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The antinociceptive effect induced by FR140423 is mediated through spinal 5-HT_{2A} and 5-HT_3 receptors

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Abstract

The involvement of 5-HT receptors in the antinociceptive effect of FR140423, 3-(difluoromethyl)-1-(4-methoxyphenyl)-5-[4-(methylsulfinyl)phenyl]pyrazole, was investigated in mice by means of the tail-pinch test. The antinociceptive effect of FR140423 injected i.t. was completely abolished by co-administration of the non-selective serotonin (5-hydroxytryptamine, 5-HT) receptor antagonist methysergide, the 5-HT_{2A} receptor antagonist ketanserin and the 5-HT_3 receptor antagonist MDL-72222 (3-tropanyl-3,5-dichlorobenzoate) but not by the 5-HT_{2B} receptor antagonist SB-204741 (*N*-(1-methyl-5-indolyl)-*N*′-(3-methylisothiazol-5-yl)urea) or the 5-HT_{2C} receptor antagonist SB-242084 (6-chloro-5-methyl-*N*-[6-(2-methylpyridin-3-yloxy)pyridin-3-yl]indoline-1-carboxamine). The antinociceptive effect of FR140423 administered orally was abolished by i.t., but not by i.c.v., injection of methysergide, ketanserin and MDL-72222. These data indicate that FR140423, unlike morphine, exerts its antinociceptive effect against a mechanical noxious stimulus, such as in the tail-pinch test, by activation of spinal 5-HT_{2A} and 5-HT_3 receptors.

Keywords: FR140423; Antinociception; Serotonergic system; 5-HT_{2A} receptor; 5-HT_3 receptor; Spinal cord; (Mice)

1. Introduction

FR140423, 3-(difluoromethyl)-1-(4-methoxyphenyl)-5-[4-(methylsulfinyl) phenyl]pyrazole, is a cyclooxygenase-2 inhibitor having an anti-inflammatory effect without gastrointestinal side effects (Ochi et al., 1999b; Ochi and Goto, 2000a). Furthermore, FR140423 injected i.t., but not i.c.v., shows an antinociceptive effect in the tail-pinch test used for testing the central analgesic action of narcotic analgesics such as morphine (Ochi et al., 1999a). Thus, we consider that the site of action of FR140423 is in the spinal cord, but not in the supraspinal site.

There are multiple pain-modulating systems within the central nervous system. The brainstem–spinal descending noradrenergic and serotonergic systems function to suppress the transmission of nociceptive information from primary afferent neurons in the spinal dorsal horn (Basbaum and Fields, 1984). The antinociceptive effects of FR140423 are also inhibited by i.t. injection of yohimbine, an α_2-adrenoceptor antagonist (Ochi and Goto, 2000b), suggesting involvement of the brainstem–spinal descending noradrenergic system. However, the role of the brainstem–spinal descending serotonergic system on the FR140423-induced antinociceptive effect remains unknown.

The present study was undertaken to clarify the antinociceptive effect of FR140423 on the serotonergic pain-modulating system in the spinal cord. We assessed the antinociceptive effect of FR140423 in the tail-pinch test in mice. We used various serotonin (5-hydroxytryptamine, 5-HT) receptor antagonists, methysergide, ketanserin, SB-204741, SB-242084 and MDL-72222, and compared these results with those for morphine and 5-HT receptor agonists.

2. Materials and methods

2.1. Animals

Ethical guidelines for the experimental use of animals were followed (Zimmermann, 1983). In addition, the ex-

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perimental work was reviewed 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 maintained in a group of 10 animals for at least 5 days on a 12-h light–dark cycle (light on from 0700 to 1900 h) in a controlled temperature (23 ± 1°C) and humidity (55 ± 5%) environment. The mice were given standard laboratory food and tap water ad libitum before the experiment.

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

The nociceptive response in the tail-pinch test was measured according to the modified Haffner's method as previously reported (Takagi et al., 1966). Briefly, mice were pretested by pinching their tail base with an artery clip (1.5 mm width, 500 g constant force), and only the mice that showed a nociceptive response such as biting the clip or vocalizing within 2 s were used for experiments. When the mice did not show the above-mentioned behaviors up to 6 s after pinching, the antinociceptive effect was regarded as positive. To prevent tissue damage, the pressure stimuli were not applied for more than 10 s. After drug treatments, the nociceptive responses in the tail-pinch test were measured at 15-min intervals for a period of 90 min. The maximal antinociceptive effects of drugs were obtained 30 min after treatment and then their effects gradually decreased. The antinociceptive effect was determined 30 min after drug administration.

2.3. Drugs

The following drugs were used: 1-(2-methoxyphenyl)piperazine HCl, α-methyl-5-HT maleate salt, 2-methyl-5-HT maleate salt, methysergide maleate salt, ketanserin tartrate salt and MDL-72222 (3-tropanyl-3,5-dichlorobenzoate) were obtained from Research Biochemicals International (Natick, MA, USA). Morphine HCl was obtained from Dainippon Pharmaceutical (Osaka, Japan). FR140423, SB-204741 (*N*-(1-methyl-5-indolyl)-*N*′-(3-methylisothiazol-5-yl)urea) and SB-242084 (6-chloro-5-methyl-*N*-[6-(2-methylpyridin-3-yloxy)pyridin-3-yl]indoline-1-carboxamine) were chemically synthesized at Fujisawa Pharmaceutical (Osaka, Japan).

Drugs were dissolved and diluted in 20% ethanol in saline for i.t. and i.c.v. injection, and were suspended and diluted in 0.5% methylcellulose for p.o. administration. Drug solutions were prepared just before experiments started. The p.o. injections were given in a volume of 10 ml/kg of animal weight, and i.t. and i.c.v. injections were given in a volume of 5 μl/mouse. To test the effects of various 5-HT receptor antagonists on the FR140423-induced antinociception, the antagonists mixed with FR140423, morphine or 5-HT agonists were injected i.t. The i.t. injection was given by a modification of the method of Hylden and Wilcox (1980). Briefly, we used a L-shaped hypodermic needle (30 gauge) curved by 90° at 4 mm from the tip. The mouse was held in one hand and the back was slightly bent to open the vertebral column. The needle was inserted into the groove at the L5 and L6 intervertebral space. The i.c.v. injection was performed with a 26-gauge hypodermic needle inserted to a depth of 3 mm into the brain ventricular system (Haley and McCormick, 1957).

2.4. Statistical analysis

Ten animals were used for each of four to five doses to determine the ED_{50} value of a drug. The ED_{50} values and their 95% confidence limits (95% C.L.) were calculated from the dose–percent inhibition relations by computer log–linear regression analysis (Litchfield and Wilcoxon, 1949).

3. Results

3.1. Antinociceptive effect of 5-HT receptor agonists in the tail-pinch test

The antinociceptive effect of various 5-HT receptor agonists given intrathecally was measured in the tail-pinch test in mice. As shown in Fig. 1, i.t. injection of the 5-HT_2 receptor agonist D-methyl-5-HT (0.1–1.6 μg/mouse) and the 5-HT_3 receptor agonist 2-methyl-5-HT (0.05–0.4

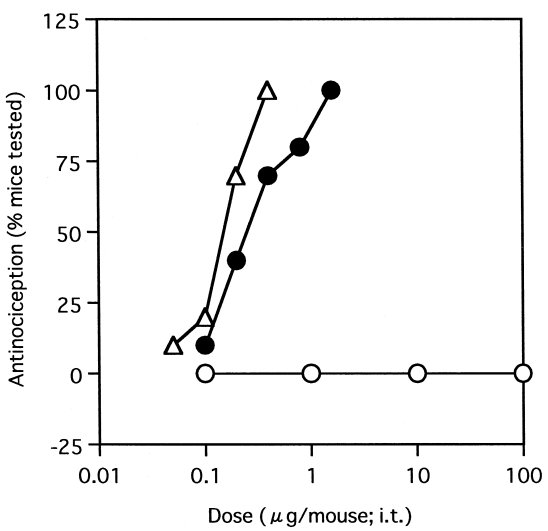

Fig. 1. Antinociceptive effects of i.t.-administered 5-HT receptor agonists in the tail-pinch test in mice. After normal nociceptive responses were measured, 1-(2-methoxyphenyl) piperazine (open circles), α-methyl-5-HT (closed circles) and 2-methyl-5-HT (open triangles) were administered i.t. The antinociceptive effect was determined by the modified Haffner's method 30 min after drug injection in mice. If mice did not show normal nociceptive responses within 6 s of pinching, the antinociceptive effect was regarded as positive ($n = 10$). *Y*-axis gives the percentage of animals showing an antinociceptive effect.

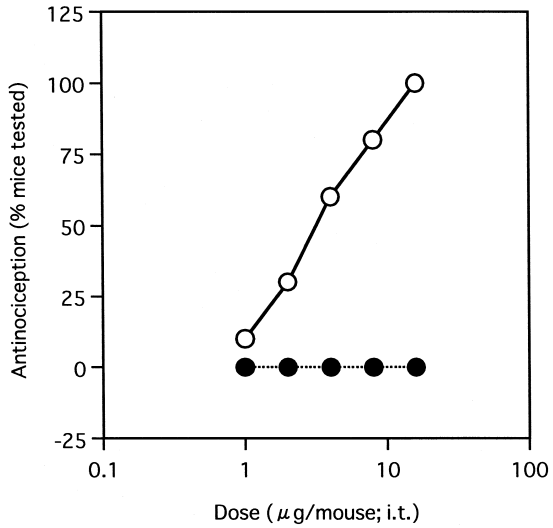

Fig. 2. Effect of methysergide on the FR140423 (i.t.)-induced antinociceptive effect. After normal nociceptive responses were measured, FR140423 was administered intrathecally. The antagonist methysergide (closed circles) at 1 μg/mouse was co-administered with FR140423 (control: open circles). The antinociceptive effect was determined by the modified Haffner's method 30 min after drug injection in mice. If mice did not show normal nociceptive responses within 6 s of pinching, the antinociceptive effect was regarded as positive ($n = 10$).

μg/mouse) had antinociceptive effects with ED_{50} values (95% C.L.) of 0.28 (0.17–0.41) and 0.14 (0.10–0.19) μg/mouse, respectively. I.t. injection of 1-(2-methoxyphenyl)piperazine (0.1–100 μg/mouse), a 5-HT_1 receptor agonist, did not have an antinociceptive effect. α-Methyl-5-HT and 2-methyl-5-HT administered p.o. at doses between 1 and 100 mg/kg did not induce an antinociceptive effect (data not shown).

3.2. Effect of 5-HT receptor antagonists on FR140423 (i.t.)-induced antinociception

The antinociceptive effect of intrathecally administered FR140423 (1–16 μg/mouse), which had an ED_{50} value (95% C.L.) of 3.2 (2.1–4.6) μg/mouse in the tail-pinch test, was blocked by co-administration (1 μg/mouse; i.t.) of methysergide, a non-selective 5-HT receptor antagonist (Fig. 2). The antinociceptive effects of i.t. administered α-methyl-5-HT (0.2–3.2 μg/mouse) and 2-methyl-5-HT (0.1–0.8 μg/mouse) were completely blocked by co-administration (1 μg/mouse; i.t.) of methysergide (Table 1). However, methysergide (10 μg/mouse; i.t.) failed to reverse the morphine (i.t.)-induced antinociception. Methysergide at doses of 1 and 10 μg/mouse i.t. did not

Table 1
Effect of methysergide on the antinociceptive effect of i.t. morphine, α-methyl-5-HT and 2-methyl-5-HT in the tail-pinch test

Drug	Antagonist	ED_{50} (95% C.L.) (μg/mouse; i.t.)
Morphine	Vehicle i.t.	0.74 (0.44–1.1)
Morphine	Methysergide 10 μg/mouse; i.t.	0.87 (0.52–1.4)
α-Methyl-5-HT	Vehicle i.t.	0.28 (0.17–0.41)
α-Methyl-5-HT	Methysergide 1 μg/mouse; i.t.	> 3.2
2-Methyl-5-HT	Vehicle i.t.	0.14 (0.10–0.19)
2-Methyl-5-HT	Methysergide 1 μg/mouse; i.t.	> 0.8

After normal nociceptive responses were measured, drugs were administered intrathecally. Methysergide were co-administered with the drugs. The antinociceptive effect was determined by the modified Haffner's method 30 min after drug injection in mice. If mice did not show normal nociceptive responses within 6 s of pinching, the antinociceptive effect was regarded as positive ($n = 10$). Vehicle–vehicle (20% ethanol in saline) alone had no effect on nociception. Vehicle did not appear to antagonize the antinociception produced by morphine or the 5-HT receptor agonists.

Table 2
Effects of 5-HT receptor antagonists on the antinociceptive effect of i.t. FR140423 and morphine in the tail-pinch test

Drug	Antagonist	ED_{50} (95% C.L.) (μg/mouse; i.t.)
FR140423	Vehicle i.t.	3.2 (2.1–4.6)
FR140423	Ketanserin 10 μg/mouse; i.t.	> 16
FR140423	SB-204741 10 μg/mouse; i.t.	3.4 (2.0–5.4)
FR140423	SB-242084 10 μg/mouse; i.t.	3.7 (2.5–5.4)
FR140423	MDL-72222 10 μg/mouse; i.t.	> 16
Morphine	Vehicle; i.t.	0.74 (0.44–1.1)
Morphine	Ketanserin 10 μg/mouse; i.t.	0.73 (0.41–1.1)
Morphine	SB-204741 10 μg/mouse; i.t.	0.74 (0.47–1.1)
Morphine	SB-242084 10 μg/mouse; i.t.	0.79 (0.43–1.3)
Morphine	MDL-72222 10 μg/mouse; i.t.	0.86 (0.56–1.3)

After normal nociceptive responses were measured, drugs were administered intrathecally. Various 5-HT receptor antagonists at 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. If mice did not show normal nociceptive responses within 6 s of pinching, the antinociceptive effect was regarded as positive ($n = 10$). Vehicle–vehicle (20% ethanol in saline) alone had no effect on nociception. Vehicle did not appear to antagonize the antinociception produced by FR140423 or morphine.

Table 3
Effects of 5-HT receptor antagonists on the antinociceptive effect of i.t. α-methyl-5-HT and 2-methyl-5-HT in the tail-pinch test

Drug	Antagonist	ED_{50} (95% C.L.) (μg/mouse; i.t.)
α-Methyl-5-HT	Vehicle i.t.	0.28 (0.17–0.41)
α-Methyl-5-HT	Ketanserin 10 μg/mouse; i.t.	> 3.2
α-Methyl-5-HT	SB-204741 10 μg/mouse; i.t.	0.29 (0.14–0.49)
α-Methyl-5-HT	SB-242084 10 μg/mouse; i.t.	0.32 (0.19–0.51)
α-Methyl-5-HT	MDL-72222 10 μg/mouse; i.t.	0.35 (0.21–0.55)
2-Methyl-5-HT	Vehicle i.t.	0.14 (0.10–0.19)
2-Methyl-5-HT	Ketanserin 10 μg/mouse; i.t.	0.16 (0.11–0.25)
2-Methyl-5-HT	SB-204741 10 μg/mouse; i.t.	0.15 (0.099–0.24)
2-Methyl-5-HT	SB-242084 10 μg/mouse; i.t.	0.15 (0.099–0.24)
2-Methyl-5-HT	MDL-72222 10 μg/mouse; i.t.	> 0.8

After normal nociceptive responses were measured, drugs were administered intrathecally. Various 5-HT receptor antagonists at 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. If mice did not show normal nociceptive responses within 6 s of pinching, the antinociceptive effect was regarded as positive ($n = 10$). Vehicle–vehicle (20% ethanol in saline) alone had no effect on nociception. Vehicle did not appear to antagonize the antinociception produced by the 5-HT receptor agonists.

produce an antinociceptive effect. To determine possible mediation through 5-HT receptor subtypes in the mechanism of the antinociceptive effect of FR140423, the effects of selective antagonists of the 5-HT_{2A}, 5-HT_{2B}, 5-HT_{2C} and 5-HT_3 receptors on the antinociception of FR140423 were examined. The antinociceptive effect of FR140423 was antagonized by co-administration (10 μg/mouse; i.t.) of the 5-HT_{2A} receptor antagonist ketanserin and the 5-HT_3 receptor antagonist MDL-72222 but not by the 5-HT_{2B} receptor antagonist SB-204741 or the 5-HT_{2C} receptor antagonist SB-242084 (Table 2). However, the antinociceptive effect of morphine was not reversed by these selective 5-HT antagonists. The antinociceptive effect of i.t. administered α-methyl-5-HT (0.2–3.2 μg/mouse) was completely blocked by co-administration (10 μg/mouse) of ketanserin but not SB-204741, SB-242084 or MDL-72222 (Table 3). The antinociceptive effect of i.t. administered 2-methyl-5-HT (0.1–0.8 μg/mouse) was completely blocked by co-administration (10 μg/mouse) of MDL-72222 but not ketanserin, SB-204741 or SB-242084. These antagonists at a dose of 10 μg/mouse i.t. did not produce an antinociceptive effect.

3.3. Effects of i.t. or i.c.v. administration of methysergide, ketanserin and MDL-72222 on FR140423 (p.o.)-induced antinociception

FR140423 (5–80 mg/kg) administered orally induced antinociception in a dose-dependent manner with an ED_{50} value (95% C.L.) of 20 (13–32) mg/kg. This antinociceptive effect was blocked by i.t. administration of methysergide at a dose of 1 μg/mouse (Table 4). However, methysergide at a dose of 10 μg/mouse i.c.v. failed to reverse the antinociceptive effect of FR140423 (5–80 mg/kg) administered orally. Furthermore, ketanserin and MDL-72222 administered i.t., but not i.c.v., at a dose of 10 μg/mouse attenuated the antinociceptive effects of p.o. administered FR140423. These antagonists at a dose of 10 μg/mouse i.c.v. did not produce an antinociceptive effect.

Table 4
Effects of i.t. or i.c.v. injected 5-HT receptor antagonists on FR140423 (p.o.)-induced antinociceptive effect

Drug	Antagonist	ED_{50} (95% C.L.) (mg/kg; p.o.)
FR140423	Vehicle i.t.	20 (13–32)
FR140423	Methysergide 1 μg/mouse i.t.	> 80
FR140423	Ketanserin 10 μg/mouse i.t.	> 80
FR140423	MDL-72222 10 μg/mouse i.t.	> 80
FR140423	Vehicle i.c.v.	20 (13–30)
FR140423	Methysergide 10 μg/mouse i.c.v.	19 (11–30)
FR140423	Ketanserin 10 μg/mouse i.c.v.	18 (9.6–33)
FR140423	MDL-72222 10 μg/mouse i.c.v.	21 (14–32)

After normal nociceptive responses were measured, FR140423 was administered orally. Various 5-HT receptor antagonists were injected immediately before treatment with FR140423. The antinociceptive effect was determined by the modified Haffner's method 30 min after drug injection in mice. If mice did not show normal nociceptive responses within 6 s of pinching, the antinociceptive effect was regarded as positive ($n = 10$). Vehicle (0.5% methylcellulose)–vehicle (20% ethanol in saline) had no effect on nociception. Vehicle did not appear to antagonize the antinociception produced by FR140423.

4. Discussion

Descending pathways originating in the brainstem–spinal monoaminergic systems play an important role in the modulation of nociceptive messages in the dorsal horn of the spinal cord. Noradrenergic and serotonergic systems comprise major components of these descending mechanisms (Yaksh et al., 1981). We previously reported that i.t. injection of the α_2-adrenoceptor antagonist yohimbine reduced the antinociceptive activity of FR140423, and suggested that the spinal α_2-adrenoceptor system was involved in the action of FR140423 (Ochi and Goto, 2000b). In the present study, we found that FR140423-induced antinociception in the tail-pinch test was antagonized by methysergide, a non-selective 5-HT receptor antagonist. This result suggests that the spinal serotonergic system plays an important role in the mechanism of antinociceptive effect of FR140423.

Multiple types of 5-HT receptors have been identified by radioligand binding techniques and molecular cloning, and each subtype of 5-HT has a distinct tissue distribution. There are several 5-HT receptors located on the spinal cord, and individual 5-HT receptor types play different roles in the control of nociception at the level of the dorsal horn. Thus, serotonergic mechanisms in the dorsal horn exert both antinociceptive and pronociceptive actions via different 5-HT receptor subtypes. For instance, Alhaider and Wilcox (1993) have shown that 5-HT_{1A} receptor agonists given i.t. are pronociceptive in the tail-flick test in mice. However, i.t. injection of 5-HT and the selective 5-HT_3 receptor agonist 2-methyl-5-HT produces antinociceptive activity that is blocked by methysergide in the tail-flick and hot-plate tests (Yaksh and Wilson, 1979; Glaum et al., 1990). We found here that spinally administered α-methyl-5-HT, a 5-HT_2 receptor agonist, and 2-methyl-5-HT, a 5-HT_3 receptor agonist, had an antinociceptive effect in the tail-pinch test. This result suggests that activation of 5-HT_2 and 5-HT_3 receptors in the spinal cord evokes antinociception. To determine the possible role of serotonergic receptor subtypes (5-HT_{2A}, 5-HT_{2B}, 5-HT_{2C} and 5-HT_3) in the mechanism of the spinal FR140423-induced antinociception that was reduced by the non-selective 5-HT receptor antagonist methysergide, the effects of the above selective antagonists of 5-HT receptors on the antinociception induced by FR140423 were tested. In our studies, the spinal antinociceptive effect of FR140423 was inhibited by co-administration of the

5-HT_{2A} receptor antagonist ketanserin and the 5-HT_3 receptor antagonist MDL-72222. However, the 5-HT_{2B} receptor antagonist SB-204741 and the 5-HT_{2C} receptor antagonist SB-242084 could not block the FR140423-induced antinociceptive effect. Furthermore, FR140423 (i.t.)-induced antinociception was not inhibited by SDZ-205,557, a 5-HT_4 receptor antagonist (data not shown). These data support the hypothesis that 5-HT_{2A} and 5-HT_3 receptors in the spinal cord are involved in the antinociception caused by FR140423 in the tail-pinch test; however, the FR140423-induced antinociceptive effect was not antagonized by i.c.v. administration of some 5-HT receptor antagonists. When administered i.c.v., FR140423 did not show any antinociceptive effect in the tail-pinch test (Ochi et al., 1999a). The site of action of FR140423 is not in the supraspinal site. In addition, the 5-HT receptor agonists, α-methyl-5-HT and 2-methyl-5-HT, after i.c.v. injection at doses between 0.1 and 100 μg/mouse did not induce antinociceptive effects (data not shown). These findings suggest that supraspinal 5-HT receptors are not involved in nociceptive transmission after mechanical noxious stimulation.

Morphine is a potent analgesic agent with many central sites of action (Yaksh et al., 1977; Kolesnikov et al., 1996). The descending serotonergic pain-modulating pathway is an important component in morphine-induced antinociception (Takagi, 1980). Microinjection of morphine into the periaqueductal gray causes the release of 5-HT from the spinal cord in a manner antagonizable by naloxone (Yaksh and Tyce, 1979). The depletion of 5-HT in the spinal cord markedly reduces the analgesic action of morphine in the tail-flick and hot-plate tests but not in the tail-pinch test (Kuraishi et al., 1983; Matsumoto et al., 1996). In agreement with these reports, our data for the tail-pinch test indicate that the serotonergic system is not involved in morphine (i.t.)-induced antinociception against mechanical noxious stimulus.

Substance P is released from the spinal dorsal horn in response to mechanical stimulation (Kuraishi et al., 1985; Oku et al., 1987; Kuraishi et al., 1989). Functional interactions between substance P and 5-HT systems have been reported such that the evoked release of substance P from the trigeminal nucleus of the rabbit is reduced by 5-HT, in a methysergide-reversible manner (Yonehara et al., 1991). In addition to this, a 5-HT receptor agonist is able to reduce the Ca^{2+}-dependent electrically evoked release of substance P from the rat spinal cord (Arvieu et al., 1996). Thus, we speculated that FR140423 may interact with endogenous 5-HT to induce its antinociceptive effect in the spinal cord. However, the effect of FR140423, which acts through spinal 5-HT_{2A} and 5-HT_3 receptors, on the release of 5-HT and substance P in the spinal nervous system remains unknown. Further studies are needed to clarify whether FR140423 inhibits the release of substance P and other transmitters mediating nociceptive information in the dorsal horn.

In conclusion, the antinociceptive effect of FR140423 in the tail-pinch test in mice was significantly blocked by i.t. but not by i.c.v. administration of 5-HT_{2A} and 5-HT_3 receptor antagonists. The present study shows that spinal but not supraspinal 5-HT_{2A} and 5-HT_3 receptors play an important role in the antinociceptive activity of FR140423 against mechanical noxious stimulation. The mechanism underlying the action of FR140423 may differ from that of morphine.

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