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FR143166 attenuates spinal pain transmission through activation of the serotonergic system

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Abstract

We investigated the antinociceptive effect of 1-(4-fluorophenyl)-3-methyl-5-[4-(methylsulfinyl)phenyl]pyrazole (FR143166) in the tail-pinch test in mice. The p.o. and i.t. injection of FR143166 exerted dose-dependent antinociceptive actions with ED_{50} values of 24 mg/kg and 15 μg/mouse, respectively. However, i.c.v. injection of FR143166 at a maximum dose of 128 μg/mouse did not show any antinociceptive effect. The antinociceptive effect of FR143166 injected i.t. was abolished by co-administration of the nonselective serotonin (5-hydroxytryptamine, 5-HT) receptor antagonist, methysergide, but not by the adrenoceptor antagonists, phentolamine and propranolol. Moreover, the effect of FR143166 was also reversed by the 5-HT_{2A} receptor antagonist, ketanserin, and the 5-HT_3 receptor antagonist, MDL-72222 (3-tropanyl-3,5-dichlorobenzoate). The effect of FR143166 was attenuated by *p*-chlorophenylalanine, but not by 6-hydroxydopamine plus nomifensine pretreatment. These results suggest that the descending serotonergic system, especially spinal 5-HT_{2A} and 5-HT_3 receptors, is involved in the antinociceptive activity of spinally administered FR143166 on noxious mechanical stimuli.

Keywords: FR143166; Antinociception; Descending serotonergic system; 5-HT_{2A} receptor; 5-HT_3 receptor; Spinal cord

1. Introduction

Analgesics that are currently in use may be broadly classified as either opiates, such as morphine, codeine and pentazocine, or nonsteroidal anti-inflammatory drugs (NSAIDs), such as aspirin and indomethacin (Rang and Urban, 1995). While NSAIDs inhibit the formation of prostaglandins induced by inflammation in peripheral sites (Vane, 1971; Ochi et al., 1999), the typical opiate, morphine, exerts a potent analgesic effect through a mechanism involving synergism of opioid systems in the many central sites (Pasternak, 1993). In addition, descending monoaminergic systems participate in the production of an antinociceptive effect by systemically or supraspinally administered opiates (Takagi, 1980; Wigdor and Wilcox, 1987).

There are multiple descending pain-modulating systems within the central nervous system (CNS). The brainstem–spinal descending noradrenergic and serotonergic systems function to suppress the pain transmission from primary afferent neurones in the spinal dorsal horn, thereby playing a modulatory role in pain processing (Fitzgerald, 1986). For example, α_2-adrenoceptor agonists exhibit antinociceptive activity (Takano and Yaksh, 1992; Kawabata et al., 1994; Ghelardini et al., 2000). Additionally, i.t. injection of serotonin (5-hydroxytryptamine, 5-HT) and of the several 5-HT receptor agonists dose-dependently exerts an antinociceptive effect in various animal models (Bardin et al., 2000; Takeshita and Yamaguchi, 1995; Obata et al., 2001).

In a screening test of antinociceptive activity of chemical compounds, we recently found 1-(4-fluorophenyl)-3-methyl-5-[4-(methylsulfinyl)phenyl]pyrazole (FR143166), a newly synthetic pyrazole derivative. The chemical structure of the compound is shown in Fig. 1. We examined the pharmacological profile focusing on the antinociceptive effect caused by FR143166 in the tail-pinch test in mice, and compared these results with those obtained with morphine. The present study was undertaken to characterize the

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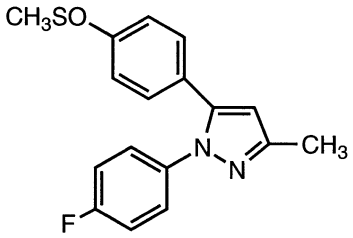

Fig. 1. Chemical structure of FR143166.

role of descending monoaminergic systems in the antinociceptive effect of FR143166.

2. Materials and methods

2.1. Animals

Experiments were conducted in accordance with the ethical guidelines of the International Association for the Study of Pain (Zimmermann, 1983). In addition, the experimental work was reviewed by the Animal Ethical Committee of Fujisawa Pharmaceutical for Animal Experimentation.

Male ddY mice (28–33 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 therefore determined 30 min after drug administration.

2.3. Monoamine depletion

The method of Matsumoto et al. (1996) was used. For central 5-HT depletion, mice were injected i.p. with three doses of the 5-HT synthesis inhibitor, *p*-chlorophenylalanine (300 mg/kg each), 72, 48 and 24 h before the experiments. For central noradrenaline depletion, 6-hydroxydopamine (50 μg/mouse), a noradrenaline depletor, was given i.c.v. 7 days before the experiments. Mice were pretreated with nomifensine (5 mg/kg, i.p.), a selective dopamine uptake blocker, to protect dopaminergic systems 30 min before 6-hydroxydopamine injection.

2.4. Drugs

The following drugs were used: methysergide maleate, mesulergine HCl, ketanserin tartrate, 3-tropanyl-3,5-dichlorobenzoate (MDL-72222; Fozard, 1984), 2-diethylaminoethyl-(2-methoxy-4-amino-5-chloro)benzoate HCl (SDZ-205,557 HCl) and nomifensine maleate were obtained from Research Biochemicals International (Natick, MA, USA). Phentolamine HCl, propranolol HCl, *p*-chlorophenylalanine methyl ester HCl and 6-hydroxydopamine HBr were obtained from Sigma (St. Louis, MO, USA). Morphine HCl was obtained from Dainippon Pharmaceutical (Osaka, Japan). FR143166, *N*-(1-methyl-5-indolyl)-*N*′-(3-methylisothiazol-5-yl)urea (SB-204741) and 6-chloro-5-methyl-*N*-[6-(2-methylpyridin-3-yloxy)pyridin-3-yl]indoline-1-carboxamine (SB-242084) were chemically synthesized at Fujisawa Pharmaceutical (Osaka, Japan).

Drugs were suspended and diluted in 0.5% methylcellulose for p.o. administration, and were dissolved and diluted in 20% ethanol in saline for i.t. and i.c.v. injections. Various monoamine depleters were dissolved in saline. Drug solutions were prepared just before experiments started. The p.o. and i.p. injection was given in a volume of 10 ml/kg of animal weight, and i.t. and i.c.v. injections in a volume of 5 μl/mouse. To test the effects of various 5-HT receptor antagonists on FR143166-induced antinociception, the antagonists mixed with these drugs were injected i.t. The i.t. injection was performed according to a modification of the method of Hylden and Wilcox (1980). Briefly, we used an L-shaped hypodermic needle (30 gauge) bent at a 90° angle 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).

2.5. 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 relation, using a computerized log-linear regression analysis (Litchfield and Wilcox, 1949).

3. Results

3.1. Antinociceptive effect of orally administered FR143166

The antinociceptive effect of FR143166 and morphine was measured in the tail-pinch test in mice. As shown in Fig. 2, FR143166 (10, 20, 40 and 80 mg/kg) and morphine (5, 10, 20 and 40 mg/kg) caused dose-dependent antinociceptive effects with ED_{50} values (95% C.L.) of 24 (14–38) and 12 (6.1–19) mg/kg, respectively. Vehicle (0.5% methylcellulose) p.o. alone had no antinociceptive effect. FR143166, at the doses used in this study, did not cause a morphine-like behavioral change such as central nervous system excitation and Straub's tail response in mice.

3.2. Antinociceptive effect of intrathecally or intracerebroventricularly administered FR143166

FR143166 (4–64 μg/mouse) and morphine (0.25–4 μg/mouse) injected i.t. exhibited dose-dependent antinociceptive effects with ED_{50} values (95% C.L.) of 15 (9.0–24) and 1.0 (0.59–2.8) μg/mouse, respectively (Fig. 3). However, i.c.v. injection of FR143166 (16–128 μg/mouse) did not show any antinociceptive effect, unlike morphine, which had an antinociceptive effect with an ED_{50} value (95% C.L.) of 0.71 (0.34–1.5) μg/mouse. Vehicle (20% ethanol in saline) i.t. or i.c.v. alone had no antinociceptive effect.

3.3. Effect of adrenoceptor antagonists on the antinociceptive effect of FR143166

The antinociceptive effect of intrathecally administered FR143166 at a dose of 80 μg/mouse in the tail-pinch test was not antagonized by co-administration (0.01–100 μg/mouse, i.t.) of phentolamine, a nonselective α-adrenoceptor antagonist (Fig. 4A). Moreover, the antinociception by FR143166 was not attenuated by co-administration (1 and 10 μg/mouse, i.t.) of propranolol, a β-adrenoceptor antagonist. However, the antinociceptive effect of i.t. administered morphine at a dose of 4 μg/mouse was completely blocked by co-administration (10 μg/mouse) of phentolamine, but not of propranolol. In addition, phentolamine at a maximum dose of 100 μg/mouse i.t. or propranolol at a maximum dose of 10 μg/mouse i.t. alone did not produce any antinociceptive effect.

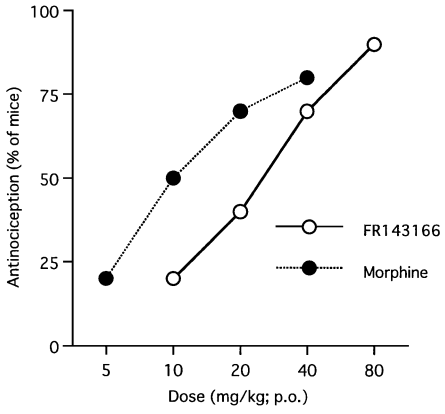

Fig. 2. Antinociceptive effect of orally administered FR143166 in the tail-pinch test in mice. After normal nociceptive responses were measured, FR143166 (open circles) or morphine (closed circles) was administered orally. The antinociceptive effect was determined by the modified Haffner's method 30 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 ($n = 10$).

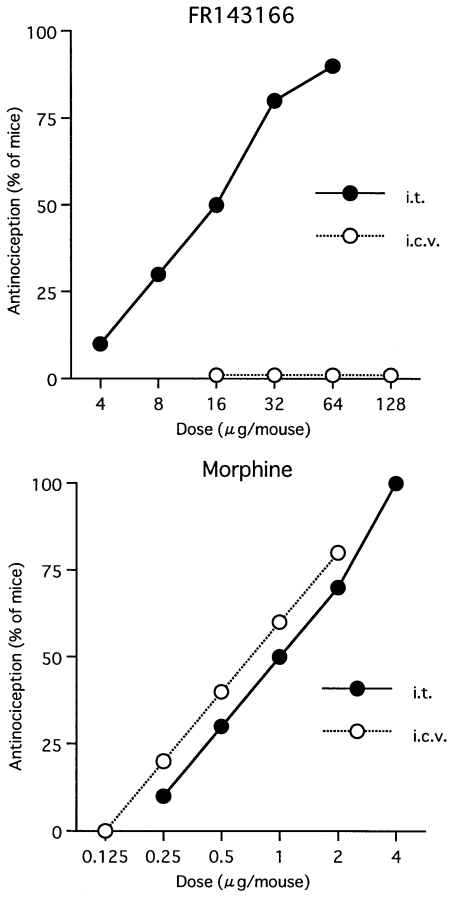

Fig. 3. Antinociceptive effect of i.t. or i.c.v. FR143166 in the tail-pinch test in mice. After normal nociceptive responses were measured, FR143166 or morphine was administered i.t. (closed circles) or i.c.v. (open circles). The antinociceptive effect was determined by the modified Haffner's method 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 ($n = 10$).

3.4. Effect of 5-HT receptor antagonists on the antinociceptive effect of FR143166

The antinociceptive effect of intrathecally administered FR143166 at a dose of 80 μg/mouse in the tail-pinch test was completely blocked by co-administration (1 μg/mouse) of methysergide, a nonselective 5-HT receptor antagonist (Fig. 4B). However, i.t. injection of methysergide

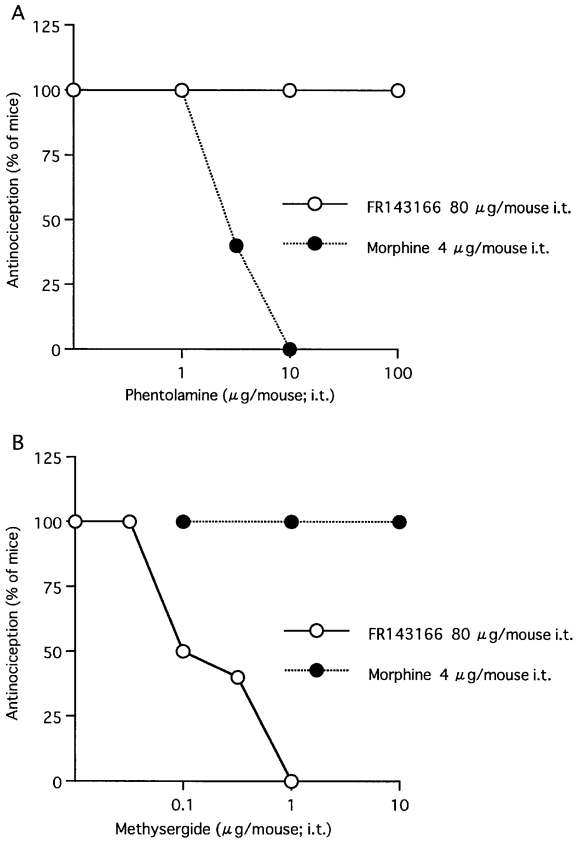

Fig. 4. Effect of phentolamine and methysergide on the antinociceptive effect of i.t. FR143166 in the tail-pinch test. After normal nociceptive responses were measured, FR143166 at a dose of 80 μg/mouse (open circles) or morphine at a dose of 4 μg/mouse (closed circles) was administered intrathecally. Phentolamine (A) or methysergide (B) was 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 ($n = 10$).

at a dose of 10 μg/mouse did not block the antinociception by morphine. In addition, methysergide (1 and 10 μg/mouse, i.t.) alone did not produce any antinociceptive effect.

To check for possible mediation through 5-HT receptor subtypes in the mechanism of the antinociceptive effect of FR143166, the effect of selective antagonists of the various 5-HT receptors on the antinociception of FR143166 was examined. The antinociceptive effect of FR143166 was blocked 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_1 receptor antagonist, mesulgine, the 5-HT_{2B} receptor antagonist, SB-204741, the 5-HT_{2C} receptor antagonist, SB-2420874, or the 5-HT_4 receptor antagonist, SDZ-205,557 (Fig. 5). However, i.t. injection of these 5-HT receptor antagonists at a dose of 1 μg/mouse did not attenuate the antinociception by FR143166. These antagonists given alone at a dose of 10 μg/mouse i.t. did not produce any antinociceptive effect.

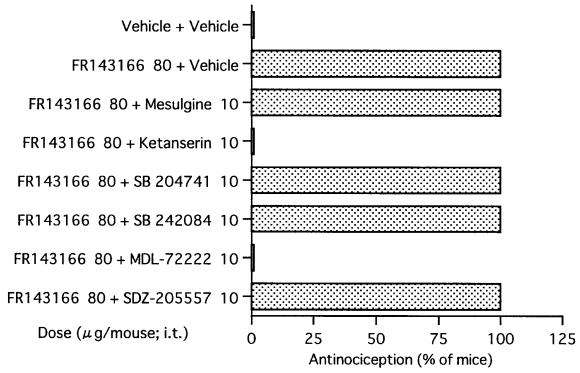

Fig. 5. Effect of 5-HT receptor antagonists on the antinociceptive effect of i.t. FR143166 in the tail-pinch test. After normal nociceptive responses were measured, FR143166 at a dose of 80 μg/mouse was administered intrathecally. Various 5-HT receptor antagonists at 10 μg/mouse i.t. were co-administered with FR143166. 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 ($n = 10$).

3.5. Effect of monoamine depletion on the antinociceptive effect of FR143166

After the treatment with the 5-HT synthesis inhibitor, *p*-chlorophenylalanine (3 × 300 mg/kg, i.p.), the antinociceptive activity of FR143166 in the tail-pinch test, decreased markedly, whereas the activity of FR143166 was not reversed by 6-hydroxydopamine plus nomifensine treatment (Fig. 6). On the other hand, 6-hydroxydopamine plus nomifensine attenuated the antinociceptive activity of morphine administered i.t. in the tail-pinch test, whereas *p*-chlorophenylalanine treatment had no effect. Pretreatment of mice with *p*-chlorophenylalanine or 6-hydroxydopamine plus nomifensine alone did not produce any antinociceptive effect. Additionally, the administration of these monoamine-depleting drugs induced no weight gain.

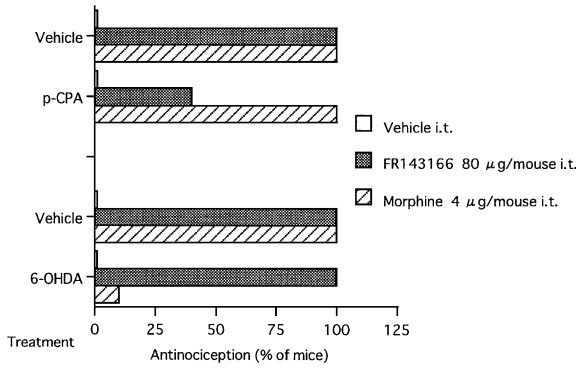

Fig. 6. Effect of *p*-chlorophenylalanine or 6-hydroxydopamine treatment on the antinociceptive effect of i.t. FR143166 in the tail-pinch test. Mice were pretreated with *p*-chlorophenylalanine (*p*-CPA) or 6-hydroxydopamine (6-OHDA) as described in the text. The antinociceptive effect was determined 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

The present results clearly indicate that the antinociceptive effect induced by FR143166 is mediated through spinal 5-HT_{2A} and 5-HT_3 receptors, and that the descending serotonergic system is involved in the antinociceptive activity of FR143166 in the mouse tail-pinch test. However, our results imply that the antinociceptive mechanism of FR143166 is independent of the descending noradrenergic system.

Systematically administered FR143166 at a dose-range of 10–80 mg/kg showed an antinociceptive effect in the tail-pinch test. In general, the tail-pinch test is a useful animal model for evaluating analgesic agents, especially opioids, affecting the CNS. To assess the action site of FR143166 in the CNS, i.t. and i.c.v. administration of FR143166 was tested in the tail-pinch test. The i.t. injection of FR143166 produced a dose-related antinociceptive effect with the ED_{50} value of 15 μg/mouse. When administered i.c.v., however, FR143166 did not show any antinociceptive effect. These results confirm that the site of action of FR143166 is in the spinal cord, but not in the supraspinal site.

In the spinal cord, monoamines such as noradrenaline and 5-HT play an important role in the modulation of nociceptive transmission. First, there is substantial evidence of dose-dependent and potent antinociceptive properties of spinal administration of clonidine, an α_2-adrenoceptor agonist, in a yohimbine-reversible manner (Ochi and Goto, 2000b). Second, i.t. administration of 5-HT in rats, rabbits and cats elicits antinociceptive effects that are antagonized by the nonselective 5-HT receptor antagonist, methysergide (Yaksh and Wilson, 1979). In the present study, the spinal antinociceptive effect of FR143166 was reversed by co-administered methysergide, but not by various adrenoceptor antagonists. 5-HT is important in the modulation of pain transmission in the spinal cord. In contrast, intraplantar injection of 5-HT induces a dose-dependent pain response (Hong and Abbott, 1994). Additionally, local administration of the selective 5-HT_{2A} receptor antagonist, sarpogrelate, shows an antinociceptive effect (Obata et al., 2000). On the other hand, supraspinal roles of the 5-HT receptor in nociception are not clear. The i.c.v. injection of the 5-HT_3 receptor agonist, 2-methyl-5-HT, is not analgesic at doses between 1 and 100 μg/rat in acute pain assays (Giordano, 1991), indicating that brain 5-HT_3 receptor systems are not involved in nociceptive control. Therefore, the spinal serotonergic system plays a key role in the mechanism of the antinociceptive effect of FR143166.

Next, the 5-HT receptors are divided into at least four subtypes based on the results of radioligand binding studies and autoradiographic studies (Hoyer et al., 1994). We previously reported that i.t. injection of the 5-HT_2 receptor agonist, α-methyl-5-HT, and the 5-HT_3 receptor agonist, 2-methyl-5-HT, dose-dependently exerts an antinociceptive effect in the tail-pinch test in mice (Ochi and Goto, 2000a). In addition, i.t. injection of the 5-HT_2 receptor agonist and the 5-HT_3 receptor agonist inhibits the number of flinches in the formalin test (Sasaki et al., 2001). These effects of the 5-HT_2 receptor agonist and the 5-HT_3 receptor agonist are reversed by co-administration of respective antagonists (Ochi and Goto, 2000a; Sasaki et al., 2001). Thus, these findings suggest that 5-HT receptor subtypes play differential roles in the modulation of nociceptive responses. To determine the possible role of serotonergic receptor subtypes (5-HT_1, 5-HT_{2A}, 5-HT_{2B}, 5-HT_{2C}, 5-HT_3 and 5-HT_4) in the mechanism of the spinal antinociceptive effect induced by i.t. FR143166 in the tail-pinch test in mice, the antagonism by the above selective antagonists of various 5-HT receptors on the antinociceptive effect of FR143166 was investigated. In the present study, the spinal antinociceptive effect of FR143166 was reversed by co-administration of the 5-HT_{2A} receptor antagonist, ketanserin, and the 5-HT_3 receptor antagonist, MDL-72222. On the other hand, the 5-HT_1 receptor antagonist, mesulgine, the 5-HT_{2B} receptor antagonist, SB-204741, the 5-HT_{2C} receptor antagonist, SB-242084, and the 5-HT_4 receptor antagonist, SDZ-205557 failed to block the antinociceptive effect induced by FR143166. Thus, we speculate that FR143166 may interact with 5-HT_{2A} and 5-HT_3 receptors to induce these antinociceptive effects in the spinal cord.

We tested the effect of pharmacological denervation with two monoamine depletors on antinociceptive activity of FR143166. Matsumoto et al. (1996) reported that pretreatment of mice with *p*-chlorophenylalanine, a 5-HT synthesis inhibitor, significantly decreases the 5-HT levels in the cortex, brainstem and spinal cord without producing a significant decrease in the levels of noradrenaline and dopamine. On the other hand, treatment with 6-hydroxydopamine plus nomifensine significantly and selectively decreases the noradrenaline levels in these brain regions without producing a significant decrease in the levels of dopamine and 5-HT (Matsumoto et al., 1996). Our speculation, mentioned above, was strongly substantiated by the present finding that pretreatment with *p*-chlorophenylalanine, but not with 6-hydroxydopamine plus nomifensine, abolished the antinociceptive activity of FR143166 in the tail-pinch test. These results suggest that FR143166 may be able to stimulate directly or indirectly the release of endogenous 5-HT from nerve terminals of the descending serotonergic neurons.

Within the spinal dorsal horn, several 5-HT receptors are located on spinipetal neurons, which may modulate the effects of substance P. Functional interactions between substance P and 5-HT in the spinal cord have been reported (Eide and Hole, 1991). 5-HT inhibits the evoked release of substance P in a methysergide-reversible manner (Yonehara et al., 1991), while the evoked release of substance P from the rat spinal cord slices is potentiated by 5-HT (Iverfeldt et al., 1986; Inoue et al., 1997). The exact role of 5-HT systems on the release of substance P in the nociceptive response is still unclear. Further studies using FR143166 are needed to clarify this point.

Morphine is a potent analgesic agent with many central sites of action (Pasternak, 1993). Morphine inhibits K^+-evoked release of substance P from superfused slices in vitro (Jessell and Iversen, 1977). Furthermore, the inhibitory effect of morphine on the noxious mechanical stimuli-induced release of substance P from the rabbit spinal dorsal horn in vivo is antagonized by the local application to the dorsal horn of the adrenoceptor antagonist, prazosin, but not of the 5-HT receptor antagonist, methysergide (Kuraishi et al., 1983a). When the tail-pinch test is used, the descending noradrenergic system is more important in the antinociceptive activity of morphine on mechanical noxious stimuli than in the descending serotonergic system (Kuraishi et al., 1983b; Ochi and Goto, 2001). In this regard, the antinociceptive activity of i.t. FR143166 may clearly differ from that of morphine.

In summary, p.o. and i.t. injection of FR143166 exerts antinociceptive activity in the tail-pinch test in mice. The spinal serotonergic system is involved in the antinociceptive activity of FR143166 against mechanical noxious stimuli.

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