

## Central antinociceptive effects of mitragynine in mice: contribution of descending noradrenergic and serotonergic systems

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Received 20 June 1996; revised 29 August 1996; accepted 3 September 1996

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### Abstract

Mitragynine is a major alkaloidal constituent of young leaves of *Mitragyna speciosa* Korth, that is known to exhibit narcotic-like activity. In this study, we investigated the roles of central monoaminergic systems in the antinociceptive action of mitragynine by means of the tail-pinch and hot-plate tests in mice. Mitragynine (1.0–10  $\mu$ g) injected i.c.v. exerted a dose-dependent antinociceptive activity in both tests. The activity of mitragynine (10  $\mu$ g, i.c.v.) in the tail-pinch test was antagonized by reserpine, 6-hydroxydopamine plus nomifensine, and *p*-chlorophenylalanine treatment, whereas the antinociceptive activity of morphine (3  $\mu$ g) given i.c.v. in this test was attenuated by 6-hydroxydopamine plus nomifensine but not by *p*-chlorophenylalanine treatment. Moreover, the activity of i.c.v. mitragynine was also antagonized by the  $\alpha_2$ -adrenoceptor antagonist, idazoxan (10  $\mu$ g), and cyproheptadine (1  $\mu$ g) administered intrathecally (i.t.). On the other hand, the antinociceptive action of i.c.v. mitragynine (10  $\mu$ g) in the hot-plate test was abolished by reserpine and 6-hydroxydopamine plus nomifensine, but not by *p*-chlorophenylalanine treatment. This action was also antagonized by i.t. injection of idazoxan (10  $\mu$ g). These results suggest that both descending noradrenergic and serotonergic systems are involved in the antinociceptive activity of supraspinally administered mitragynine on the mechanical noxious stimulation, while the descending noradrenergic system predominantly contributes to the effect of supraspinal mitragynine on the thermal noxious stimulation. The mechanisms underlying the suppressive action of mitragynine on the nociceptive response may differ from those of morphine in mice.

**Keywords:** Mitragynine; Antinociception; (Mouse); Noradrenergic system;  $\alpha_2$ -Adrenoceptor; Serotonergic system

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### 1. Introduction

Mitragynine, an alkaloid, accounts for about 66% of the total alkaloids extracted from the young leaves of *Mitragyna speciosa* Korth (Ponglux et al., 1994; Shellard, 1974). The leaves of this plant are known to produce narcotic-like actions when smoked, chewed, or drunk as a suspension (Jansen and Prast, 1988a,b). We have previously found that mitragynine possesses an antinociceptive action when administered intraperitoneally (i.p.) or intracerebroventricularly (i.c.v.) in mice, and that the effects of mitragynine administered i.p. and i.c.v. are blocked by i.c.v. naloxone,

an opioid receptor antagonist, indicating involvement of opioid receptors in the action of mitragynine (Matsumoto et al., 1996b). These findings seem to explain the medicinal use of this plant in Thailand to replace morphine in addict detoxification and treatment programs (Jansen and Prast, 1988b).

There are multiple pain-modulating systems in the central nervous system. The bulbospinal descending noradrenergic and serotonergic pathways play important roles in the transmission of nociceptive information from primary afferent neurons in the spinal cord dorsal horn (Proudfit, 1988; Reddy and Yaksh, 1980; Yaksh et al., 1981; Yaksh and Wilson, 1979). These systems also participate in opioid-induced antinociception in rodents. For example, intrathecal (i.t.) injection of an  $\alpha_2$ -adrenoceptor antagonist reduces the antinociceptive activity of morphine (Proudfit,

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1988). However, the extent of involvement of these bulbospinal monoaminergic systems in opioid-induced antinociception appears to vary with the type of nociceptive stimulus (Howe and Ziegler, 1984; Kuraishi et al., 1983; Wigdor and Wilcox, 1987).

Our previous study demonstrated that mitragynine, as well as the selective 5-HT<sub>2A</sub> receptor antagonist, ritanserin, suppressed the 5-methoxy-*N,N*-dimethyltryptamine-induced head-twitch behavior but not head-weaving behavior or spontaneous motor activity in mice, and that the action of mitragynine was attenuated by the  $\alpha_2$ -adrenoceptor antagonists, yohimbine and idazoxan, suggesting a role of noradrenergic systems in the action of mitragynine (Matsumoto et al., 1996a). Thus, it is possible that these pharmacological profiles of mitragynine may account for its antinociceptive activity. In the present study, to clarify this possibility, we examined the effects of monoamine depletors, an  $\alpha_2$ -adrenoceptor antagonist, and a 5-HT receptor antagonist on the antinociception caused by mitragynine in the tail-pinch and hot-plate tests.

## 2. Materials and methods

### 2.1. Animals

Male *ddY* mice (Japan SLC, Shizuoka, Japan) were obtained at the age of 4 weeks. The mice were housed in groups of 15 per cage (35 × 30 × 16 cm), on a 12 h light/dark cycle (lights on: 07:30–19:30 h) at 25 ± 1°C for at least 1 week before the experiments and given free access to food and water. The present studies were conducted in accordance with the standards established by the Guide for the Care and Use of Laboratory Animals of Toyama Medical and Pharmaceutical University.

### 2.2. Measurement of nociceptive responses in the tail-pinch and hot-plate tests

#### 2.2.1. Tail-pinch test

The nociceptive response in the tail-pinch test was measured according to Haffner's method as previously reported (Huong et al., 1995; Takagi et al., 1966). Briefly, mice were pretested by pinching their tails with hemostatic forceps (3 mm width, 500 g constant pressure), and only the mice that showed nociceptive responses such as biting the forceps within 2 s were used for the experiments. The latency of nociceptive responses in these animals was expressed as the tail-pinch latency. To minimize tissue damage, a cut-off time of 6 s was selected.

#### 2.2.2. Hot-plate test

In the hot-plate test, an animal was placed on a metal plate maintained at 55 ± 0.5°C and the latency of nociceptive responses, such as hind paw licking, hind paw flicking or jumping, was measured according to the method of

Eddy and Leimbach (1953) and Hunskaar et al. (1986). Only the mice that showed the nociceptive response within 18 s were used for the experiments. The latency of nociceptive responses in these animals was expressed as the hot-plate latency. To prevent tissue damage, a cut-off time of 45 s was selected.

### 2.3. Monoamine depletion

For endogenous monoamine depletion, reserpine (5 mg/kg) was injected i.p. 3 h before the experiments (Starr et al., 1987). The 5-HT depletion was achieved by the method of Dursun and Handley (1993). Briefly, mice were injected i.p. with 3 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), a noradrenaline depletor, was given i.c.v. (contralateral to the site for i.c.v. mitragynine injection) 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 (Ojima et al., 1995). Noradrenaline, dopamine and 5-HT levels in the cortex, brainstem and spinal cord of mice were determined using high performance liquid chromatography with electrochemical detector as previously reported (Matsumoto et al., 1995; Ojima et al., 1995).

### 2.4. Drugs

Mitragynine was purified from the alkaloidal fraction extracted from the young leaves of *Mitragyna speciosa* Korth, as previously described (Ponglux et al., 1994). The following drugs were used: idazoxan HCl, *p*-chlorophenylalanine methyl ester HCl and 6-hydroxydopamine HBr (Sigma, St. Louis, MO, USA), nomifensine maleate (Research Biochemicals, Natick, MA, USA), yohimbine HCl (Nacalai Tesque, Kyoto, Japan), reserpine (Apoplone Inj., Daiichi Pharmaceutical, Tokyo, Japan), morphine HCl (Dainippon Pharmaceutical, Tokyo, Japan) and cyproheptadine HCl (Merck, Tokyo, Japan).

Mitragynine was dissolved in 1% acetic acid, and the pH was adjusted up to 4.7 with 1 M NaOH. 6-Hydroxydopamine solution was freshly prepared with ice-cold saline containing 0.2% ascorbic acid. Cyproheptadine was dissolved in 0.5% (v/v) dimethyl sulfoxide, and diluted with saline. Other test drugs were dissolved in saline. Drug solutions were prepared just before the start of the experiments.

Intracerebroventricular (i.c.v.) and intrathecal (i.t.) injections of test agents were performed according to the methods described by Haley and McCormick (1957) and Hylden and Wilcox (1980), respectively. Mitragynine (10 µg) and morphine (3 µg) were injected i.c.v. 15 min before the experiments.

## 2.5. Statistical analysis

The effects of drugs on the nociceptive response were analyzed statistically with the Kruskal-Wallis analysis of variance followed by the Mann-Whitney *U*-test for multiple comparisons. Neurochemical data were analyzed by means of a two-tailed Student's *t*-test. Differences with  $P < 0.05$  were considered statistically significant.

## 3. Results

### 3.1. Antinociceptive action of i.c.v. administered mitragynine

As shown in Fig. 1, when administered i.c.v., mitragynine (1–10  $\mu\text{g}$ ) and morphine (0.3–3  $\mu\text{g}$ ) dose dependently prolonged the latency of nociceptive responses in the tail-pinch and hot-plate tests. The ability of mitragynine to cause antinociception was about one-third that of morphine in both tests (mitragynine 10  $\mu\text{g}$ : approximately 25.1 nmol; morphine 3  $\mu\text{g}$ : approximately 9.3 nmol). However, there was no difference in the sensitivity in these tests after either mitragynine or morphine. We previously found that the effects of mitragynine and morphine administered i.c.v. were maximal at about 15 min after administration (Matsumoto et al., 1996b). Thus, in the present study, we compared the nociceptive responses among different groups at 15 min after administration of mitragynine or morphine.

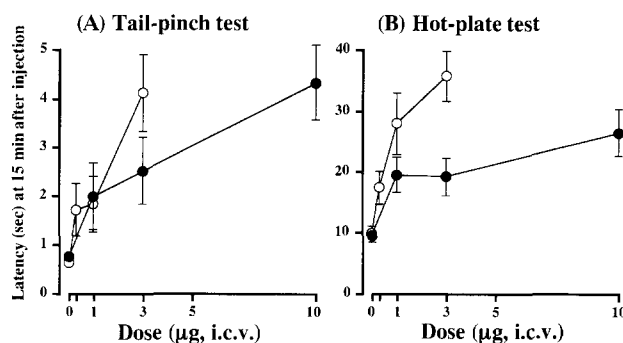


Fig. 1. Dose-dependent antinociceptive activities of mitragynine and morphine administered i.c.v. in the tail-pinch and hot-plate tests in mice. Mitragynine (closed circles; 1–10  $\mu\text{g}$ ), morphine (open circles; 0.3–3  $\mu\text{g}$ ) or vehicle was administered i.c.v. 15 min before the experiment, and the nociceptive latency was then measured in the tail-pinch (A) and hot-plate tests (B). The pre-test values in the tail-pinch and hot-plate tests were  $0.87 \pm 0.07$  and  $12.13 \pm 0.78$  s (mean  $\pm$  S.E.M.,  $n = 40$ ), respectively. Cut-off times in the tail-pinch test and hot-plate test were 6 and 45 s, respectively.

### 3.2. Effects of monoamine depletion on the antinociceptive activity of mitragynine

As summarized in Table 1, pretreatment of mice with 5 mg/kg reserpine (i.p.), a monoamine depletor, significantly decreased the contents of noradrenaline, dopamine and 5-HT. Treatment with *p*-chlorophenylalanine ( $3 \times 300$  mg/kg, i.p.), a 5-HT synthesis inhibitor, significantly and selectively decreased the 5-HT contents in the cortex, brainstem and spinal cord without producing a significant

Table 1

Effects of reserpine, *p*-chlorophenylalanine and 6-hydroxydopamine on the levels of noradrenaline, dopamine and serotonin in the cortex, brainstem and spinal cord of mice

Treatment	NA ( $\mu\text{g/g}$ tissue)		DA ( $\mu\text{g/g}$ tissue)		5-HT ( $\mu\text{g/g}$ tissue)	
<b>Cortex</b>						
Control	$0.336 \pm 0.035$		$0.142 \pm 0.048$		$0.812 \pm 0.125$	
Reserpine	$0.012 \pm 0.007^a$	(–96.4)	$0.001 \pm 0.000^a$	(–100)	$0.113 \pm 0.028^a$	(–86.1)
<i>p</i> -CPA	$0.254 \pm 0.026$	(–24.4)	$0.161 \pm 0.083$	(13.3)	$0.313 \pm 0.087^a$	(–61.5)
Control	$0.349 \pm 0.079$		$0.144 \pm 0.037$		$0.611 \pm 0.051$	
6-OHDA	$0.030 \pm 0.006^a$	(–91.4)	$0.098 \pm 0.027$	(–31.9)	$0.566 \pm 0.029$	(–7.4)
<b>Brainstem</b>						
Control	$0.677 \pm 0.030$		$0.078 \pm 0.023$		$1.039 \pm 0.071$	
Reserpine	$0.076 \pm 0.018^a$	(–88.8)	$0.020 \pm 0.003^a$	(–74.4)	$0.146 \pm 0.016^a$	(–85.9)
<i>p</i> -CPA	$0.636 \pm 0.053$	(–6.1)	$0.052 \pm 0.012$	(–33.3)	$0.318 \pm 0.036^a$	(–69.4)
Control	$0.825 \pm 0.051$		$0.117 \pm 0.012$		$0.962 \pm 0.059$	
6-OHDA	$0.595 \pm 0.049^a$	(–27.9)	$0.178 \pm 0.015^a$	(+52.1)	$0.950 \pm 0.058$	(–1.2)
<b>Spinal cord</b>						
Control	$0.378 \pm 0.025$		$0.033 \pm 0.004$		$0.912 \pm 0.041$	
Reserpine	$0.005 \pm 0.003^a$	(–98.7)	$0.004 \pm 0.004^a$	(–87.9)	$0.151 \pm 0.051^a$	(–83.4)
<i>p</i> -CPA	$0.390 \pm 0.028$	(3.2)	$0.026 \pm 0.003$	(–21.2)	$0.175 \pm 0.016^a$	(–80.2)
Control	$0.319 \pm 0.026$		$0.023 \pm 0.002$		$0.790 \pm 0.040$	
6-OHDA	$0.100 \pm 0.041^a$	(–68.7)	$0.021 \pm 0.004$	(–8.7)	$0.817 \pm 0.068$	(3.4)

Each value represents the mean  $\pm$  S.E.M. ( $n = 5$ ). The numbers in parentheses are the percentage changes. *p*-CPA: *p*-chlorophenylalanine; 6-OHDA: 6-hydroxydopamine; NA: noradrenaline; 5-HT: serotonin. <sup>a</sup>  $P < 0.05$  compared with respective control.

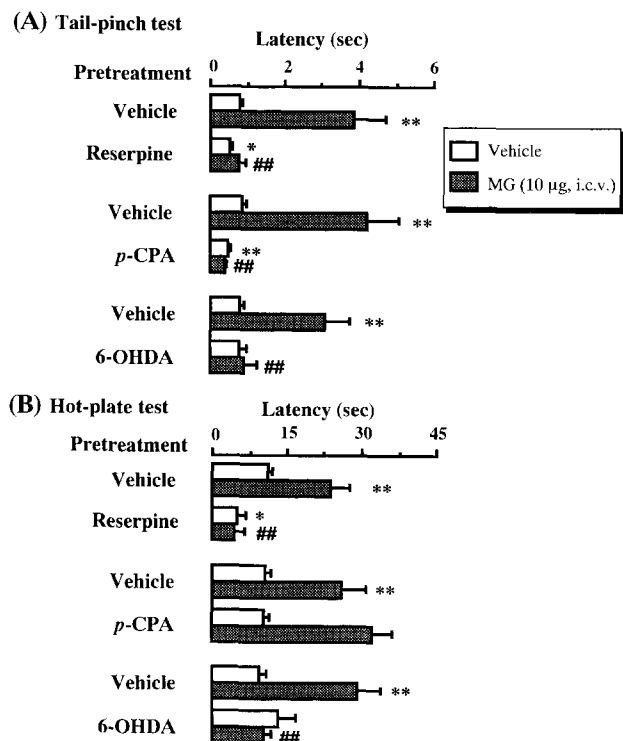


Fig. 2. Effects of reserpine, *p*-chlorophenylalanine and 6-hydroxydopamine treatment on the mitragynine-induced antinociception in the tail-pinch and hot-plate tests. Animals were pretreated with reserpine, *p*-chlorophenylalanine (*p*-CPA) or 6-hydroxydopamine (6-OHDA) as described in the text. Fifteen minutes after i.c.v. injection of mitragynine (MG, 10 µg), the nociceptive latency in the tail-pinch (A) and hot-plate tests (B) was measured. Each column represents the mean latency  $\pm$  S.E.M. ( $n = 7-9$ ). \*  $P < 0.05$ , \*\*  $P < 0.01$  compared with the respective vehicle control. ##  $P < 0.01$  compared with the group treated with mitragynine alone.

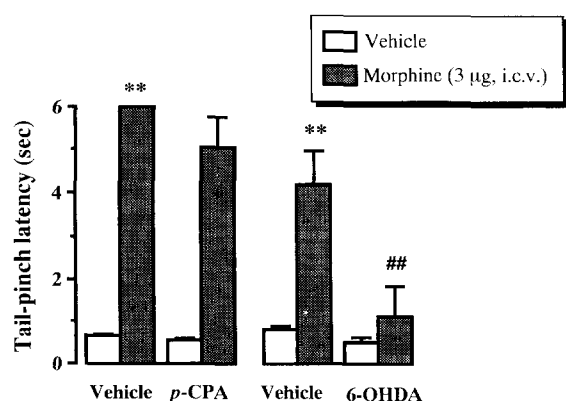


Fig. 3. Effects of *p*-chlorophenylalanine and 6-hydroxydopamine treatment on the morphine-induced antinociception in the tail-pinch test. Animals were pretreated with *p*-chlorophenylalanine (*p*-CPA) or 6-hydroxydopamine (6-OHDA) as described in the text. Fifteen minutes after i.c.v. injection of morphine (3 µg), the nociceptive latency in the tail-pinch test was measured. Each column represents the mean latency  $\pm$  S.E.M. ( $n = 8$ ). \*  $P < 0.01$  compared with the respective vehicle control. ##  $P < 0.01$  compared with the group treated with morphine alone.

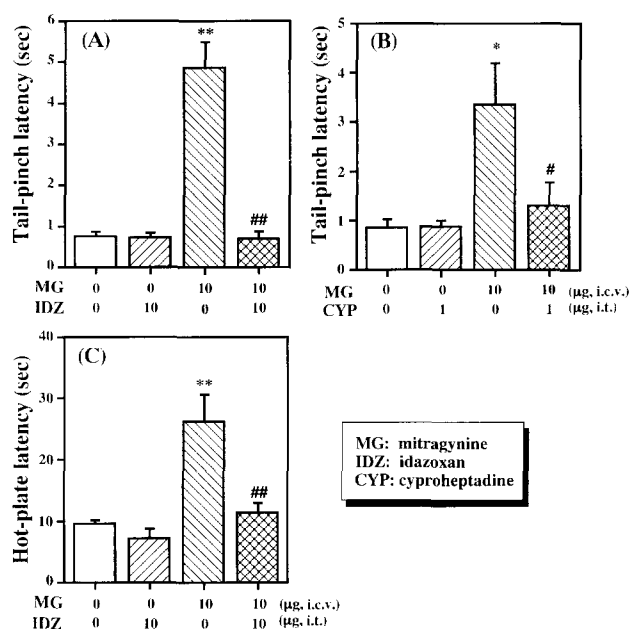


Fig. 4. Effect of i.t. administered idazoxan and cyproheptadine on the mitragynine-induced antinociception. Mitragynine (10 µg) or vehicle was injected i.c.v. at time 0. Idazoxan (10 µg), cyproheptadine (1 µg) or vehicle was administered i.t. immediately before mitragynine. The latency to show a nociceptive response in the tail-pinch (A, B) and hot-plate tests (C) was measured at 15 min after mitragynine injection. Each value represents the mean latency  $\pm$  S.E.M. ( $n = 7-8$ ). \*\*  $P < 0.01$ , \*  $P < 0.05$  vs. saline + vehicle, ##  $P < 0.01$  vs. saline + mitragynine (Mann-Whitney *U*-test).

decrease in the contents of noradrenaline or dopamine. On the other hand, treatment with 6-hydroxydopamine plus nomifensine significantly decreased the noradrenaline contents in these brain regions, but this treatment caused a significant increase in the dopamine level in the brainstem.

After the treatment with reserpine, *p*-chlorophenylalanine or 6-hydroxydopamine plus nomifensine, the antinociceptive activity of i.c.v. mitragynine (10 µg) in the tail-pinch test decreased markedly (Fig. 2A), while the activity in the hot-plate test was attenuated by reserpine, 6-hydroxydopamine plus nomifensine but not by *p*-chlorophenylalanine treatment (Fig. 2B). These results indicate that both descending noradrenergic and serotonergic systems contribute to the antinociceptive action of mitragynine administered i.c.v. in the tail-pinch test, while only the noradrenergic system plays a important role in the action in the hot-plate test. On the other hand, 6-hydroxydopamine plus nomifensine significantly attenuated the antinociceptive activity of morphine (3 µg) administered i.c.v. in the tail-pinch test, whereas *p*-chlorophenylalanine treatment had no effect (Fig. 3).

### 3.3. Effects of i.t. administration of idazoxan and cyproheptadine on the i.c.v. mitragynine-induced antinociception

Idazoxan (10 µg), an  $\alpha_2$ -adrenoceptor antagonist, given i.t. slightly but non-significantly suppressed the basal noci-

ceptive responses in the tail-pinch and hot-plate tests. This treatment markedly attenuated the antinociception caused by i.c.v. injection of mitragynine (10  $\mu$ g) in both tests (Fig. 4A,C). I.t. administration of cyproheptadine (1  $\mu$ g), a 5-HT receptor antagonist, also significantly antagonized the antinociceptive action of i.c.v. mitragynine (10  $\mu$ g) in the tail-pinch test (Fig. 4B).

#### 4. Discussion

We previously reported that mitragynine dose dependently exerted antinociceptive activity in the tail-pinch and hot-plate tests in mice following i.p. or i.c.v. administration, and suggested that the supraspinal opioid system was at least partly involved in the action of mitragynine, since the action of mitragynine was antagonized by i.c.v. naloxone, an opioid receptor antagonist (Matsumoto et al., 1996b). In the present study, we found that the antinociceptive activity of supraspinally administered mitragynine in the tail-pinch test could be attributed to both descending noradrenergic and serotonergic systems, while the mitragynine-induced antinociception in the hot-plate test was predominantly due to the descending noradrenergic system.

Evidence indicates that the descending noradrenergic, serotonergic and methionine-enkephalinergic systems play important roles in the action of analgesics such as morphine (Wigdor and Wilcox, 1987) and that the descending noradrenergic system is more important in the antinociceptive action of morphine on mechanical noxious stimulation than is the descending serotonergic system (Kuraishi et al., 1983). Moreover, i.t. administration of  $\alpha_2$ -adrenoceptor agonists causes antinociception, while  $\alpha_2$ -adrenoceptor antagonists administered i.t. block the antinociception caused by i.t. injection of noradrenaline or stimulation of the descending noradrenergic system (Fleetwood-Walker et al., 1985; Kawabata et al., 1994; Wigdor and Wilcox, 1987; Wilcox et al., 1987), indicating involvement of the spinal  $\alpha_2$ -adrenoceptors in antinociception. In the present study, the antinociceptive activity of mitragynine in the tail-pinch test was completely abolished by 6-hydroxydopamine plus nomifensine, *p*-chlorophenylalanine, and reserpine, treatment with which significantly depleted endogenous noradrenaline, 5-HT, and both, respectively, in the brain. On the other hand, the activity of morphine in the tail-pinch test was attenuated by 6-hydroxydopamine plus nomifensine but not by *p*-chlorophenylalanine treatment. These results indicate that the effect of mitragynine on mechanical noxious stimulation is mediated by both descending noradrenergic and serotonergic systems. This hypothesis can be further supported by the fact that i.t. administration of the  $\alpha_2$ -adrenoceptor antagonist, idazoxan, and the 5-HT receptor antagonist, cyproheptadine, significantly antagonized the action of i.c.v. mitragynine in the tail-pinch test. Together, these results suggest that mitragynine may be able to directly or indirectly stimulate

the release of endogenous noradrenaline and 5-HT from nerve terminals of the descending monoaminergic neurons, resulting in transmission blockade of the nociceptive information in the spinal cord. However, it remains unclear whether the descending noradrenergic and serotonergic systems contribute to the same extent to the action of mitragynine on the mechanical stimulus-induced nociceptive response. To answer this question requires further investigation of whether the dose-response curve of mitragynine is shifted following the lesion of these neuronal systems.

Our previous study demonstrated that the antinociceptive activity of mitragynine administered i.c.v. was antagonized by i.c.v. naloxone (3–10  $\mu$ g), an opioid receptor antagonist, suggesting the possible involvement of supraspinal opioid systems in the action of mitragynine (Matsumoto et al., 1996b). In the present study, the antinociceptive activity of mitragynine and morphine exhibited different sensitivity to 5-HT depletion in the tail-pinch test. Thus, it would be expected that mitragynine may interact with the supraspinal opioid receptor subtypes which are involved in modulation of the descending serotonergic activity but have a low affinity for morphine.

It is of interest that the mitragynine-induced antinociception in the hot-plate test exhibited a sensitivity to 5-HT depletion different from that observed in the tail-pinch test. The hot-plate response is reported to be organized supraspinally and affected by ascending 5-HT systems (Roberts, 1988). For instance, i.t. administration of a 5-HT receptor antagonist blocks inhibition of the tail-flick response by stimulation of the periaqueductal gray matter but this treatment does not block the same inhibition of the hot-plate response (Jensen and Yaksh, 1984). Moreover, Suh et al. (1989) have reported that, given i.t., the  $\alpha_2$ -adrenoceptor antagonist antagonizes the antinociception caused by i.c.v. morphine in the hot-plate test, while given i.t., the 5-HT receptor antagonist fails. However, there is a conflicting report on the roles of the 5-HT systems in the nociceptive response caused by thermal noxious stimulation. Narita et al. (1993) have demonstrated that both noradrenergic and serotonergic systems are involved in the  $\mu$  receptor-mediated antinociceptive action of i.c.v. morphine in the hot-plate test. Although the exact role of 5-HT systems in the nociceptive response in this test is still unclear, the present findings suggest that the descending noradrenergic system plays a predominant role in the antinociceptive action of i.c.v. mitragynine on the thermal noxious stimulation, while the contribution of spinal 5-HT transmission to this action, if any, is slight.

We previously reported that mitragynine dose dependently suppresses the 5-HT<sub>2A</sub> receptor-mediated head-twitch response in mice, and that the suppressive action is reversed by a selective  $\alpha_2$ -adrenoceptor antagonist. In addition, both noradrenaline and 5-HT depletion fail to attenuate the effect of mitragynine, suggesting that suppression of the head-twitch response by mitragynine may

be due to postsynaptic  $\alpha_2$ -adrenoceptor stimulation, or blockade of 5-HT<sub>2A</sub> receptor sites by mitragynine, or both (Matsumoto et al., 1996a). Together, these results make it likely that mitragynine causes antinociception by stimulating  $\alpha_2$ -adrenoceptors and/or blocking 5-HT<sub>2A</sub> receptors in mice. Indeed, both  $\alpha_2$ -adrenoceptor agonists (Nabeshima et al., 1987) and 5-HT<sub>2A</sub> receptor antagonists (Alhaider, 1991; Barber et al., 1989; Lin and Su, 1992) are known to have analgesic activity in the brain. The discrepancy in the sensitivity to monoamine depletion between suppression of the nociceptive responses and that of the 5-HT<sub>2A</sub> receptor-mediated head-twitch responses by mitragynine remains unclear but it may be due to differences in the brain area involved in these behavioral responses. The head-twitch response is reportedly induced by activation of central 5-HT<sub>2A</sub> receptors (Handley and Singh, 1986), while antinociception can be induced from spinal and supraspinal levels (Fields, 1993).

In summary, supraspinally administered mitragynine exerts antinociceptive activity in the tail-pinch and hot-plate test. Both descending noradrenergic and serotonergic systems are involved in the antinociceptive activity of mitragynine on mechanical noxious stimulation, while the descending noradrenergic system contributes predominantly to the action of mitragynine on thermal noxious stimulation. Although the antinociceptive action of i.c.v. mitragynine is sensitive to an opioid receptor antagonist, the mechanism(s) underlying the action may differ from that of the morphine action.

## Acknowledgements

This work was supported in part by the Monbusho International Scientific Research Program for Joint Research (No. 06044035) from the Ministry of Education, Science and Culture, Japan. We thank Prof. Y. Kuraishi (Toyama Medical and Pharmaceutical University) for his technical advice concerning the i.t. injection procedure.

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