the selective μ-, δ- and κ-opioid receptor antagonists, naloxonazine and β-funaltrexamine (β-FNA), naltorindole and nor-binaltorphimine, respectively, and compared these results with those of morphine.

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2. Materials and methods

2.1. Animals

Ethical guidelines for the experimental use of animals were followed (Zimmermann, 1983). In addition, all experiments were carried out according to protocol approved by the Fujisawa Pharmaceutical Animal Experiment Committee for animal experimentation.

Male ddY mice (25–35 g, Japan SLC, Hamamatsu, Japan) were used at the age of 6 weeks. The animals were housed for at least 5 days in a controlled environment and were allowed food and water ad libitum.

2.2. Measurement of nociceptive response in the tail-pinch test

Nociceptive response in the tail-pinch test was performed according to the modified Haffner's method as previously reported (Takagi et al., 1966). Briefly, mice were pretested by pinching their tail base with a 4 cm long artery clip, exerting a pressure of 500 g, and only the mice that showed a nociceptive response such as biting the clip or vocalizing within 2 s were used for the experiments. When the mice did not show the above mentioned behaviors within 6 s after pinching, the antinociceptive effect was regarded as positive. To prevent tissue damage, a cut-off time of 10 s was selected. After drug treatments, the nociceptive responses in the tail-pinch test were measured at 15 min intervals for a period of 90 min. The antinociceptive effect was determined 30 min after drug administration in mice.

2.3. Opioid receptor binding assay

Mouse spinal membranes were prepared as previously described (Chen et al., 1993). The receptor sites were characterized by utilizing the specific radiolabeled μ-, δ- and κ-opioid ligand [^{3}H] DAMGO ([D-Ala^2, *N*-Me-Phe^4, Gly5-ol]enkephalin) 2 nM, [^{3}H] DPDPE ([D-Pen^{2,5}]-enkephalin) 2 nM and [^{3}H] U-69,593 ((5α, 7α, 8β)-(+)-*N*-methyl-*N*-[7-(1-pyrrolidinyl)-1-oxaspiro(4, 5)dec-8-yl]be-nzeneacetamide) 2 nM, respectively. The obtained membranes were suspended in 30 mM Tris–HCl buffer, pH 7.4, containing 1 mM EDTA, aprotinin 0.025 u/ml, leupeptin 5 μM and pepstatin A 0.5 μM. Drugs were dissolved in 1% dimethylsulfoxide in the above-mentioned buffer. The system was incubated for 1 h at 25°C. Non-specific binding was determined in the presence of 1 μM of the unlabeled ligand. Free ligand was separated from bound ligand by vacuum filtration onto Whatman GF/B glass filters which had been preincubated in 0.5% polyethylimine for 2 h to reduce non-specific binding. Radioactivity was determined in the presence of 3 ml aqueous scintillant.

2.4. Naloxone-induced jumping and diarrhea tests

The jumping and diarrhea tests were performed according to the procedure described by Kamei et al. (1973). Mice were treated with FR140423 (50–200 mg/kg p.o.) or morphine (25–100 mg/kg p.o.) twice a day at 0900 and 1900 for 5 days, a total of nine times. Mice were challenged with naloxone at a dose of 10 mg/kg s.c. 6 h after the last administration of drugs. For measuring the jumping and diarrhea responses, 10 animals were simultaneously placed on a round platform 30 cm in diameter and 35 cm in height. The number of animals which jumped off the platform and produced diarrhea was counted within 30 min after naloxone injections.

2.5. Drugs

The following drugs were used: indomethacin, naloxone HCl, naltorindole HCl and nor-binaltorphimine diHCl were obtained from Sigma (St. Louis, MO, USA). Naloxonazine diHCl and β-FNA HCl were obtained from Research Biochemicals International (Natick, MA, USA). Morphine HCl was obtained from Dainippon Pharmaceutical (Osaka, Japan). [^{3}H] DAMGO, [^{3}H] DPDPE and [^{3}H] U-69,593 were from New England Nuclear (Wilmington, DE, USA). FR140423 (3-(difluoromethyl)-1-(4-methyoxyphenyl)-5-[4-(methylsulfinyl)phenyl]pyrazole) was chemically synthesized at Fujisawa Pharmaceutical (Osaka, Japan).

FR140423 was suspended and diluted in 0.5% methylcellulose for p.o. administration and was dissolved and diluted in 20% ethanol in saline for i.t. and i.c.v. injection. Opioid receptor antagonists were dissolved in saline. Drug solutions were prepared just before starting experiments. P.o. and s.c. injection was performed in a volume of 10 ml/kg of animal weight, and i.t. and i.c.v. injection was done in a volume of 5 μl/mouse. To test the effects of various opioid antagonists on the FR140423-induced antinociception, antagonists were injected s.c., i.t. or i.c.v. immediately before treatment of animals with FR140423. I.t. injection was performed according to the method of Hylden and Wilcox (1980). I.c.v. injection was performed according to the method of Haley and McCormick (1957).

2.6. Statistical analysis

Ten animals were used at each of three to five dose levels to determine the ED_{50} value of a drug. The ED_{50} values and their 95% confidence limits (95% C.L.) were determined by computer using log-linear regression analysis (Litchfield and Wilcoxon, 1949).

3. Results

3.1. Antinociceptive effect of oral administered FR140423

The antinociceptive effect of FR140423 given orally was measured in the tail-pinch test in mice. As shown in

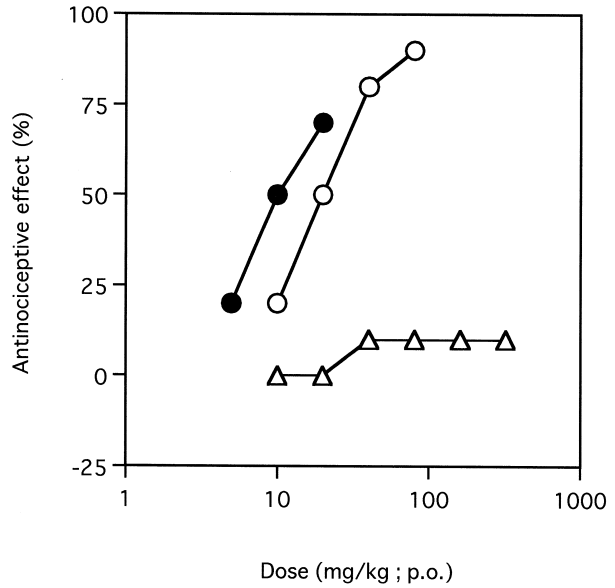

Fig. 1. Antinociceptive effect of orally administered FR140423 in the tail-pinch test in mice. After measuring the normal nociceptive responses, FR140423 (open circle), morphine (closed circle) or indomethacin (open triangle) was administered p.o. The antinociceptive effect was determined by the modified Haffner's method 30 min after drug injection in mice. When the mice did not show the normal nociceptive responses within 6 s after pinching, the antinociceptive effect was regarded as positive ($n = 10$).

Fig. 1, FR140423 was administered p.o. at doses of 10, 20, 40 and 80 mg/kg. At these doses, FR140423 showed a dose-dependent antinociceptive effect with an ED_{50} value (95% C.L.) of 21 (11–32) mg/kg. The ED_{50} value (95% C.L.) for morphine given orally was 11 (4.9-43) mg/kg. On the other hand, indomethacin did not show any antinociceptive effect in the tail-pinch test in mice, even at the highest doses tested, 320 mg/kg. FR140423, at the doses used in this study, did not cause a morphine-like behavioral change such as CNS excitation and Straub's tail response in mice (data not shown).

3.2. Antinociceptive effect of intrathecally or intracerebroventricularly administered FR140423

I.t. injection of FR140423 (1–16 μg/mouse) exhibited an antinociceptive effect in the tail-pinch test with an ED_{50} value (95% C.L.) of 3.1 (1.8–5.7) μg/mouse as shown in Fig. 2A. However, i.c.v. injection of FR140423 (8–64 μg/mouse) did not show an antinociceptive effect (Fig. 2B). The ED_{50} value (95% C.L.) of i.t. and i.c.v. injection of morphine were 1.0 (0.59–2.8) μg/mouse and 0.71 (0.34–1.5) μg/mouse, respectively.

3.3. Effect of naloxone on the FR140423-induced antinociception in the tail-pinch test

The antinociceptive effect of orally administered FR140423 (5–80 mg/kg) in the tail-pinch test was blocked by spinal (100 ng/mouse, i.t.) administration of naloxone, an opioid receptor antagonist (Table 1). However, the antinociception caused by p.o. administered FR140423 was not antagonized by i.c.v. injected naloxone (10 ng/mouse). The antinociceptive effect of orally adminis-

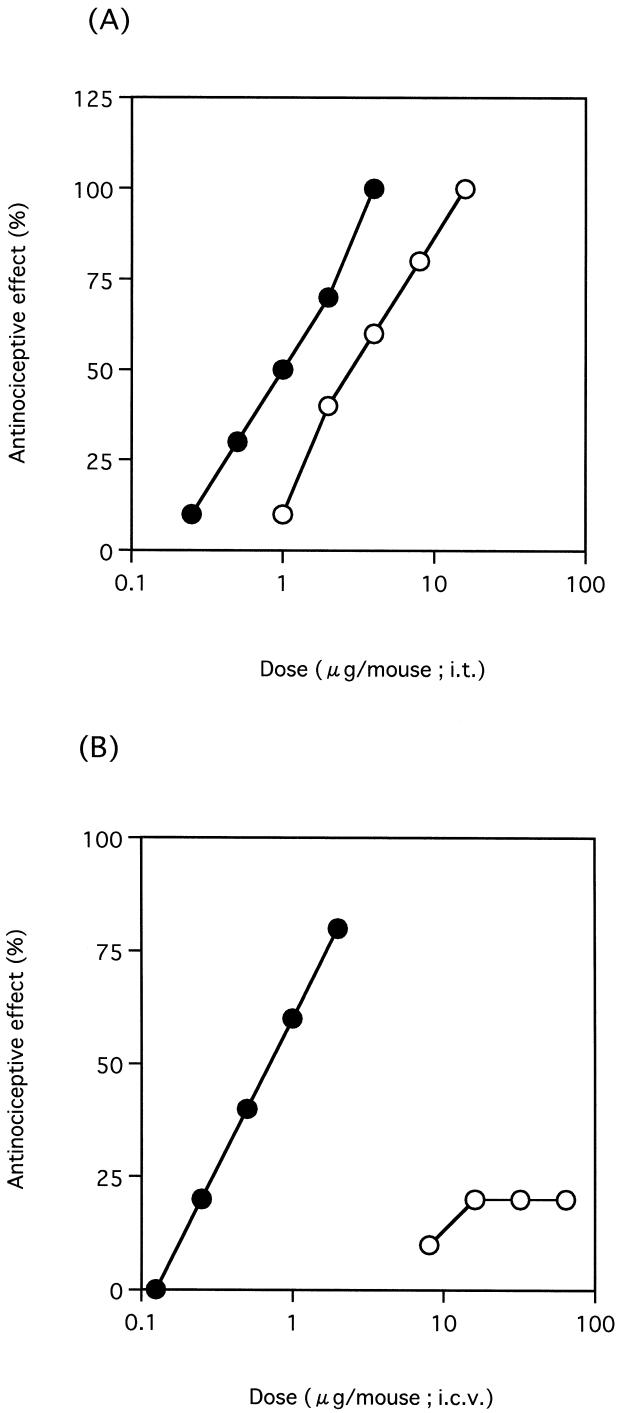

Fig. 2. Antinociceptive effects of i.t. (A) and i.c.v. (B) administered FR140423 in the tail-pinch test in mice. After measuring the normal nociceptive responses, FR140423 (open) or morphine (closed) was administered. The antinociceptive effect was determined by the modified Haffner's method 30 min after drug injection in mice. When the mice did not show the normal nociceptive responses within 6 s after pinching, the antinociceptive effect was regarded as positive ($n = 10$).

Table 1
Effect of i.t. or i.c.v. injected naloxone on FR140423 (p.o.)-induced antinociception in the tail-pinch test
After measuring the normal nociceptive responses, FR140423 or morphine was administered orally. Antagonist naloxone at 100 ng/mouse i.t. or 10 ng/mouse i.c.v. was injected immediately before treatment of drugs. The antinociceptive effect was determined by the modified Haffner's method 30 min after drug injection in mice. When the mice did not show the normal nociceptive responses within 6 s after pinching, the antinociceptive effect was regarded as positive ($n = 10$).

Drug	Antagonist	ED_{50} (95% C.L.) (mg/kg; p.o.)
FR140423	Saline i.t.	20 (9.5–42)
FR140423	Naloxone 100 ng/mouse i.t.	> 80
Morphine	Saline i.t.	10 (5.9–17)
Morphine	Naloxone 100 ng/mouse i.t.	> 80
FR140423	Saline i.c.v.	17 (9.1–41)
FR140423	Naloxone 10 ng/mouse i.c.v.	17 (8.0–31)
Morphine	Saline i.c.v.	11 (5.1–27)
Morphine	Naloxone 10 ng/mouse i.c.v.	> 80

tered morphine (5–40 mg/kg) was completely blocked by central (100 ng/mouse, i.t. or 10 ng/mouse, i.c.v.) administration of naloxone. Moreover, the antinociceptions caused by i.t. injected FR140423 (1–16 μg/mouse) and morphine (0.25–8 μg/mouse) were also antagonized by naloxone 0.2 mg/kg s.c. and 100 ng/mouse i.t. (Table 2).

3.4. Effects of naloxonazine and β-FNA on the FR140423-induced antinociception in the tail-pinch test

I.t. injection of naloxonazine 10 μg/mouse and β-FNA 10 μg/mouse failed to reverse the antinociceptive effects of p.o. or i.t. administered FR140423 (Tables 3 and 4). However, the antinociceptive effect of morphine was antagonized by i.t. injection of naloxonazine 1 μg/mouse and β-FNA 1 μg/mouse.

3.5. Effect of naltorindole on the FR140423-induced antinociception in the tail-pinch test

As shown in Table 5, the antinociceptive effect caused by p.o. FR140423 (5–80 mg/kg) was also antagonized by spinal (10 μg/mouse, i.t.) but not by supraspinal (10 μg/mouse, i.c.v.) administration of naltorindole. Moreover, the antinociceptive effect of i.t. administered FR140423 (1–16 μg/mouse) was blocked by systemic (0.2 mg/kg, s.c.) and spinal (10 μg/mouse, i.t.) administration of naltorindole (Table 6). The antinociceptive effect of morphine was not abolished by naltorindole (10 μg/mouse i.t., 10 μg/mouse i.c.v. and 20 mg/kg s.c.).

Table 2
Effect of s.c. or i.t. injected naloxone on antinociceptive effect of i.t. FR140423 in the tail-pinch test
After measuring the normal nociceptive responses, FR140423 or morphine was administered intrathecally. Antagonist naloxone at 0.2 mg/kg s.c. was injected immediately before treatment of drugs, and naloxone 100 ng/mouse i.t. was co-administered with drugs. The antinociceptive effect was determined by the modified Haffner's method 30 min after drug injection in mice. When the mice did not show the normal nociceptive responses within 6 s after pinching, the antinociceptive effect was regarded as positive ($n = 10$).

Drug	Antagonist	ED_{50} (95% C.L.) (μg/mouse; i.t.)
FR140423	Saline s.c.	3.1 (1.7–5.2)
FR140423	Naloxone 0.2 mg/kg s.c.	> 16
Morphine	Saline s.c.	0.91 (0.37–2.0)
Morphine	Naloxone 0.2 mg/kg s.c.	> 8
FR140423	Saline i.t.	3.4 (1.9–5.8)
FR140423	Naloxone 100 ng/mouse i.t.	> 16
Morphine	Saline i.t.	0.77 (0.35–1.4)
Morphine	Naloxone 100 ng/mouse i.t.	> 8

Table 3
Effects of i.t. injected naloxonazine and β-FNA on FR140423 (p.o.)-induced antinociception in the tail-pinch test
After measuring the normal nociceptive responses, FR140423 or morphine was administered orally. Antagonists, naloxonazine and β-FNA at 1 or 10 μg/mouse i.t., were injected immediately before treatment of drugs. The antinociceptive effect was determined by the modified Haffner's method 30 min after drug injection in mice. When the mice did not show the normal nociceptive responses within 6 s after pinching, the antinociceptive effect was regarded as positive ($n = 10$).

Drug	Antagonist	ED_{50} (95% C.L.) (mg/kg; p.o.)
FR140423	Saline i.t.	19 (12–28)
FR140423	Naloxonazine 10 μg/mouse i.t.	23 (14–41)
FR140423	β-FNA 10 μg/mouse i.t.	17 (11–26)
Morphine	Saline i.t.	11 (6.3–20)
Morphine	Naloxonazine 1 μg/mouse i.t.	> 80
Morphine	β-FNA 1 μg/mouse i.t.	> 80

Table 4
Effects of i.t. co-administered naloxonazine and β-FNA on antinociceptive effect of i.t. FR140423 in the tail-pinch test
After measuring the normal nociceptive responses, FR140423 or morphine was administered intrathecally. Antagonists, naloxonazine and β-FNA at 1 or 10 μg/mouse i.t., were co-administered with drugs. The antinociceptive effect was determined by the modified Haffner's method 30 min after drug injection in mice. When the mice did not show the normal nociceptive responses within 6 s after pinching, the antinociceptive effect was regarded as positive ($n = 10$).

Drug	Antagonist	ED_{50} (95% C.L.) (μg/mouse; i.t.)
FR140423	Saline i.t.	3.0 (1.9–4.4)
FR140423	Naloxonazine 10 μg/mouse i.t.	3.2 (2.1–4.6)
FR140423	β-FNA 10 μg/mouse i.t.	2.8 (1.7–4.1)
Morphine	Saline i.t.	0.80 (0.50–1.2)
Morphine	Naloxonazine 1 μg/mouse i.t.	> 8
Morphine	β-FNA 1 μg/mouse i.t.	> 8

Table 5
Effect of i.t. or i.c.v. injected naltrindole on FR140423(p.o.)-induced antinociception in the tail-pinch test
After measuring the normal nociceptive responses, FR140423 or morphine was administered orally. Antagonist, naltrindole at 10 μg/mouse i.t. or 10 μg/mouse i.c.v., was injected immediately before treatment of drugs. The antinociceptive effect was determined by the modified Haffner's method 30 min after drug injection in mice. When the mice did not show the normal nociceptive responses within 6 s after pinching, the antinociceptive effect was regarded as positive ($n = 10$).

Drug	Antagonist	ED_{50} (95% C.L.) (mg/kg; p.o.)
FR140423	Saline i.t.	22 (13–39)
FR140423	Naltrindole 10 μg/mouse i.t.	> 80
Morphine	Saline i.t.	15 (9.9–24)
Morphine	Naltrindole 10 μg/mouse i.t.	12 (4.8–22)
FR140423	Saline i.c.v.	16 (8.5–25)
FR140423	Naltrindole 10 μg/mouse i.c.v.	11 (4.7–18)
Morphine	Saline i.c.v.	11 (7.1–16)
Morphine	Naltrindole 10 μg/mouse i.c.v.	10 (6.3–15)

3.6. Effect of nor-binaltorphimine on the FR140423-induced antinociception in the tail-pinch test

I.t. injection of nor-binaltorphimine 10 μg/mouse did not attenuate the antinociceptive effects of p.o. or i.t. administered FR140423 and morphine (data not shown).

3.7. Effect of FR140423 on opioid receptor binding

Binding studies were also carried out with membranes prepared from mouse spinal cord. FR140423 (at concentrations of 1–100 μM) was only weakly inhibitory towards [^{3}H] DPDPE binding (9.1% inhibition at 100 μM) to δ-opioid receptors, indicating that FR140423 does not bind with the specific δ-opioid receptors (Fig. 3). Unlike

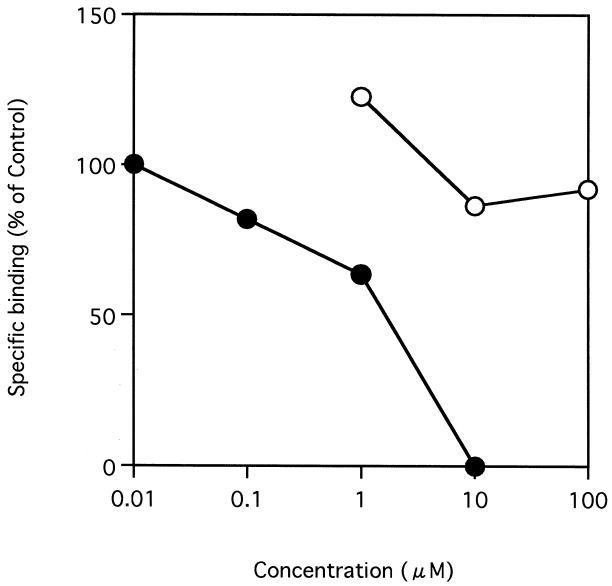

Fig. 3. Effect of FR140423 on specific [^{3}H] DPDPE binding to mouse spinal δ-opioid receptors. Mouse spinal membranes were characterized by utilizing the following specific radiolabeled δ-opioid ligand [^{3}H] DPDPE 2 nM after incubation for 1 h at 25°C. The binding of [^{3}H] DPDPE (2 nM) to the δ-opioid receptors was displaced by the indicated concentrations of FR140423 (open) or morphine (closed) ($n = 3$).

FR140423, morphine, which is a μ-opioid receptor agonist, effectively replaced the [^{3}H] DAMGO, DPDPE and U-69,593 binding with IC_{50} values (95% C.L.) of 3.4 (0.029–87), 950 (110–20000) and 180 (18–1800) nM, respectively. In addition, FR140423 did not bind to the μ- and κ-opioid receptors (data not shown).

3.8. Naloxone-induced jumping and diarrhea tests in mice treated with FR140423

In the jumping and diarrhea tests, morphine-treated mice jumped and produced diarrhea after injection of

Table 6
Effect of s.c. or i.t. injected naltrindole on antinociceptive effect of i.t. FR140423 in the tail-pinch test
After measuring the normal nociceptive responses, FR140423 or morphine was administered intrathecally. Antagonist, naltrindole at 0.2 or 20 mg/kg s.c., was injected immediately before treatment of drugs, and naltrindole 10 μg/mouse i.t. was co-administered with drugs. The antinociceptive effect was determined by the modified Haffner's method 30 min after drug injection in mice. When the mice did not show the normal nociceptive responses within 6 s after pinching, the antinociceptive effect was regarded as positive ($n = 10$).

Drug	Antagonist	ED_{50} (95% C.L.) (μg/mouse; i.t.)
FR140423	Saline s.c.	3.1 (1.4–5.5)
FR140423	Naltrindole 0.2 mg/kg s.c.	> 16
Morphine	Saline s.c.	0.83 (0.40–1.5)
Morphine	Naltrindole 20 mg/kg s.c.	0.83 (0.31–1.7)
FR140423	Saline i.t.	2.9 (1.4–4.9)
FR140423	Naltrindole 10 μg/mouse i.t.	> 16
Morphine	Saline i.t.	0.79 (0.43–1.3)
Morphine	Naltrindole 10 μg/mouse i.t.	0.90 (0.41–1.8)

Table 7
Naloxone-induced jumping and diarrhea tests in mice
Mice were treated with FR140423 (50–200 mg/kg, p.o.) or morphine (25–100 mg/kg, p.o.) twice a day at 0900 and 1900, a total of nine times. Six hours after the final administration of test compounds, naloxone at a dose of 10 mg/kg was injected subcutaneously to the mice and the incidence of jumping and diarrhea was observed for 30 min ($n = 10$).

Drug (mg/kg p.o.)		Incidence of jumping (%)	Incidence of diarrhea (%)
Control		0	0
FR140423	50	0	0
	100	0	0
	200	0	0
Morphine	25	0	50
	50	10	60
	100	50	90

naloxone. On the other hand, no FR140423-treated mice jumped and produced diarrhea, even after injection with 10 mg/kg naloxone (Table 7).

4. Discussion

In the present study, oral administration of FR140423 and morphine produced antinociceptive effect in the tail-pinch test in mice; however, indomethacin, which shows anti-inflammatory and analgesic effects mediated by inhibiting prostaglandins formation in the peripheral site, did not have any effect in this assay. This suggests that cyclooxygenase does not play an important role in nociceptive transmission in the tail-pinch test. FR140423 was discovered as a drug with cyclooxygenase-2 inhibition, but unlike indomethacin FR140423 showed an antinociceptive effect in the tail-pinch test. In general, the tail-pinch test is a useful animal model for evaluating narcotic analgesic agents affecting the CNS, and morphine and codein produce antinociceptive actions in this assay (Takagi et al., 1966). It was strongly suggested that FR140423 exerted another mechanism of action, which was different from cyclooxygenase-2 inhibition, against pain response.

To evaluate the site of action of FR140423 in the tail-pinch test, i.t. and i.c.v. administration of FR140423 were tested. I.t. injection of a small dose of FR140423 was also effective in the tail-pinch assay with the ED_{50} value of 3.1 μg/mouse, which was 200 times lower than the dose required for systemic administration. This technique for i.t. injection in mice appears to be a good method for studying spinally mediated actions of drugs. In the case of i.t. injected [^{3}H] morphine, it has been reported that [^{3}H] morphine is not found in significant quantities in either the midbrain or forebrain, and distributes more than 95% in the spinal cord (Hylden and Wilcox, 1980). When administered i.c.v., FR140423 did not show any antinociceptive effect in the tail-pinch test. These results suggest that the site of action of FR140423 is in the spinal cord, but not in the supraspinal site. Under the same conditions, morphine, a well-known specific μ-opioid receptor agonist, had a potent antinociceptive effect in both i.t. and i.c.v. administeration with ED_{50} values of 1.0 and 0.71 μg/mouse, respectively, which are in complete agreement with the literature (Shimomura et al., 1971; Hylden and Wilcox, 1980). In the supraspinal action, thus, FR140423 clearly differs from morphine, which shows antinociceptions in both spinal and supraspinal site.

To evaluate the mechanism of action of FR140423 in the tail-pinch test, the effect of naloxone, a non-selective opioid antagonist, on the FR140423-induced antinociceptive effect was tested. The FR140423-induced antinociceptive effect was reversed by s.c. administration of naloxone. Moreover, the antinociceptive effect of FR140423 was abolished by i.t. administeration of naloxone. These findings suggest that spinal opioid systems are at least involved in the antinociceptive action of FR140423. On the other hand, FR140423-induced antinociception was not antagonized by i.c.v. administration of naloxone unlike morphine. Therefore, FR140423 does not act through supraspinal opioid receptors.

Morphine inhibited spinal μ-opioid receptor binding using a specific ligand, [^{3}H] DAMGO, with an IC_{50} value of 3.4 nM. However, FR140423 did not have any affinity for any of the opioid receptors in the spinal cord. In other words, FR140423 induces an antinociceptive effect through indirect action on opioid receptors unlike morphine, a μ-opioid receptor agonist. Moreover, FR140423 did not cause a morphine-like behavioral change such as CNS excitation and Straub's tail response at the doses which exerted an antinociceptive effect. For this point, we consider FR140423 to be a unique compound.

The antinociceptive effect of FR140423 in the tail-pinch test was abolished by naltrindole 0.2 mg/kg s.c. and 10 μg/mouse i.t., a selective δ-opioid receptor antagonist. This suggests that the naltrindole-sensitive antinociceptive effect of FR140423 involves interaction with δ-receptors. However, the selective μ-opioid receptor antagonists naloxonazine and β-FNA and κ-opioid receptor antagonist nor-binaltorphimine did not block the FR140423-induced antinociceptive effect. In addition, FR140423 did not bind to any of the opioid receptors in in vitro assays. A possible explanation for these results would be that FR140423 acts indirectly on δ-opioid receptors in mouse spinal cord by causing the release of endogenous opioids such as [Met^5]enkephalin to produce the spinal antinociceptive effect. Thus, the mechanism of action of FR140423 was found to be clearly different from that of morphine which directly binds to μ-opioid receptors. There is, however, no direct evidence that [Met^5]enkephalin is released from opioid nerve terminals by FR140423.

Morphine has been the principal agent in controlling postoperative pain for a long time (Pasternak, 1993); however, the problems of development of tolerance, physical and psychological dependence in long term therapy, respiratory depressant effects, sedation and withdrawal symptoms as potentially serious side effects have arisen (Yaksh et al., 1977). Kamei et al. (1973) reported that physical dependence was developed by sustained or repeated exposure to morphine, and jumping behavior elicited by naloxone was observed in mice to which morphine was administered for a long-term treatment. The supraspinal μ-opioid receptors play a more important role than spinal μ-opioid receptors in withdrawal jumping which may reflect an escape behavior (Miyamoto and Takemori, 1993). Naloxonazine, a specific μ_1-opioid antagonist, selectively blocks morphine analgesia without affecting withdrawal signs such as body weight loss and wet-dog shakes precipitated by naloxone in morphine dependent mice (Ling et al., 1984; Ling et al., 1986). Thus, the withdrawal syndrome might be considered as actions mediated through μ_2-opioid receptors. The reason why mice chronically treated with

FR140423 did not produce the morphine-like physical dependence and withdrawal syndrome such as jumping and diarrhea precipitated by naloxone is that FR140423 indirectly acts on spinal δ-opioid receptors unlike morphine. Nevertheless, to clarify the exact mechanism underlying the antinociceptive effect of FR140423 requires further investigation.

In conclusion, the results of this study provide evidence for involvement of spinal δ-opioid mechanisms in the FR140423-induced antinociception. FR140423 may induce the release of endogenous opioid peptides to produce spinal antinociception.

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