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Antinociceptive effect of 7-hydroxymitragynine in mice: Discovery of an orally active opioid analgesic from the Thai medicinal herb *Mitragyna speciosa*

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Abstract

Mitragynine is an indole alkaloid isolated from the Thai medicinal plant *Mitragyna speciosa*. We previously reported the morphine-like action of mitragynine and its related compounds in the in vitro assays. In the present study, we investigated the opioid effects of 7-hydroxymitragynine, which is isolated as its novel constituent, on contraction of isolated ileum, binding of the specific ligands to opioid receptors and nociceptive stimuli in mice. In guinea-pig ileum, 7-hydroxymitragynine inhibited electrically induced contraction through the opioid receptors. Receptor-binding assays revealed that 7-hydroxymitragynine has a higher affinity for μ-opioid receptors relative to the other opioid receptors. Administration of 7-hydroxymitragynine (2.5–10 mg/kg, s.c.) induced dose-dependent antinociceptive effects in tail-flick and hot-plate tests in mice. Its effect was more potent than that of morphine in both tests. When orally administered, 7-hydroxymitragynine (5–10 mg/kg) showed potent antinociceptive activities in tail-flick and hot-plate tests. In contrast, only weak antinociception was observed in the case of oral administration of morphine at a dose of 20 mg/kg. It was found that 7-hydroxymitragynine is a novel opioid agonist that is structurally different from the other opioid agonists, and has potent analgesic activity when orally administered.

Keywords: Mitragynine; 7-Hydroxymitragynine; Morphine; Opioid receptor; Analgesic; Ileum

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Introduction

The leaf of *Mitragyna speciosa* (*kratom* in Thai) has been used in Thailand for its opium-like effect (Burkill, 1935) and its coca-like stimulant ability to combat fatigue and enhance tolerance to for hard work under a scoring sun (Grewal, 1932; Suwanlert, 1975). Additionally, it has been used to treat diarrhea and to wean addicts off morphine (Suwanlert, 1975; Jansen and Prast, 1988).

We have studied the pharmacological activities of an indole alkaloid isolated from Uncaria hook (*Uncaria rhynchophylla*) that is used clinically for hypertension (Yano et al., 1991; Horie et al., 1992). Mitragynine (Fig. 1), the main component of the leaves of *Mitragyna speciosa*, is an indole alkaloid and structurally similar to Uncaria alkaloid. Mitragynine was reported to be comparable to codeine as an analgesic in dog, but it was not considered to act on opioid receptors (Macko et al., 1972; Jansen and Prast, 1988). We studied the effect of mitragynine on guinea-pig ileum contraction and found that mitragynine is an opioid receptor agonist (Watanabe et al., 1992, 1997). Some pharmacological investigations of mitragynine have also revealed that it has an antinociceptive action through the supraspinal opioid receptors, and that its action is dominantly mediated by μ- and δ-receptor subtypes in in vivo and in vitro studies (Matsumoto et al., 1996a,b; Tohda et al., 1997; Thongpradichote et al., 1998).

Our previous study demonstrated a potent opioid agonistic property of mitragynine and its derivative compound mitragynine pseudoindoxyl in in vitro experiments (Yamamoto et al., 1999). In guinea-pig ileum, mitragynine and mitragynine pseudoindoxyl inhibit the vagally stimulated twitch contraction through opioid receptors. The effect of mitragynine pseudoindoxyl was 20-fold more potent than that of morphine. In mouse vas deferens, the effect of mitragynine pseudoindoxyl was 35-fold more potent than that of morphine. Mitragynine pseudoindoxyl was found to be a very potent μ- and δ-opioid agonist in both functional and binding assays (Yamamoto et al., 1999). In spite of its potent opioid effect, mitragynine pseudoindoxyl induced only a weak antinociceptive effect in the mouse tail-flick test in comparison with morphine (Takayama et al., 2002).

We previously compared the antinociceptive effect of *Mitragyna speciosa* and mitragynine in in vivo experiments, but the antinociceptive effect of mitragynine was less potent than that of the crude extract of *Mitragyna speciosa* (Watanabe et al., 1992, 1999). This finding means that minor constituents of *Mitragyna speciosa* may have a very potent antinociceptive effect. We surveyed the other constituents of *Mitragyna speciosa* and found a new compound 7-hydroxymitragynine (Fig. 1), as a minor constituent of this plant (Ponglux et al., 1994). 7-Hydroxymitragynine exhibited a higher

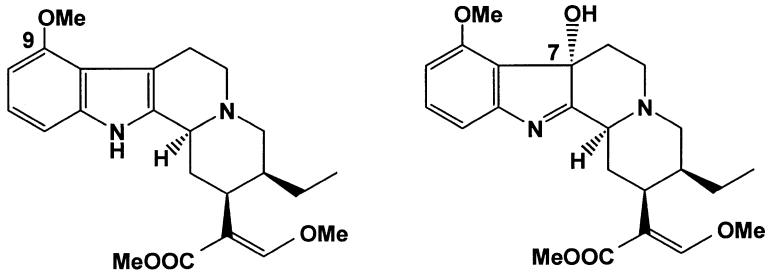

Fig. 1. Chemical structures of mitragynine and 7-hydroxymitragynine.

potency than mitragynine in the guinea-pig ileum test and a high affinity for μ-opioid receptors in binding assays (Takayama et al., 2002).

In the present study, we examined the opioid effect of 7-hydroxymitragynine on isolated guinea-pig ileum contraction and receptor binding assays. In addition, we investigated the antinociceptive activity of subcutaneous and oral administration of 7-hydroxymitragynine in tail-flick and hot-plate tests, comparing its activity with morphine.

Methods

Animals

Male albino Dunkin-Hartley guinea-pigs (320–570 g) purchased from Takasugi Lab. Animals Co., Ltd. (Saitama, Japan) and male ddY mice (22–38 g) from Japan SLC Inc. (Shizuoka, Japan) were used. Animals were housed under conditions with controlled temperature (24 ± 2°C) and a 12-h light/dark cycle (lighting on at 0700), for at least 1 week before the start experiment. Food and water were available ad libitum. The experiments were performed in compliance with "Guiding Principles for the Care and Use of Laboratory Animals" approved by the Japanese Pharmacological Society and the guidelines approved by the Ethical Committee on Animal Care and Animal Experiment of Graduate School of Pharmaceutical Sciences, Chiba University. This study was carried out in accordance with the guidelines of the Ethics Committee of the International Association for the Study of Pain (Zimmermann, 1983).

Isolated tissue preparations

Male guinea-pigs were killed by stunning and exsanguination. Segments of the ileum were removed and placed in Krebs-Henseleit solution (mM): NaCl, 112.08; KCl, 5.90; $CaCl_2$, 1.97; $MgCl_2$, 1.18; NaH_2PO_4, 1.22; $NaHCO_3$, 25.00; and glucose, 11.49. The ileum was placed under 1 g tension in a 5 ml organ bath containing the nutrient solution. The bath was maintained at 37°C and continuously bubbled with a mixture of 95% O_2 and 5% CO_2. Tissues were stimulated by a platinum needle-ring (a ring was placed 20 mm above the base of a needle 5 mm in length) electrode. After 60 min equilibration in Krebs-Henseleit solution, the ileum was transmurally stimulated (Cox and Weinstock, 1966) with monophasic pulses (0.2 Hz and 0.1 ms duration) by a stimulator (SEN-7203, Nihon Kohden, Tokyo, Japan). Contractions were isotonically recorded by using a displacement transducer (NEC Type 45347, San-ei Instruments Ltd., Tokyo, Japan). Effects of drug treatments were examined on the twitch contractions evoked by transmural stimulation elicited through ring electrodes. At the start of each experiment a maximum response to acetylcholine (3 μM) was obtained in each tissue to check its stability. The mean amplitude of the electrically stimulated contraction was about 30% of the maximal response to acetylcholine (3 μM). The electrically-induced twitch contraction was almost abolished by tetrodotoxin (1 μM) and atropine (0.1 μM) as described previously (Watanabe et al., 1997). Thus the electrical stimulation induces cholinergic contraction in guinea-pig ileum (Brookes et al., 1991). All concentration-response curves were constructed in a cumulative manner. The height of the twitch response to transmural stimulation was measured before and after drug challenge. Contraction (%) is expressed as a percentage of twitch response to the transmural stimulation before the drug challenge.

Receptor binding assay

Male guinea-pigs were killed by stunning and cervical dislocation. The whole brain (excluding cerebellum) was quickly removed, weighed, placed in ice cold 50 mM Tris HCl buffer, pH 7.4, and frozen immediately. Frozen brains were stored at −70 °C until the assay. For each experiment, frozen brains from two animals were thawed and homogenized with a Polytron homogenizer (PT 10-35, Kinematica, Littau, Switzerland) for 60 sec in 50 mM Tris HCl (pH 7.4) and centrifuged at 49,000 g for 10 min (Childers et al., 1979). The pellet was re-homogenized and centrifuged again. For the binding assays, membrane fractions were suspended in assay buffer. Protein content was measured by using a DC-protein assay kit (Bio-Rad, Richmond, USA).

Saturation-binding isotherms were produced by incubating membrane proteins with radiolabeled compounds in different concentrations. Using the above solution, 0.1 ml aliquots of protein were added to 0.9 ml mixture solution of the labeled assay sample with unlabeled competing ligands in appropriate concentrations, which was solved in 50 mM Tris-HCl (pH 7.4) assay buffer. The assay solution contained one of the followings; 3 nM of [^{3}H][D-Ala^2, N-MePhe^4, Gly-ol^5]-enkephalin ([^{3}H]DAMGO), 3 nM of [^{3}H](5α,7α,8β)-(+)-*N*-(7-[1-pyrrolidinyl]-1-oxaspirol[4,5]dec-8-yl) benzeneacetamide ([^{3}H]U69593) or 1 nM of [^{3}H][D-Pen^2, D-Pen^5]-enkephalin ([^{3}H]DPDPE). The incubation periods were 3, 4, and 1 hr for [^{3}H]DAMGO, [^{3}H]DPDPE, and [^{3}H]U69593, respectively, at 25 °C. The reaction was terminated by rapid filtration under reduced pressure through glass fiber filters (Whatman GF/B, presoaked in 0.3% polyethyleneimine) followed by the addition of 4 ml of ice-cold Tris-HCl buffer. Filters were further washed with 4 ml ice-cold buffer and left allowed to dry. Radioactivity bound to the filters was measured by liquid scintillation spectrometry (Aloka LSC-5100, Tokyo, Japan). Nonspecific binding for [^{3}H]DAMGO, [^{3}H]DPDPE or [^{3}H]U69593 was determined in the presence of 1 μM unlabeled DAMGO, naltrindole hydrochloride, and U69593, respectively. All values were presented as the mean ± s.e.mean. The apparent dissociation constant (K_D) and maximum binding site density (B_{max}) for radioligands were estimated by Scatchard analysis of the saturation. The ability of unlabeled drugs to inhibit specific radioligand binding was expressed as the IC_{50} value, which was the molar concentration of unlabeled drug necessary to displace 50% of the specific binding. Inhibition constants (Ki) of unlabeled compounds were calculated as described by Cheng and Prusoff (1973).

Tail-flick test

The method was adapted from that of D'Amour and Smith (1941). Mice responded to a focused heat stimulus by flicking or moving their tail from the path of the stimulus, thereby exposing a photocell located in the tail-flick analgesia meter (Ugo Basile Tail-flick Unit 7360, Ugo Basile, Comerio, Italy) immediately below the tail. The reaction time was automatically recorded. Prior to dosing, the nociceptive threshold was measured three times, and the mean of the reaction times was used as pre-drug latency for each mouse. The cut-off time of 10 sec was used to prevent tissue damage.

Hot-plate test

In the hot-plate test, an animal was placed in a glass cylinder of 24 cm diameter on a metal plate maintained at 55 ± 0.2 °C, and the latency period until nociceptive responses such as licking, shaking of the limbs or jumping was measured. Prior to dosing, the nociceptive threshold was measured three times,

and the mean of the reaction times was used as the pre-drug latency for each mouse. The cut-off time of 45 sec was used to prevent tissue damage.

Antinociception in tail-flick and hot-plate tests was quantified as the maximum possible effect (MPE) using the following formula:

$$\text{MPE } (\%) = \frac{\text{Post} - \text{drug latency} - \text{Pre} - \text{drug latency}}{\text{Cut} - \text{off time} - \text{Predrug latency}} \times 100 \quad (1)$$

Drugs

The drugs used in this study were acetylcholine chloride (Dai-ichi Pharmaceutical Co., Tokyo, Japan), atropine sulfate (Nacalai tesque Inc., Tokyo, Japan), tetrodotoxine (Sankyo, Tokyo, Japan), morphine hydrochloride (Takeda Chemical Industries, Osaka, Japan), naloxone hydrochloride, DAMGO, U-69593, naltrindole hydrochloride (Sigma Chemical Co., St. Louis, USA) and [^{3}H]DAMGO, [^{3}H]DPDPE and [^{3}H]U69593 (NEN Life Science Products Inc., Boston, USA). Mitragynine was isolated from extract of leaves of *Mitragyna speciosa* and 7-hydroxymitragynine was synthesized as described previously (Ponglux et al., 1994; Takayama et al., 2002). The purity (>99%) of these compounds was checked by HPLC and ^{1}H-NMR (500 MHz) analysis (Takayama et al., 2002).

For bioassay and binding assay, mitragynine and 7-hydroxymitragynine were first dissolved in 100% dimethylsulfoxide to yield a 10 mM solution and then subsequently diluted with distilled water. Other drugs were dissolved in distilled water.

For antinociceptive tests, morphine and 7-hydroxymitragynine were first dissolved in 100% dimethylsulfoxide and then subsequently diluted with 0.5% carboxyl methylcellulose. The final concentration of dimethylsulfoxide was 4.8%. The vehicle did not affect the pain response in tail-flick and hot-plate tests (data not shown). All drug solutions were prepared just before the experiments. Solution was injected in a volume of 10 ml/kg body weight. Feeding probe was used when drugs and their vehicle were given p.o.

Statistical analysis

The data are expressed as the mean ± s.e.mean. Statistical analyses were performed with two-tailed Student's *t*-test for comparison of two groups, and by a one-way analysis of variance followed by a Bonferroni multiple comparison test for comparison of more than two groups. A P value < 0.05 was considered statistically significant.

Results

Effect of 7-hydroxymitragynine on electrically induced contraction in guinea-pig ileum

The inhibitory effect of 7-hydroxymitragynine on twitch contraction induced by electrical stimulation in guinea-pig ileum is shown in Fig. 2. The addition of 7-hydroxymitragynine inhibited

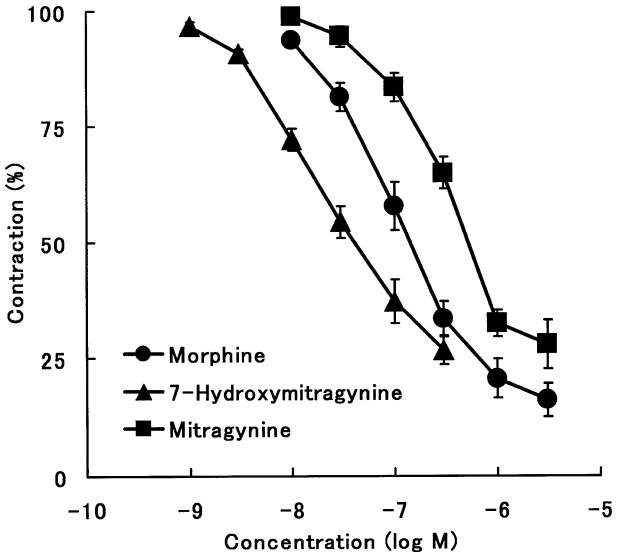

Fig. 2. Concentration response curves for inhibitory effects of 7-hydroxymitragynine and mitragynine on electrical stimulation-induced contraction in guinea-pig ileum. Each value is expressed as contraction percentage of the transmurally stimulated twitch contraction before the addition of samples. Data represent mean ± s.e.mean of five animals.

the electrically stimulated twitch contraction in a concentration-dependent manner as mitragynine and morphine did. The pD_2 values were 7.78 ± 0.08 for 7-hydroxymitragynine, 6.50 ± 0.06 for mitragynine and 7.02 ± 0.08 for morphine. Consequently, 7-hydroxymitragynine exhibits about 13- and 46-fold higher potency than morphine and mitragynine, respectively. Naloxone reversed the inhibitory effect of 7-hydroxymitragynine (control, 67.2 ± 5.3%, n = 5; naloxone 10 nM, 48.3 ± 10.2%, n = 5; naloxone 300 nM, −8.0 ± 5.3%, n = 5, $P < 0.001$ vs. control) as well as that of morphine (control, 77.7 ± 7.8%, n = 5; naloxone 10 nM, 63.0 ± 9.8%, n = 5; naloxone 300 nM, −33.0 ± 13.5%, n = 5, $P < 0.001$ vs. control). 7-Hydroxymitragynine (300 nM) did not affect the concentration-response curve for acetylcholine in the ileum (data not shown). These results suggest that 7-hydroxymitragynine has an opioid agonistic activity in the guinea-pig ileum.

Effect of 7-hydroxymitragynine on opioid-receptor binding in brain

Competition binding assays revealed that 7-hydroxymitragynine bound to opioid receptors in homogenates of guinea-pig brain membrane (Table 1). The affinities of these compounds for three opioid receptor types were determined by evaluating the inhibition of binding of ligands to μ-, δ- and κ-opioid receptors. Specific bindings of these radioligands for the opioid receptor types were saturable, and Scatchard plots were linear. The K_D values of [^{3}H]DAMGO, [^{3}H]DPDPE and [^{3}H]U69593 were 1.07 ± 0.06, 0.66 ± 0.05 and 0.87 ± 0.05 nM, respectively. Further, their B_{max} values were 88.2 ± 15, 41.2 ± 0.74 and 78.5 ± 9.8 fmol/mg protein, respectively.

Fig. 3 shows displacement curves for specific binding of [^{3}H]DAMGO, [^{3}H]DPDPE and [^{3}H]U69593 with various concentrations of 7-hydroxymitragynine. 7-Hydroxymitragynine interacted with all three

Table 1
Binding affinities (pKi) of 7-hydroxymitragynine on μ-, δ- and κ-opioid receptors in homogenates of guinea-pig brain

	[^{3}H]DAMGO (μ-sites)	[^{3}H]DPDPE (δ-sites)	[^{3}H]U69593 (κ-sites)
7-Hydroxymitragynine	8.01 ± 0.03	6.84 ± 0.12	6.71 ± 0.11
DAMGO	8.73 ± 0.04	ND	ND
Naltrindole	ND	8.61 ± 0.01	ND
U-69593	ND	ND	8.77 ± 0.03

The values are expressed as the mean ± s.e.mean of five or six separate displacement curves, each assayed in triplicate. The μ-binding sites were labeled with [^{3}H]DAMGO (3 nM), δ-sites with [^{3}H]DPDPE (1 nM) and κ-sites with [^{3}H]U69593 (3 nM). ND: not determined.

opioid sites but bound preferentially to μ-opioid receptors with pKi values of 8.01 ± 0.03, and displayed about 5 times lower affinity than DAMGO (pKi values of 8.73 ± 0.04). The relative affinities of 7-hydroxymitragynine for μ-, δ- and κ-opioid receptors were 89.8%, 5.6% and 4.6%, respectively.

Antinociceptive effects of subcutaneously and orally administered 7-hydroxymitragynine in mice

Antinociceptive effects of 7-hydroxymitragynine and morphine were compared in acute thermal pain tests in mice. Fig. 4 shows the antinociceptive effects of subcutaneous administration of these compounds. The administration of 7-hydroxymitragynine induced antinociception in a dose-dependent manner (2.5–10 mg/kg) in both tests. In the tail-flick test, the MPE value of 7-hydroxymitragynine (5 mg/kg, s.c.) reached 100% between 15–30 min after its administration. On the other hand, the maximum

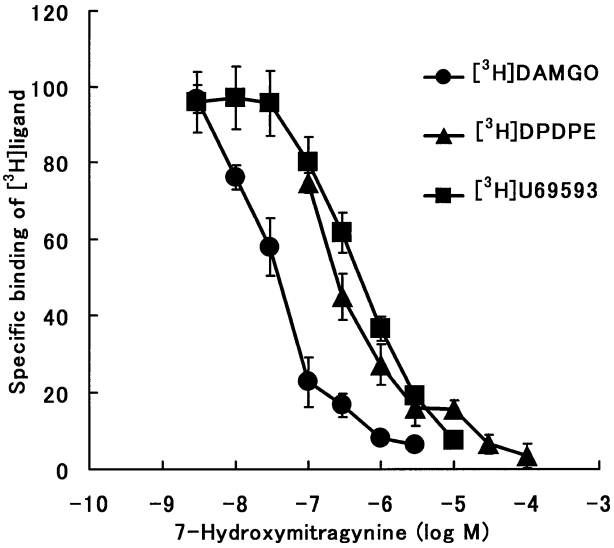

Fig. 3. Displacement curves for 7-hydroxymitragynine on specific binding of [^{3}H]DAMGO, [^{3}H]DPDPE and [^{3}H]U69593 in guinea-pig brain homogenates. Each value is expressed as a percentage of the specific binding in the absence of 7-hydroxymitragynine. Data represent mean ± s.e.mean of five or six separate experiments performed in triplicate.

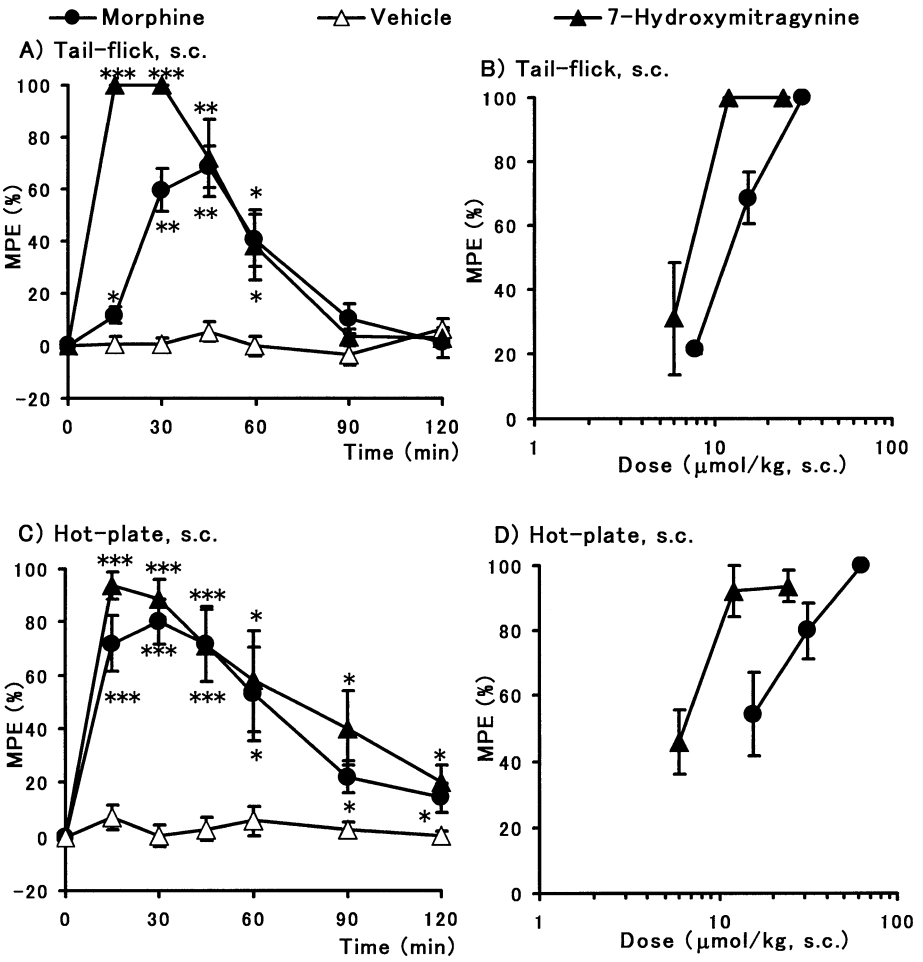

Fig. 4. Antinociceptive effects of 7-hydroxymitragynine and morphine in mice. All compounds were administered subcutaneously. A) Time course of the antinociceptive effect of 7-hydroxymitragynine (5 mg/kg) and morphine (5 mg/kg) in the tail-flick test. B) Dose-response curves for antinociceptive effects of 7-hydroxymitragynine and morphine in the tail-flick test. Each point represents maximum effect of the drugs at each dose. C) Time course of the antinociceptive effect of 7-hydroxymitragynine (10 mg/kg) and morphine (10 mg/kg) in the hot-plate test. D) Dose-response curves for the antinociceptive effect of 7-hydroxymitragynine and morphine in the hot-plate test. Each point represents maximum effect of the drugs at each dose. Each point represents the mean ± s.e.mean of six mice. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$, versus the corresponding control group.

MPE value of morphine (5 mg/kg, s.c.) was only about 69% at 45 min after its administration. In the hot-plate test, a similar effect of 7-hydroxymitragynine was observed. The MPE values of 7-hydroxymitragynine (10 mg/kg, s.c.) and morphine (10 mg/kg, s.c.) peaked at 94% at 15 min and 79% at 30 min, respectively, after their administration. The effect of 7-hydroxymitragynine was more efficacious than that of morphine at the same dose in both tests. Naloxone (2 mg/kg, s.c.) inhibited the antinociceptive effect of 7-hydroxymitragynine (10 mg/kg, s.c.) in tail-flick (control, 100 ± 0%, $n = 6$; naloxone, 8.4 ± 4.9%, $n = 6$, $P < 0.001$) and hot-plate tests (control, 94 ± 5.0%, $n = 6$; naloxone, 9.5 ± 4.9%, $n = 6$, $P < 0.001$).

The results about oral administration are noteworthy in tail-flick and hot-plate tests. Fig. 5 shows the antinociceptive effects of oral administration of 7-hydroxymitragynine and morphine. In the tail-flick test, 7-hydroxymitragynine (2.5–10 mg/kg, p.o.) showed a dose-dependent inhibition of pain response. The MPE value of 7-hydroxymitragynine (10 mg/kg, p.o.) reached 100% between 15–30 min after its administration, and a significant antinociceptive effect lasted for 90 min. On the contrary, the MPE value of morphine (20 mg/kg, p.o.) was only about 49% at 45 min after administration. In the hot-plate test, the MPE value of 7-hydroxymitragynine (20 mg/kg, p.o.) reached 87% at 15 min after administration. In

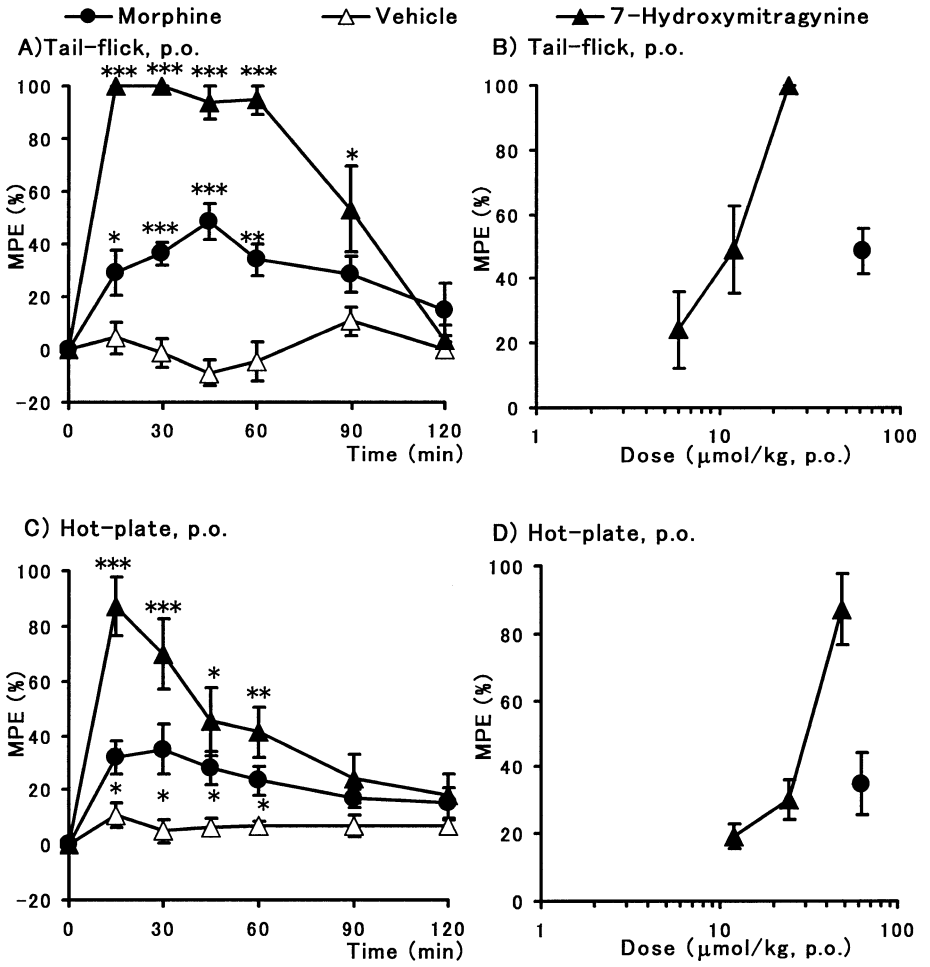

Fig. 5. Antinociceptive effects of 7-hydroxymitragynine and morphine in mice. All compounds were administered orally. A) Time course of the antinociceptive effect of 7-hydroxymitragynine (10 mg/kg) and morphine (20 mg/kg) in the tail-flick test. B) Dose-response curves for antinociceptive effects of 7-hydroxymitragynine and morphine in the tail-flick test. Each point represents maximum effect of the drugs at each dose. C) Time course of the antinociceptive effect of 7-hydroxymitragynine (20 mg/kg) and morphine (20 mg/kg) in the hot-plate test. D) Dose-response curves for the antinociceptive effect of 7-hydroxymitragynine and morphine in the hot-plate test. Each point represents maximum effect of the drugs at each dose. Each point represents the mean ± s.e.mean of six mice. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$, versus the corresponding control group.

contrast, only weak antinociception was observed when morphine was orally administered at a dose of 20 mg/kg.

Discussion

We isolated a new compound, 7-hydroxymitragynine, as a minor constituent of the Thai medicinal herb *Mitragyna speciosa* (Ponglux et al., 1994). In the present study, we investigated its opioid effects in an isolated ileum contraction test, a receptor binding assay and antinociceptive tests, and found it to be a potent opioid agonist.

The electrically stimulated ileal preparation from guinea-pig was used as a model of the action of 7-hydroxymitragynine in its putative strong analgesic effects in mice. As morphine did, 7-hydroxymitragynine inhibited the electrically stimulated ileum contraction in a concentration-dependent manner as reported previously (Takayama et al., 2002). On the other hand, 7-hydroxymitragynine did not affect contraction induced by direct stimulation of the receptor on the smooth muscle by acetylcholine. Taken together, it is supposed that 7-hydroxymitragynine acts on the nerve endings to inhibit the release of neurotransmitters. The guinea-pig ileum contains populations of functional μ- and κ-opioid receptors (Lord et al., 1977; Chavkin and Goldstein, 1981). The inhibitory effect of 7-hydroxymitragynine was antagonized by the opioid receptor antagonist naloxone. Taken together, 7-hydroxymitragynine is found to have an opioid agonist property on μ- and/or κ-opioid receptors. In this ileum test, 7-hydroxymitragynine exhibits about 13-fold higher potency than morphine as reported previously (Takayama et al., 2002). It is noteworthy that 7-hydroxymitragynine, a novel alkaloid structurally different from the other opioid agonists, has a more potent opioid agonistic effect than morphine in guinea-pig ileum. In addition, 7-hydroxymitragynine exhibits about 46-fold higher potency than mitragynine as reported previously (Takayama et al., 2002). It is supposed that the introduction of a hydroxy group at the C7 position in the chemical structure of mitragynine is a suitable functional group for pharmacophore binding to opioid receptors.

Guinea-pig brain homogenates are commonly used as means of assessing the multiple opioid receptor binding spectra of narcotic analgesics. A close correlation between in vitro functional systems and opioid receptor binding in the brain has also been suggested (Pert and Snyder, 1973; Lord et al., 1977). Competition binding assays revealed that 7-hydroxymitragynine bound to opioid receptors in homogenates of guinea-pig brain membrane. Its affinities for three opioid receptor types were determined by evaluating the inhibition of binding of ligands to μ-, δ- and κ-opioid receptors. As a result, 7-hydroxymitragynine interacted with all three opioid sites, but bound preferentially to μ-opioid receptors as reported previously (Takayama et al., 2002).

The antinociceptive effect of mitragynine on noxious mechanical and thermal stimulation has been already reported in the tail-pinch and hot-plate tests in mice (Matsumoto et al., 1996a,b; Tohda et al., 1997; Thongpradichote et al., 1998; Watanabe et al., 1999), although its antinociceptive effect was less potent than that of morphine. In the present study, we found potent antinociceptive action of 7-hydroxymitragynine on thermal stimulation in tail-flick and hot-plate tests. The afferent nociceptive stimulus is similar in both tail-flick and hot-plate tests. However, spinal transection of the animal abolishes the pain response in the hot-plate test, while the pain response in tail-flick test is not affected. Therefore, the former is considered to be a supraspinally mediated response, and the latter response is considered a spinally mediated one (Yaksh, 1999).

7-Hydroxymitragynine showed a dose-dependent antinociceptive action when subcutaneously and orally administered to mice. The antinociceptive effect of 7-hydroxymitragynine was more potent than morphine in tail-flick and hot-plate tests in mice. Furthermore, the inhibitory effect of naloxone on antinociception induced by 7-hydroxymitragynine in mice indicates the involvement of opioid receptor systems in its action. Taken together, 7-hydroxymitragynine elicited antinociception in mice through its action on supraspinal and/or spinal opioid receptors. We demonstrated for the first time that 7-hydroxymitragynine has opioid receptor agonistic action sufficiently potent to produce antinociception. It is reported that pseudo-akuammigine, an alkaloid from *Picralima nitida* with structural similarity to mitragynine, exerts antinociceptive activity by oral administration in the tail-flick test (Menzies et al., 1998; Duwiejua et al., 2002).

It is noteworthy that 7-hydroxymitragynine possesses a potent antinociceptive action after oral administration in tail-flick and hot-plate tests. On the contrary, orally administered morphine did not show antinociceptive effects in either test. These results suggest that 7-hydroxymitragynine may be well absorbed when given by the oral route. It is well known that morphine, administered orally, is rapidly metabolized in the liver and excreted in urine (Iwamoto and Klaassen, 1977; Glare and Walsh, 1991). Consequently, a large amount of morphine is orally administered in the clinical use as analgesics. However, the repeated administration of morphine leads to induction of tolerance, and high doses of morphine may elicit undesirable side effects.

Conclusion

The Mitragyna alkaloid, 7-hydroxymitragynine induced dose-dependent antinociceptive effects in tail-flick and hot-plate tests in mice. This compound is structurally different from other opioid agonists, and has a more potent antinociceptive activity than morphine, especially with oral administration. 7-Hydroxymitragynine may be a seed for new analgesics due to its unique structure and a potent activity.

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