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Suppressive Effect of Mitragynine on the 5-Methoxy-N,N-dimethyltryptamine-Induced Head-Twitch Response in Mice

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MATSUMOTO, K., M. MIZOWAKI, H. TAKAYAMA, S.-I. SAKAI, N. AIMI, AND H. WATANABE. *Suppressive effect of mitragynine on the 5-methoxy-N,N-dimethyltryptamine-induced head-twitch response in mice.* PHARMACOL BIOCHEM BEHAV **57**(1/2) 319–323, 1997.—We investigated the effects of mitragynine, a major alkaloid isolated from the leaves of *Mitragyna speciosa* Korth (Rubiaceae), on the 5-HT_{2A} receptor-mediated head-twitch response in mice. Intraperitoneal injection of mitragynine (5–30 mg/kg), as well as intraperitoneal injection of 5-HT_{2A} receptor antagonist ritanserin, inhibited the 5-methoxy-N,N-dimethyltryptamine (5-MeO-DMT; 16 mg/kg, IP)-induced head-twitch response in a dose-dependent manner. In contrast, mitragynine affected neither head-weaving caused by 5-MeO-DMT, nor drug-free spontaneous motor activity. Pretreatment of mice with reserpine (5 mg/kg, IP), *p*-chlorophenylalanine (*p*-CPA, 300 mg/kg × 3 times, IP), or 6-hydroxydopamine (6-OHDA, 50 μg/mouse, ICV) plus nomifensine (5 mg/kg, IP) did not change the suppressant effect of mitragynine on the head-twitch response caused by 5-MeO-DMT. On the other hand, the α_2-adrenoceptor antagonists yohimbine (0.5 mg/kg, IP), and idazoxan (0.2 mg/kg, IP), significantly attenuated the suppressant effect of mitragynine. Lesion of central noradrenergic systems by 6-OHDA plus nomifensine did not alter the effect of idazoxan (0.2 mg/kg) on mitragynine-induced suppression of the head-twitch response. These results indicate that stimulation of postsynaptic α_2-adrenoceptor, blockade of 5-HT_{2A} receptors, or both, are involved in suppression of 5-HT_{2A} receptor-mediated head-twitch response by mitragynine. © 1997 Elsevier Science Inc.

Mitragynine Head-twitch Mice Noradrenaline α_2-Adrenoceptor 5-HT_{2A} receptor

MITRAGYNA speciosa Korth (Thai name: 'kratom', Rubiaceae) is a tree grown widely in the Southeast Asia, and its leaves are known to produce narcotic-like actions when smoked, chewed, or drunk as a suspension (16,17). Mitragynine (Fig. 1), which accounts for about 66% of the total alkaloids extracted from the young leaves of the plant (26), appears to be a psychoactive drug and produces analgesic and antitussive actions comparable to codeine (16,17,19). Mitragynine has a methoxyl group at position 4 of its indole structure, rendering this compound analogous to the 4-substituted indole psychedelics such as psilocybin and lysergic acid diethylamide (16,17). Although the neuropharmacological actions of these 4-substituted indole compounds are thought to be associated with the central serotonergic systems, little information is available on the effect of mitragynine on serotonergic function in the brain.

5-HT_{2A} receptor in the central nervous system is known to participate in various psychiatric disorders such as depression, anxiety, schizophrenia, sleep disorders, hallucinations, etc., in man (3,7,9,25). In rodents, 5-HT_{2A} receptor agonists and 5-HT precursors produce a "head-twitch response" (4), and this behavior provides an experimental model with which to study 5-HT_{2A} receptor function in the brain (12,22,23). Previous studies have implicated the tonic stimulation of α_2-adrenoceptors by endogenous noradrenaline in the suppression of the 5-HT_{2A} receptor-mediated head-twitch response in mice

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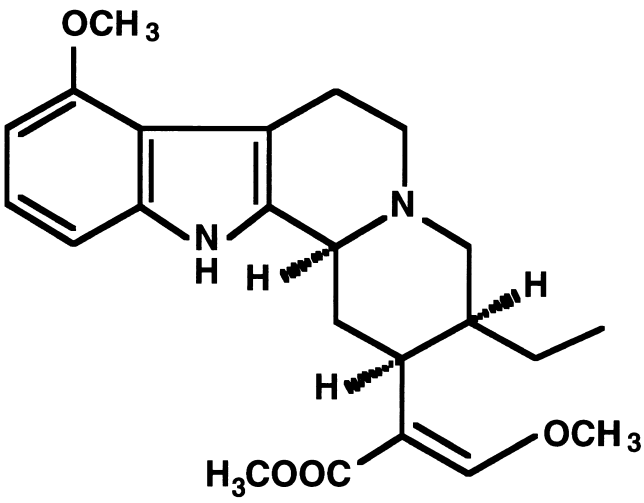

FIG. 1. The chemical structure of mitragynine, a major constituent of the alkaloidal fraction extracted from the leaves of *Mitragyna speciosa* Korth.

(5,15). In the present study, to clarify the ability of mitragynine to interact with central serotonergic systems, we investigated the effect of mitragynine on the head-twitch response caused by 5-MeO-DMT, and the possible mechanisms of action of this alkaloid.

MATERIALS AND METHODS

Animals

Male ddY mice (Japan SLC, Shizuoka, Japan) were obtained at the age of 4 weeks. They were housed in groups of 15 per cage (35 × 30 × 16 cm), on a 12 h L:D cycle (lights on: 0730–1930 h) at 25 ± 1 °C for at least 1 week before the experiments. Food and water were given ad libitum.

Measurement of 5-MeO-DMT-Induced Head-Twitch and Head-Weaving Responses

Mice were pretreated with test drugs or corresponding vehicles 30 min before the start of the experiments, and then were individually placed in the observation cages (24 × 17 × 12 cm) with a thin sawdust floor covering. Immediately after 5-MeO-DMT injection (16 mg/kg, IP.), behavioral changes were videotaped for later analysis. We chose 16 mg/kg 5-MeO-DMT in this study, because our preliminary study indicated that 5-MeO-DMT caused head-twitch and head-weaving responses in a dose-dependent manner, and that the drug, at 16 mg/kg, produced a median level of the behavioral responses. Head-twitch and head-weaving responses were counted for 10 min immediately after 5-MeO-DMT injection. The 5-HT_{2A} receptor antagonist (ritanserin) and the α_2-adrenoceptor antagonists (idazoxan and yohimbine) were administered either SC or IP, respectively, 30 min before 5-MeO-DMT injection.

Monoamine Depletion

Monoamines were depleted by an intraperitoneal (IP) injection of reserpine (5 mg/kg) 3 h before the start of the experiments (27).

Inhibition of 5-HT Synthesis in the Brain

Inhibition of 5-HT synthesis in the brain was achieved according to the method described by Dursun and Handley et al. (6). Briefly, mice were injected intraperitoneally with 3 doses of *p*-CPA (300 mg/kg, each) 24, 48, and 72 h before the experiments.

Lesion of Noradrenergic Systems in the Brain

Lesion of central noradrenergic systems in mice was performed as previously described (21). Mice were pretreated with nomifensine (5 mg/kg, IP), a selective dopamine uptake blocker, to protect dopaminergic systems. Thirty min later, mice were injected intracerebroventricularly with 6-OHDA (50 μg/mouse) or the corresponding vehicle. Intracerebroventricular (ICV) injection was accomplished by inserting a specifically designed injection needle into the lateral ventricle of mouse brain (about 2 mm lateral and 2 mm caudal to bregma) according to the method of Haley and McCormick (13). The injection needle consisted of an intradermic injection needle and a polyethylene tube which covered the needle and served as a "stopper" to give a tip length of 3 mm. Injection volume was adjusted to 5 μl/mouse. Seven days after ICV injection, mice were used for the behavioral experiments. Our previous data (21) indicated that this treatment significantly decreased noradrenaline contents in the cortex and hypothalamus by about 80 and 40%, respectively.

Measurement of Spontaneous Motor Activity

Spontaneous motor activity of the mice was measured as previously reported (1,2) using a Scanet SV-10 (Toyo Sangyo Co., Ltd., Toyama, Japan). Briefly, the photosensors were set at a height of 2.5 cm above the floor. Immediately after IP injection of vehicle or mitragynine (30 mg/kg), each mouse was placed individually in the Plexiglas cage (25 × 18 × 24 cm), which was fixed at the center of the Scanet SV-10 system, and spontaneous motor activity was measured over a 30 min period and calculated from the scanning data obtained.

Drugs

Mitragynine was isolated from the alkaloidal fraction extracted from the young leaves of *Mitragyna speciosa* Korth (Rubiaceae) as described previously (24). Drugs were obtained from the following sources: 5-methoxy-N,N-dimethyltryptamine (5-MeO-DMT), idazoxan HCl, p-chlorophenylalanine methyl ester HCl (*p*-CPA) and 6-hydroxydopamine HBr (6-OHDA) (Sigma Chemical Co., St. Louis, MO), ritanserin and nomifensine maleate (Research Biochemicals Inc., Natick, MA), yohimbine HCl (Nacalai Tesque Inc., Kyoto, Japan), reserpine (Apoplon Inj., Daiichi Pharmaceutical Co., Ltd., Tokyo, Japan). Mitragynine was dissolved in 1% acetic acid and adjusted pH up to 4.7 with 1N-NaOH. 5-MeO-DMT was dissolved in saline by adding a few drops of 1N-HCl, and adjusted pH up to 4.7 with 1N-NaOH. Ritanserin was dissolved in a small amount of ethanol and then diluted with saline. 6-OHDA solution was freshly prepared by dissolving in ice-cold saline containing 0.2% ascorbic acid. Other test drugs were dissolved in saline. Drug solutions were prepared just before the start of the experiments.

Statistics

Drug effects were analyzed with the Kruskal–Wallis analysis of variance followed by the Mann–Whitney's U-test for multiple comparisons. The ED_{50} values, with 95% confidence

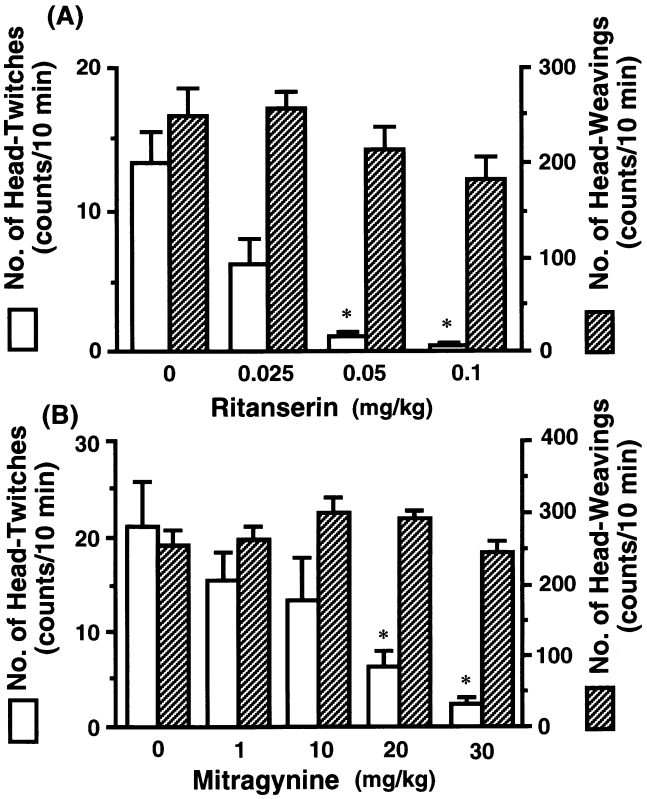

FIG. 2. Effects of ritanserin (A) and mitragynine (B) on the 5-MeO-DMT-induced head-twitch response. Mice were pretreated with vehicle or different doses of ritanserin or mitragynine (IP) 30 min before 5-MeO-DMT. 5-MeO-DMT (16 mg/kg) was injected IP. Head-twitch and head-weaving responses were counted during a 10 min observation period. Each data point represents the mean ± SEM ($n = 7$). *$p < 0.01$ vs. respective vehicle-pretreated group.

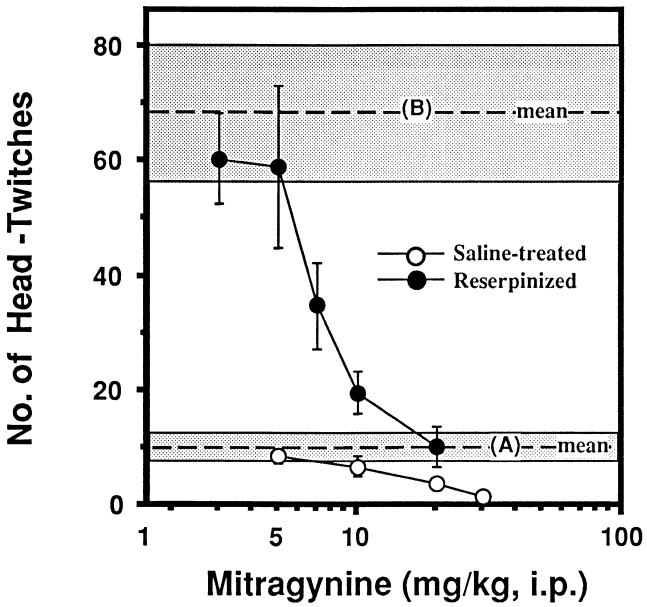

FIG. 3. Effect of reserpine treatment on suppression of the 5-MeO-DMT-induced head-twitch response by mitragynine. Mice were pretreated with reserpine (5 mg/kg, IP) 3 h before the experiments. Mitragynine (30 mg/kg) or vehicle were injected (IP) 30 min before 5MeO-DMT (16 mg/kg, IP). The 5-MeO-DMT-induced head-twitch response was observed as described in Fig. 2. The dotted areas indicated by A and B represent the vehicle control values (mean ± SEM) in saline-pretreated and reserpine-pretreated mice, respectively. The ED_{50} values (95% confidence limits) were 12.5 (19.3–7.7) and 8.1 (13.3–5.4) mg/kg in control and reserpinized mice, respectively.

limits, of mitragynine doses that produced a 50% suppression of the 5-MeO-DMTinduced head-twitch response in normal and reserpine-pretreated mice were calculated by Probit Analysis (10) using a computerized program. Values of $p < 0.05$ were considered statistically significant.

RESULTS

Effects of Mitragynine on 5-MeO-DMT-Induced Head-Twitch and Head-Weaving Responses

As shown in Fig. 2, the 5-HT agonist 5-MeO-DMT (16 mg/kg, IP) caused headtwitch and head-weaving responses in mice. Pretreatment with ritanserin (0.025–0.1 mg/kg, SC), a selective 5-HT_{2A} receptor antagonist, significantly suppressed the 5-MeO-DMT-induced head-twitch response in a dose-dependent manner. Mitragynine (1–30 mg/kg, IP) also dose-dependently attenuated the head-twitch response caused by 5-MeO-DMT in mice, although the effective dose of mitragynine was larger than that of ritanserin. On the other hand, mitragynine, as well as ritanserin, exhibited no significant effect on the 5-MeO-DMT-induced head-weaving response.

Effects of Mitragynine on Spontaneous Motor Activity in Mice

Mitragynine, at the dose (30 mg/kg, IP) that maximally suppressed the 5-MeO-DMT-induced head-twitch response, did not significantly change spontaneous motor activity in mice [spontaneous motor activity levels in vehicle-treated mice and mitragynine-treated mice were 5,536 ± 1,583 and 4,099 ± 677 counts/30 min (mean ± SEM, $n = 7$-8; $p > 0.05$), respectively]. Mitragynine, at the doses tested in this study, did not induce any behavioral changes.

Effects of Reserpine and p-CPA Treatments on Mitragynine Suppression of Head-Twitch Response

Pretreatment with reserpine (5 mg/kg, IP) markedly increased the number of head-twitches caused by 5-MeO-DMT (Fig. 3). Mitragynine (3–20 mg/kg, IP) exhibited a dose-dependent suppressant action on the 5-MeO-DMT-induced head-twitch response in reserpinized mice. The apparent ED_{50} value of mitragynine to suppress the head-twitch response did not change following reserpine treatment [ED_{50} (95% confidence limits): 12.5 (19.3–7.7) and 8.1 (13.3–5.4) mg/kg in control and reserpinized mice, respectively]. Moreover, the suppressive effect of 30 mg/kg mitragynine on 5-MeO-DMT-induced head-twitch response did not change following pretreatment with saline [21.2 ± 4.6 and 2.3 ± 0.8 counts/10 min in vehicle control and 30 mg/kg mitragynine-treated group, respectively (mean ± SEM, $n = 10$, $p < 0.01$)] or with *p*-CPA [16.6 ± 4.2 and 1.7 ± 0.7 counts/10 min in vehicle control and 30 mg/kg mitragyninetreated group ($n = 7$, $p < 0.01$), respectively].

Effects of α_2-Adrenoceptor Antagonists on Mitragynine Inhibition of the Head-Twitch Response

Yohimbine (0.5 mg/kg) and idazoxan (0.2 mg/kg) significantly reversed the mitragynine (20 mg/kg, IP)-induced inhibi-

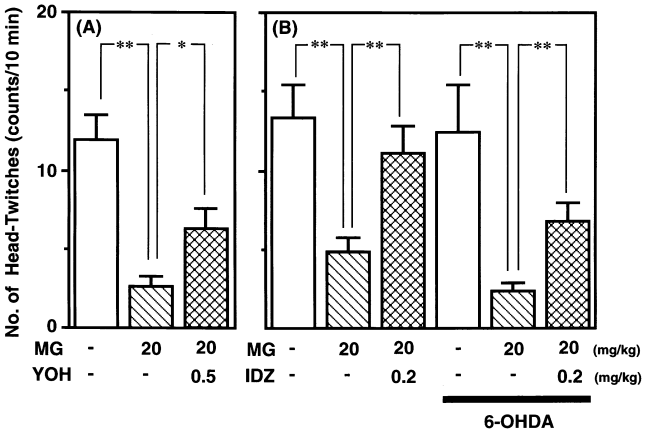

FIG. 4. Effect of idazoxan and yohimbine on mitragynine-induced suppression of the 5-MeO-DMT-induced head-twitch response in normal and 6-OHDA-pretreated mice. Yohimbine (YOH; 0.5 mg/kg, A), idazoxan (IDZ; 0.2 mg/kg; B), or saline were injected (IP) 30 min before 5-MeO-DMT (16 mg/kg). Mitragynine (MG; 20 mg/kg, A and B) or vehicle was injected (IP) immediately after administration of α_2-adrenoceptor antagonists. The 5-MeO-DMT-induced head-twitch response was observed as described in Fig. 2. Each data point represents the mean ± SEM. ($n = 10$). *$p < 0.05$ and **$p < 0.01$.

tion of the head-twitch response caused by 5-MeO-DMT (Fig. 4). We chose the doses of yohimbine and idazoxan that were shown to have no effect on 5MeO-DMT-induced head-twitches by themselves in our previous study (20). Consistent with our previous data (20), pretreatment with 6-OHDA did not significantly change the basal head-twitch response or the suppressive effect of mitragynine on the head-twitch response. 6-OHDA-treatment slightly but non-significantly attenuated the antagonistic effect of idazoxan (0.2 mg/kg) on mitragynine-induced suppression of the head-twitch response.

DISCUSSION

The present results demonstrate that mitragynine suppresses the 5-MeO-DMT-induced head-twitch response by stimulating postsynaptic α_2-adrenoceptors and/or by directly attenuating 5-HT_{2A} receptor function. 5-HT_{2A} and 5-HT_{1A} receptor subtypes are known to be implicated in the head-twitch and head-weaving responses caused by 5-HT agonists, respectively (11,30). In this study, mitragynine, as well as the selective 5-HT_{2A} receptor antagonist ritanserin, dose-dependently attenuated the head-twitch response caused by 5-MeO-DMT without affecting 5-MeO-DMT-induced head-weaving behavior. Moreover, mitragynine by itself did not induce a head-weaving response or an alteration in spontaneous motor activity in mice. Thus, these findings indicate that mitragynine possibly modulates 5-HT_{2A} receptor-mediated behavioral response.

The 5-HT_{2A} receptor-mediated head-twitch response is reportedly modulated by drugs such as clonidine, a selective α_2-adrenoceptor agonist, and 8-hydroxy-2(dipropylamino)tetraline (8-OH-DPAT), a selective 5-HT_{1A} receptor agonist (5,6,8,14,15). There is evidence indicating that tonic stimulation of postsynaptic α_2-adrenoceptors by endogenous noradrenaline negatively regulates the appearance of the 5-HT_{2A} receptor-mediated head-twitch response in mice (5,15). Moreover, endogenous 5-HT appears to be partly involved in 8-OH-DPAT's suppression of the head-twitch response caused by a selective 5-HT_{2A} receptor agonist (6). Thus, it is possible that either noradrenaline or 5-HT is involved in the suppressive action of mitragynine on the 5-MeO-DMT-induced head-twitch response in mice. However, involvement of noradrenaline and 5-HT seems unlikely, since pretreatment with reserpine, *p*-CPA, or 6-OHDA plus nomifensine did not change the suppressive action of mitragynine.

At least two mechanisms could account for the action of mitragynine: 1) direct blockade of the 5-HT_{2A} receptor subtype and/or 2) modulation of 5-HT_{2A} receptor function through stimulation of either the 5-HT_{1A} receptor or the postsynaptic α_2-adrenoceptor. It remains to be clarified whether mitragynine can directly interact with the 5-HT_{2A} receptor subtype. On the other hand, involvement of the 5-HT_{1A} receptor in mitragynine-induced suppression of the head-twitch response seems to be excluded, since as previously discussed, mitragyine neither produces the head-weaving response by itself nor increases the head-weavings induced by 5-MeO-DMT. In this study, the α_2-adrenoceptor antagonists yohimbine and idazoxan, at doses that alone had no effect on the head-twitch response, significantly reversed the inhibition of 5-MeO-DMT-induced head-twitch by mitragynine. The effect of idazoxan was slightly decreased following lesion of central noradrenergic systems, but was still statistically significant. Thus, these findings indicate that postsynaptic α_2-adrenoceptor stimulation is at least partly involved in the suppression of the head-twitch response by mitragynine. This hypothesis seems to be further supported by the recent findings in this laboratory and other laboratories that postsynaptic α_2-adrenoceptor mechanisms play an important role in the antinociceptive action of mitragynine (29; Matsumoto et al, unpublished data). Nevertheless, it remains unclear whether postsynaptic α_2-adrenoceptor receptor stimulation represents the major contribution to the suppression of the head-twitch response by mitragynine, since our previous data showed that the antagonistic effect of ritanserin and ketanserin on 5-MeO-DMT-induced head-twitch was also apparently blocked by idazoxan, at a dose which had no effect on head-twitches caused by 5-MeO-DMT. Further, our previous data showed that the effect of idazoxan was attenuated by lesion of noradrenergic systems in the brain (20).

5-HT_{2A} receptor agonists are reported to possess hallucinogenic activity in humans that correlates significantly with the affinities of those agonists for the 5-HT_{2A} receptor subtype (7,9). Moreover, their affinity for the 5-HT_{2A} receptor appears to correlate with their ability to produce head-twitch behavior in rodents (18). Although a clinical study has shown that chronic intake of the leaves of *Mitragyna speciosa* ('kratom') produces psychotic symptoms such as hallucinations in some cases (28), very few systematic studies have investigated whether mitragynine causes hallucination. Mitragynine possesses the chemical structure one might expect of a psychedelic hallucinogen (17). However, taking into account the present results, it can be speculated that mitragynine may suppress the occurrence of hallucinations triggered by stimulation of 5-HT_{2A} receptor in humans.

In conclusion, mitragynine-induced suppression of 5-MeO-DMT-induced head-twitch may be due to its agonistic property at postsynaptic α_2-adrenoceptors, or its antagonistic property at 5-HT_{2A} receptor, or both.

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