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The antinociceptive effect of FR140423 in mice: involvement of spinal α_2-adrenoceptors

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Abstract

We investigated the role of the spinal noradrenergic system in the antinociceptive effect of FR140423, 3-(difluoromethyl)-1-(4-methoxyphenyl)-5-[4-(methylsulfinyl)phenyl]pyrazole, by using the tail-pinch test in mice and various adrenoceptor antagonists. The antinociceptive effect of FR140423 injected i.t. was completely abolished by co-administration of the non-selective α-adrenoceptor antagonist phentolamine and the α_2-adrenoceptor antagonist yohimbine but not by the α_1-adrenoceptor antagonist prazosin or the β-adrenoceptor antagonist propranolol. Oral administration of FR140423, at doses of 5–80 mg/kg, produced a dose-dependent antinociceptive effect with an ED_{50} value of 19 mg/kg. This antinociception was abolished by i.t., but not i.c.v., injection of phentolamine and yohimbine (10 μg/mouse). These results suggest that α_2-adrenoceptors in the spinal cord are involved in the antinociceptive effect of FR140423 against mechanical noxious stimulus as they are in the effect of morphine and clonidine.

Keywords: FR140423; Antinociception; Noradrenergic system; α_2-Adrenoceptor; Spinal cord; (Mouse)

1. Introduction

FR140423 (3-(difluoromethyl)-1-(4-methoxyphenyl)-5-[4-(methylsulfinyl)phenyl]pyrazole) is a potent analgesic compound discovered during the screening of a series of novel pyrazole derivatives with the Randall–Selitto method (Ochi et al., 1999b). We have previously found that FR140423 has an antinociceptive effect when administered p.o. and i.t. but not i.c.v. in the tail-pinch test in mice, and that the effects of FR140423 administered p.o. and i.t. are blocked by s.c. and i.t. naloxone, an opioid receptor antagonist, thus indicating the involvement of spinal opioid receptors in the action of FR140423 (Ochi et al., 1999a).

The brainstem–spinal monoaminergic systems contribute to the antinociceptive effect of opioids (Takagi, 1980; Wigdor and Wilcox, 1987). The brainstem–spinal monoaminergic systems play important roles in the transmission of nociceptive information from primary afferent neurons in the spinal dorsal horn, having a modulatory role in pain processing (Basbaum and Fields, 1984). For example, i.t. injection of an α_2-adrenoceptor antagonist reduces the antinociceptive effect of morphine, which suggests that the brainstem–spinal noradrenergic system is involved (Proudfit, 1988).

The present study was undertaken to clarify the antinociceptive effect of FR140423 on the noradrenergic pain-modulating system in the spinal cord. We assessed the antinociceptive effect of FR140423 in the tail-pinch test in mice. We used various adrenoceptor antagonists, phentolamine, prazosin, yohimbine and propranolol, and compared these results with those of morphine and adrenoceptor agonists.

2. Materials and methods

2.1. Animals

Ethical guidelines for the experimental use of animals were followed (Zimmermann, 1983). In addition, the experimental work was reviewed by the Fujisawa Pharma-

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ceutical 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

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 a 1.5-mm wide artery clip, exerting a force 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 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 antinociceptive effect was determined 30 min after drug administration.

2.3. Drugs

The following drugs were used: L-phenylephrine HCl, clonidine HCl, (−)-isoproterenol (+)-bitartrate, phentolamine HCl, prazosin HCl and propranolol HCl were obtained from Sigma (St. Louis, MO, USA). Yohimbine HCl was obtained from Nacalai Tesque (Kyoto, Japan). Morphine HCl was obtained from Dainippon Pharmaceutical (Osaka, Japan). FR140423 was chemically synthesized at Fujisawa Pharmaceutical.

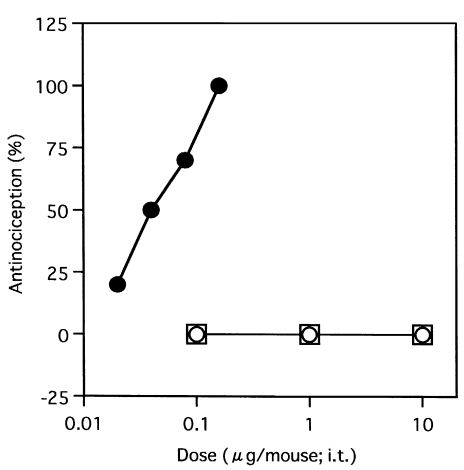

Fig. 1. Antinociceptive effects of i.t. administered adrenoceptor agonists in the tail-pinch test in mice. After normal nociceptive responses were measured, phenylephrine (open circle), clonidine (closed circle) and isoproterenol (open square) were administered i.t. The antinociceptive effect was determined 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 antinoceceptive effect.

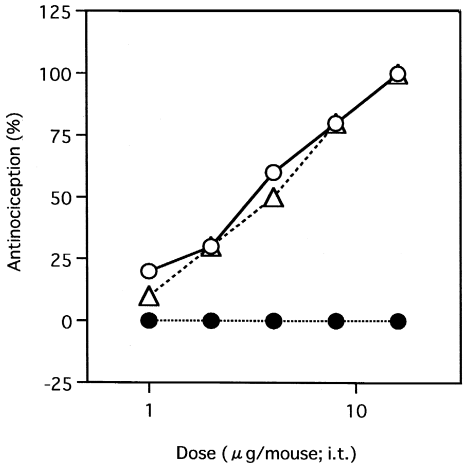

Fig. 2. Effects of adrenoceptor antagonists on the FR140423 (i.t.)-induced antinociceptive effect. After normal nociceptive responses were measured, FR140423 was administered intrathecally. Antagonists phentolamine (closed circle) and propranolol (open triangle) at 10 μg/mouse were co-administered with FR140423 (control: open circle). 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 ($n = 10$).

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. P.o. 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 adrenoceptor antagonists on the FR140423-induced antinociception, antagonists were injected i.t. or i.c.v. immediately before treatment of animals with FR140423. I.t. injection was given by a modification of the method of Hylden and Wilcox (1980). Briefly, we used an 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. 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

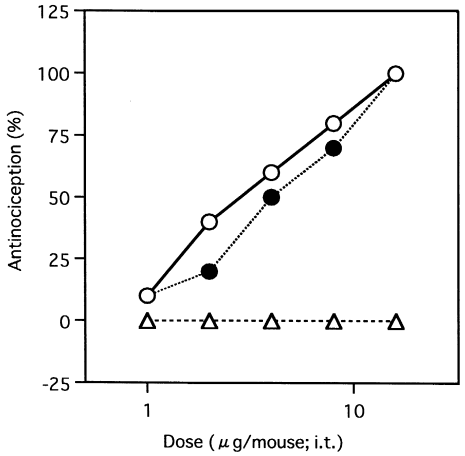

Fig. 3. Effects of α-adrenoceptor antagonists on the FR140423 (i.t.)-induced antinociceptive effect. After normal nociceptive responses were measured, FR140423 was administered intrathecally. Antagonists prazosin (closed circle) and yohimbine (open triangle) at 10 μg/mouse were co-administered with FR140423 (control: open circle). 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 ($n = 10$).

from the dose-percent inhibition relations by computer log–linear regression analysis (Litchfield and Wilcoxon, 1949).

3. Results

3.1. Antinociceptive effect of intrathecally administered adrenoceptor agonists in the tail-pinch test

The antinociceptive effect of adrenoceptor agonists given intrathecally was measured in the tail-pinch test in mice. As shown in Fig. 1, i.t. injection of clonidine (0.02–0.16 μg/mouse) had an antinociceptive effect with an ED_{50} value (95% C.L.) of 0.032 (0.020–0.044) μg/mouse. I.t. injection of phenylephrine and isoproterenol (0.1–10 μg/mouse) did not have an antinociceptive effect.

Table 1
Effects of adrenoceptor antagonists on antinociceptive effect of i.t. morphine and clonidine in the tail-pinch test

Antagonist	Drug: ED_{50} (95% C.L.) (μg/mouse; i.t.)	
	Morphine	Clonidine
Vehicle, i.t.	0.93 (0.59–1.4)	0.036 (0.022–0.052)
Phentolamine 10 μg/mouse, i.t.	> 8	> 0.32
Prazosin 10 μg/mouse, i.t.	0.85 (0.46–1.4)	0.033 (0.016–0.048)
Yohimbine 10 μg/mouse, i.t.	> 8	> 0.32
Propranolol 10 μg/mouse, i.t.	1.0 (0.66–1.5)	0.034 (0.022–0.047)

After normal nociceptive responses were measured, drugs were administered intrathecally. Adrenoceptor 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 ($n = 10$).

3.2. Effect of adrenoceptor antagonists on the 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 3.0 (1.9–4.4) μg/mouse in the tail-pinch test, was blocked by co-administration (10 μg/mouse; i.t.) of phentolamine, a non-selective α-adrenoceptor antagonist (Fig. 2). However, this antinociception was not antag-

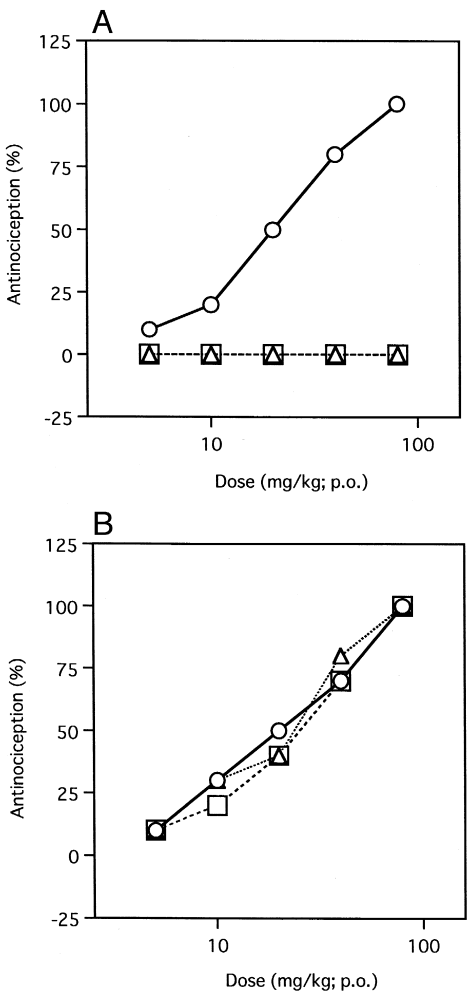

Fig. 4. Effects of i.t. or i.c.v. injected yohimbine and phentolamine on FR140423 (p.o.)-induced antinociceptive effect. After normal nociceptive responses were measured, FR140423 was administered orally. Antagonists, yohimbine (square) and phentolamine (triangle) at 10 μg/mouse i.t. (A) or 10 g/mouse i.c.v. (B), were injected immediately before treatment of FR140423 (control: circle). 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 ($n = 10$).

onized by co-administration (10 μg/mouse; i.t.) of propranolol, a β-adrenoceptor antagonist. To determine possible mediation through α-adrenoceptors in the mechanism of the antinociceptive effect of FR140423, the effects of selective antagonists of α_1- and α_2-adrenoceptors on the antinociception of FR140423 were examined. The antinociceptive effect of FR140423 was antagonized by co-administration of the α_2-adrenoceptor antagonist yohimbine but not the α_1-adrenoceptor antagonist prazosin (Fig. 3). The antinociceptive effects of i.t. administered morphine (0.5–8 μg/mouse) and clonidine (0.04–0.32 μg/mouse) were completely blocked by co-administration (10 μg/mouse) of phentolamine and yohimbine but not prazosin or propranolol (Table 1). 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 α_2-adrenoceptor antagonists yohimbine and phentolamine on the 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 19 (12–27) mg/kg. This antinociceptive effect was blocked by i.t. administration of yohimbine at a dose of 10 μg/mouse (Fig. 4A). However, yohimbine at a dose of 10 μg/mouse i.c.v. failed to reverse the antinociceptive effect of FR140423 (5–80 mg/kg) administered orally (Fig. 4B). The antinociceptive effects of p.o. administered morphine (2.5–40 mg/kg) and clonidine (0.002–0.064 mg/kg), which had ED_{50} values (95% C.L.) of 8.6 (5.6–13) and 0.0091 (0.0056–0.013) mg/kg, respectively, were completely antagonized by i.t., but not i.c.v., administration of yohimbine at a dose of 10 μg/mouse (Table 2). Furthermore, phentolamine administered i.t., but not i.c.v., attenuated the antinociceptive effects of p.o. administered of FR140423, morphine and clonidine. These antagonists at a dose of 10 μg/mouse i.c.v. did not produce an antinociceptive effect.

Table 2
Effects of adrenoceptor antagonists on antinociceptive effect of p.o. morphine and clonidine in the tail-pinch test

Antagonist	Drug: ED_{50} (95% C.L.) (mg/kg; p.o.)	
	Morphine	Clonidine
Vehicle, i.t.	8.6 (5.6–13)	0.0091 (0.0056–0.013)
Phentolamine 10 μg/mouse, i.t.	> 80	> 0.064
Yohimbine 10 μg/mouse, i.t.	> 80	> 0.064
Vehicle, i.c.v.	9.9 (6.7–14)	0.0098 (0.0067–0.014)
Phentolamine 10 μg/mouse, i.c.v.	10 (6.4–15)	0.011 (0.0073–0.015)
Yohimbine 10 μg/mouse, i.c.v.	8.6 (5.2–12)	0.0096 (0.0053–0.016)

After normal nociceptive responses were measured, drugs were administered orally. Adrenoceptor antagonists at 10 μg/mouse i.t. or i.c.v. were injected immediately before drug treatment. 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 ($n = 10$).

4. Discussion

We previously reported that FR140423 dose dependently exerted antinociceptive activity in the tail-pinch test in mice following p.o. or i.t. but not i.c.v. administration, and suggested that the spinal δ-opioid receptor system was involved in the action of FR140423, since the action of FR140423 was antagonized by i.t. naltrindole, a δ-opioid receptor antagonist (Ochi et al., 1999a). In the present study, we found that the antinociceptive effect of spinally and systemically administered FR140423 in the tail-pinch test could be attributed to the spinal noradrenergic system.

In general, the major spinal noradrenergic system mediating antinociception is believed to be of the α_2-subtype, on the basis of reports that i.t. administered of α_2-adrenoceptor agonists have powerful antinociceptive effects and that α_2-adrenoceptor antagonists administered i.t. block the antinociception produced by i.t. injection of noradrenaline and activation of the descending noradrenergic system, indicating involvement of the spinal α_2-adrenoceptors in antinociception (Wilcox et al., 1987; Takano and Yaksh, 1992). Moreover, α_2-adrenoceptor agonists such as dexmedetomidine, clonidine and ST-91, but not α_1-adrenoceptor agonists, dose dependently reverse the allodynia (Yaksh et al., 1995). In our present study, spinally administered clonidine, an α_2-adrenoceptor agonist, but neither phenylephrine nor isoproterenol showed dose-dependent and robust antinociceptive properties in the tail-pinch test, and this effect was antagonized by yohimbine. This is believed to reflect an α_2-adrenoceptor interaction, supporting the above evidence.

The dorsal horn of the spinal cord is a major locus of the antinociceptive actions of α_2-adrenoceptor agonists. The antinociceptive effect of FR140423 administered i.t. in the tail-pinch test was abolished by co-administration of yohimbine, an α_2-adrenoceptor antagonist. However, the α_1-adrenoceptor antagonist prazosin and the β-adrenoceptor antagonist propranolol did not block the FR140423-induced antinociceptive effect. These results indicate that α_2-adrenoceptors in the spinal cord are involved in the production of antinociception by FR140423. There is, however, no direct evidence whether FR140423 activates the descending pathway by acting in medullary adrenergic cell bodies or acts on their spinal nerve terminals of the descending modulatory pathway to release endogenous noradrenaline.

Morphine is a potent analgesic agent with many central sites of action (Rang and Urban, 1995). At the spinal level, morphine acts through the μ-opioid receptor localized within the dorsal horn (Stevens, 1996). The noradrenergic descending system has also been strongly implicated in

morphine-induced antinociception. Wigdor and Wilcox (1987) have reported that phentolamine and yohimbine are both more effective than the α_1-adrenoceptor antagonist prazosin in blocking antinociception produced by systemically administered morphine, as measured in the tail-flick test in rats. In agreement with this report, our data indicate that the spinal noradrenergic system activated by i.t. administered morphine may act via α_2-adrenoceptors to cause antinociception. The spinal antinociceptive profile of the μ-opioid receptor agonist morphine, whose effects are antagonized by yohimbine, is similar to that of FR140423, which acts through spinal δ-opioid receptors. These results suggest that interactions of opioid receptors with the spinal noradrenergic system in mice are mediated by not only the μ-opioid receptor subtype but also the δ-opioid receptor subtype.

The release of substance P from spinal cord slices induced by veratridine or high K^+-evoked depolarization is reduced by noradrenaline, α_2-adrenoceptor agonist and opioids such as morphine (Jessell and Iversen, 1977; Pang and Vasko, 1986; Ono et al., 1991). Further, in experiments using a push–pull cannula implanted into the rabbit spinal dorsal horn in situ, Kuraishi et al. (1985) reported that noradrenaline inhibited the release of substance P provoked by a noxious mechanical stimulus in a yohimbine-reversible manner. However, the effect of FR140423, which acts through spinal α_2-adrenoceptors, on the release of substance P in the spinal nervous system remains unknown. To clarify whether FR140423 inhibits the release of substance P and other transmitters mediating nociceptive information in the dorsal horn requires further investigation.

In conclusion, the antinociception of FR140423 in the tail-pinch test in mice was significantly blocked by i.t. but not by i.c.v. administration of α_2-adrenoceptor antagonists. The present study shows that spinal but not supraspinal α_2-adrenoceptors play an important role in the antinociceptive activity of FR140423 against mechanical noxious stimulation.

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