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Suppressive effects of isorhynchophylline on 5-HT_{2A} receptor function in the brain: Behavioural and electrophysiological studies

Kinzo Matsumoto[a,*], Ryo Morishige[a], Yukihisa Murakami[a], Michihisa Tohda[a], Hiromitsu Takayama[b], Iwao Sakakibara[c], Hiroshi Watanabe[a]

[a]*Division of Medicinal Pharmacology, Institute of Natural Medicine, Toyama Medical and Pharmaceutical University, 2630 Sugitani, Toyama 930-0194, Japan*
[b]*Graduate School of Pharmaceutical Sciences, Chiba University, Chiba 263-8522, Japan*
[c]*Research Division, Tsumura & Co., 3586 Yoshiwara, Ibaraki 300-1192, Japan*

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Abstract

Isorhynchophylline is a major oxindole alkaloid found in *Uncaria* species which have long been used in traditional Chinese medicine. Here, we investigated the effects of isorhynchophylline and isorhynchophylline-related alkaloids on 5-hydroxytryptamine (5-HT) receptor-mediated behavioural responses in mice and 5-HT-evoked current responses in *Xenopus* oocytes expressing 5-HT_{2A} or 5-HT_{2C} receptors. Isorhynchophylline dose-dependently inhibited 5-HT_{2A} receptor-mediated head-twitch but not 5-HT_{1A} receptor-mediated head-weaving responses evoked by 5-methoxy-N,N-dimethyltryptamine. Pretreatment with reserpine, a monoamine-depleting agent, enhanced the head-twitching, but did not influence the effect of isorhynchophylline on the behavioural response. Isocorynoxeine, an isorhynchophylline-related alkaloid in which the configuration of the oxindole moiety is the same as in isorhynchophylline, also reduced the head-twitch response in reserpinized mice over the same dose range as isorhynchophylline, while both rhynchophylline and corynoxeine, stereoisomers of isorhynchophylline and isocorynoxeine, did not. None of the alkaloids tested had an effect on meta-chlorophenylpiperazine-induced hypolocomotion, a 5-HT_{2C} receptor-mediated behavioural response. In experiments in vitro, isorhynchophylline and isocorynoxeine dose-dependently and competitively inhibited 5-HT-evoked currents in *Xenopus* oocytes expressing 5-HT_{2A} receptors, but had less of a suppressive effect on those in oocytes expressing 5-HT_{2C} receptors. These results indicate that isorhynchophylline and isocorynoxeine preferentially suppress 5-HT_{2A} receptor function in the brain probably via a competitive antagonism at 5-HT_{2A} receptor sites and that the configuration of the oxindole moiety of isorhynchophylline is essential for their antagonistic activity at the 5-HT_{2A} receptor.

Keywords: Isorhynchophylline; Isocorynoxeine; *Uncaria* alkaloid; 5-HT_{2A} receptor-mediated head twitch response; 5-HT_{2A} receptor-mediated current response; *Xenopus* oocyte

1. Introduction

Several lines of evidence indicate that the 5-HT_2 receptors in the central nervous system (CNS) are involved in psychiatric disorders such as depression, anxiety, schizophrenia, sleep disorders, and hallucinations (Baxter et al., 1995; Glennon, 1990; Schmidt et al., 1995). The 5-HT_{2A} receptor subtype in particular appears to play an important role in neuropsychiatric disorders and the blockade of this subtype has been hypothesized to result in antipsychotic activity since the actions of indoleamine and phenethylamine hallucinogens are mediated by this receptor (Aghajanian and Marek, 2000; Meltzer et al., 2003). In rodents, the stimulation of 5-HT_2 receptors in the CNS produces a variety of distinctive behavioural alterations such as head-twitching via 5-HT_{2A} receptors (Green and Heal, 1985) and hypolocomotion via 5-HT_{2C} receptors (Gleason et al., 2001; Gleason and Shannon, 1998). These

* Corresponding author. Fax: +81 76 434 5056.
E-mail address: mkinzo@ms.toyama-mpu.ac.jp (K. Matsumoto).

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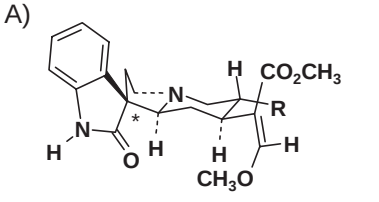
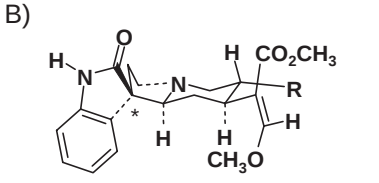
behavioural changes provide experimental models with which to study the functions of each receptor subtype in the brain.

The *Uncaria* species, such as *Uncaria rhynchophylla* (MIQ) Jackson and *Uncaria sinensis* (Oliv.) Havil, have long been used in Chinese medicine as antipyretic, antihypertensive and anticonvulsant medications for the treatment of headache, vertigo and epilepsy (Tang and Eisenbrand, 1992). A double-blind and placebo-controlled study performed by Terasawa et al. (1997), demonstrated that Choto-san (Diao-Teng-San in Chinese), a kampo (Japanese herbal) prescription in which a *Uncaria* plant (Rubiaceae) has been believed to play a key role, significantly improved psychiatric symptoms of patients with vascular dementia such as hallucination and delusion. However, the neuronal mechanism(s) and the herbal component(s) contributing to the effect remain to be clarified.

Isorhynchophylline and rhynchophylline (Fig. 1) are the major tetracyclic oxindole alkaloids in *Uncaria* species. Previously (Kang et al., 2002a), we demonstrated that these alkaloids exhibited antagonistic activity toward 5-HT_2 receptor-mediated current responses in *Xenopus* oocytes injected with rat brain total RNA. Together with the putative role of 5-HT_2 receptors in neuropsychiatric disorders, these preliminary findings prompted us to consider the possibility that *Uncaria* plant alkaloids may attenuate experimental neuropsychiatric symptoms which occur via 5-HT_2 receptors. Here we examined the effects of isorhynchophylline and isorhynchophylline-related oxindole alkaloid components (Fig. 1) on 5-HT_{1A}-, 5HT_{2A} and 5-HT_{2C} receptor-mediated behavioural alterations in mice and current responses in *Xenopus* oocytes, an in vitro receptor expression system.



	-R
Isorhynchophyllin (IRHY)	-CH_2H_5
Isocorynoxeine (ICOX)	-CH=CH_2



	-R
Rhynchophyllin (RHY)	-CH_2H_5
Corynoxeine (ICOX)	-CH=CH_2

Fig. 1. Chemical structures of isorhynchophylline and related oxindole alkaloids, rhynchophylline, isocorynoxeine and corynoxeine. The asterisk indicates the spiro C-7 position of a tetracyclic oxindole.

2. Materials and methods

2.1. Behavioural experiments

2.1.1. Animals

Male ICR mice (Japan SLC, Shizuoka, Japan) were obtained at the age of 5–6 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) at 25 ± 1 °C for at least 1 week before the experiments. Food and water were given ad libitum.

2.1.2. Measurement of head-twitch and head-weaving responses

The measurement of 5-methoxy-N,N-dimethyltryptamine (5-MeO-DMT)-induced head-twitch and head-weaving behaviour was performed according to our previous methods (Matsumoto et al., 1997a,b). Briefly, mice were pretreated with test drugs or corresponding vehicles 30 min before the start of the experiments, and then were individually placed in observation cages (24 × 17 × 12 cm) with a thin sawdust floor covering. Immediately after 5-MeO-DMT was injected (16 mg/kg, i.p.), behavioural changes were videotaped for later analysis. We chose 16 mg/kg of 5-MeO-DMT in this study, because it can induce head-twitch and head-weaving responses during the same observation period and produces a medial level of behavioural responses (Matsumoto et al., 1997a). Head-twitch and head-weaving responses were counted for 10 min immediately after the 5-MeO-DMT injection. Test drugs were administered i.p. 30 min before the injection.

2.1.3. Monoamine depletion

To exclude tonic influences of monoaminergic systems on head-twitch responses (Darmani et al., 1991; Heal et al., 1986; Matsumoto et al., 1997a), monoamines were depleted with an intraperitoneal injection of reserpine (5 mg/kg) 3 h before the start of the experiments according to our previous reports (Matsumoto et al., 1996, 1997a).

2.1.4. 5-HT_{2C} receptor-mediated suppression of spontaneous motor activity

Spontaneous motor activity was measured as reported (Asakura et al., 1994; Matsumoto et al., 1997a) using Scanet® (SV-10, Toyo Sangyo Co., Ltd., Toyama, Japan). Immediately after the i.p. injection of vehicle or the 5-HT_{2C} receptor agonist meta-chlorophenylpiperazine (mCPP), each mouse was placed individually in a Plexiglass cage (40 × 30 × 20 cm), which was fixed at the center of the Scanet SV-10 system, and spontaneous motor activity was measured over a 30 min period. Test drugs were administered i.p. 30 min before the mCPP injection.

2.2. Electrophysiological experiments

2.2.1. 5-HT_{2A} and 5-HT_{2C} receptor preparations

Rat-derived 5-HT_{2A} and 5-HT_{2C} cDNA clones inserted in *p*Bluescript KS(−) were kindly provided by Dr. D. J. Julius (Department of Cellular and Molecular Pharmacology, University of California, San Francisco, CA, USA). The receptor cDNAs for 5-HT_{2A} and 5-HT_{2C} were linearized with *Hin*dIII and *Xho*I,

respectively, extracted with phenol chloroform, and precipitated in ethanol and sodium acetate. cRNA was synthesized using a transcription kit (Stratagene, Cedar Creek, TX, USA) with T7 RNA polymerase, extracted with phenol-chloroform, and precipitated in ethanol and sodium acetate.

2.2.2. Whole cell voltage clamping of injected oocytes

The isolation and microinjection of *Xenopus* oocytes (Hamamatsu Seibutsu, Shizuoka, Japan) were performed as previously described (Kang et al., 2002a,b,c). *Xenopus* oocytes were injected with 50 ng of cRNA coding for the 5-HT_{2A} or 5-HT_{2C} receptor. An oocyte placed in a 50-μl chamber was perfused with modified Barth's saline (MBS) containing (in mM): NaCl, 88; KCl, 1; $CaCl_2$, 0.41; $Ca(NO_3)_2$, 0.33; $MgSO_4$, 0.82; $NaHCO_3$, 2.4; sodium pyruvate, 2.5; and HEPES, 5, pH 7.4 at 1.5 ml/min at room temperature (22–25 °C). Electrodes for recording and clamping were pulled from capillary tubing (outside diameter, 1.5 mm) and filled with 3 M KCl. 5-HT-induced current responses of oocytes with 5-HT_{2A} or 5-HT_{2C} receptors were measured at a holding potential of −60 mV using a two-electrode voltage clamp setup (Gene Clamp 500, Axon instruments, Foster City, CA, USA) as previously described (Kang et al., 2002a,b,c).

Considering the expected desensitization of current responses evoked by repeated application of each neurotransmitter, the currents measured before and after test drug treatment were averaged as control responses. The data are expressed as percentages of control responses to compensate for variability in the level of receptors in different oocytes.

All experiments were conducted in accordance with the National Institutes of Health guide for the care and use of Laboratory animals (NIH Publications No. 8023, revised 1978) and had the approval of the Institutional Animal Use and Care Committee of Toyama Medical and Pharmaceutical University.

2.3. Drugs

Tetracyclic oxindole alkaloids, isorhynchophylline, rhynchophylline, isocorynoxeine, and corynoxeine were isolated from *Uncaria* species and identified as reported (Abdel-fattah Mohamed et al., 2000). The following drugs were obtained from commercial sources: 5-MeO-DMT, ketanserin tartrate, 4-iodo-N-(2-[4-(methoxyphenyl)-1-piperazinyl]ethyl)-N-2-pyridinylbenzamide (p-MPPI) HCl, 1-(3-chlorophenyl) piperazine HCl (mCPP), and mianserin HCl (Sigma Chem., St. Louis, MO), and reserpine (Apoplon®, Daiichi Pharmaceutical Co., Ltd., Tokyo, Japan). In behavioural experiments, the tetracyclic oxindole alkaloids, 5-MeO-DMT and ketanserin were dissolved in an equi-molar amount of HCl and then diluted with saline. The pH of each solution was adjusted to 5–7 with 1N NaOH. Mianserin and mCPP were dissolved in saline. In electrophysiological experiments, the

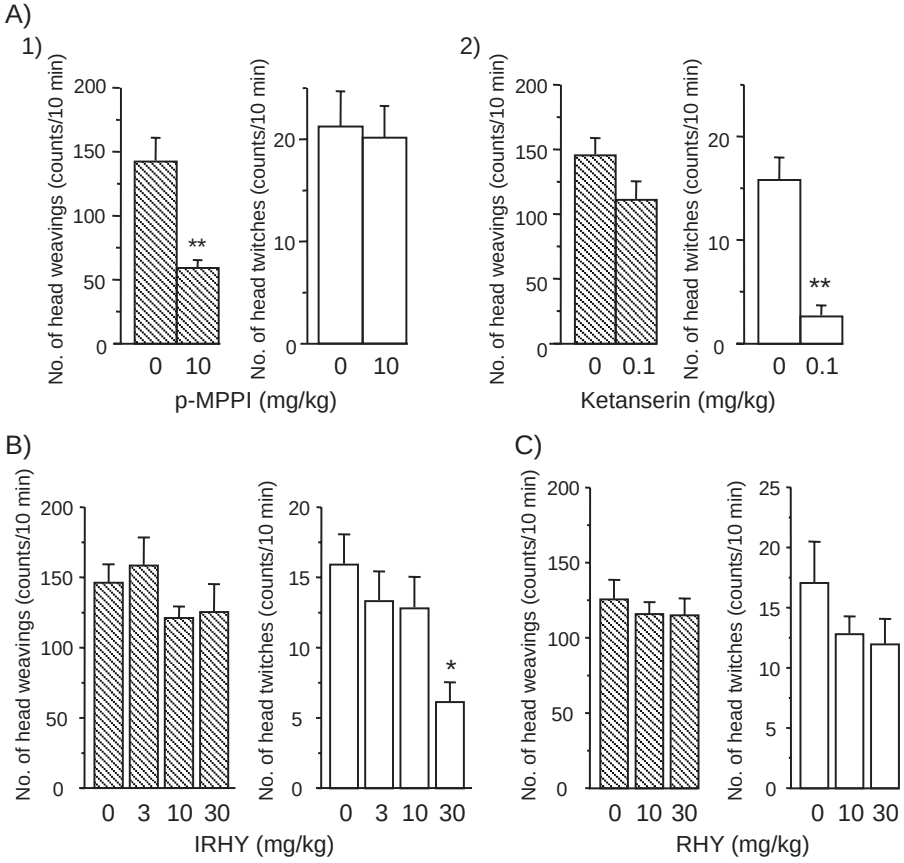


Fig. 2. Effects of the 5-HT_{1A} antagonist p-MPPI, the mixed 5-$HT_{2A/2C}$ receptor antagonist ketanserin and oxindole alkaloids (isorhynchophylline [IRHY]and rhynchophylline [RHY]) on the 5-MeO-DMT-induced head-weaving and head-twitch responses in mice. Mice received an i.p. injection of 5-MeO-DMT (16 mg/kg) just before the start of the experiments. Test drugs were injected i.p. 30 min before 5-MeO-DMT (n=9–11). Each datum represents the mean±S.E.M. *P<0.05, **P<0.01 vs. vehicle-treated group.

alkaloids dissolved in DMSO were diluted with normal MBS. The final concentration of DMSO used in the electrophysiological experiments was ≤0.1%. DMSO at this concentration did not induce any current responses in oocytes expressing 5-HT_{2A} or 5-HT_{2C} receptors. Drug solutions were prepared just before the start of the experiments.

2.4. Data analysis

In the behavioural experiments, head-twitch and head-weaving data were analysed with the Mann–Whitney Rank Sum-test for two-group comparisons or with the Kruskal–Wallis analysis of variance followed by Dunn's test for multiple comparisons. Changes in spontaneous motor activity were analysed with the one-way analysis of variance (ANOVA) followed by Student–Newman–Keuls test for multiple comparisons. In the electrophysiological experiments, the results are presented as percentages of control responses in order to compensate for variability in the level of receptors in different oocytes. The *n* values are the number of different oocytes studied. Values are expressed as the mean±S.E.M. except where noted otherwise. Each experiment was carried out with oocytes from at least two different frogs. Data analyses were performed with Student's *t*-test or the paired *t*-test. Curve fitting and the estimation of EC_{50} values and Hill coefficients from concentration–response curves were performed using PRISM® (GraphPad Software Inc., San Diego, CA, USA).

3. Results

3.1. Effects of isorhynchophylline on 5-MeO-DMT-induced head-twitch and head-weaving responses

Consistent with our previous reports (Matsumoto et al., 1997a,b) the systemic administration of 16 mg/kg of 5-MeO-DMT, a 5-HT receptor agonist, induced head-twitch and head-weaving responses in mice. p-MPPI, a selective in vivo and in vitro 5-HT_{1A} receptor antagonist (Allen et al., 1997; Kung et al., 1994, 1995), significantly attenuated the 5-MeO-DMT-induced head-weaving [$T=48$, $P<0.001$] but not head-twitching [$T=87$, $P=0.838$] (Fig. 2A-1), while the mixed 5-$HT_{2A/2C}$ antagonist ketanserin, significantly attenuated the head-twitch behaviour [$T=151$, $P<0.001$] without affecting the head-weaving [$T=127$, $P=0.096$] (Fig. 2A-2), indicating that 5-MeO-DMT-induced head-weaving and head-twitch responses are mediated by stimulation of 5-HT_{1A} and 5-HT_{2A} receptors, respectively. Isorhynchophylline, as well as ketanserin, dose-dependently reduced the head-twitch response [$H=13.202$, $P<0.01$] (Fig. 2B), while rhynchophylline, a stereoisomer altered at the C-7 position of the oxindole structure, did not affect the response to 5-MeO-DMT [$H=1.020$, $P=0.600$] (Fig. 2C). In contrast, neither alkaloid had any effect on the 5-MeO-DMT-induced head-weaving response in mice [isorhynchophylline: $H=3.164$, $P=0.367$; rhynchophylline: $H=0.304$, $P=0.859$] (Fig. 2B and C).

Head-twitch behaviour induced by stimulation of the 5-HT_{2A} receptor is known to be negatively regulated via the stimulation of α_2-adrenoceptors by endogenous noradrenaline. We next examined using reserpinized mice whether or not endogenous monoamines are involved in the suppressive effect of isorhynchophylline on the head-twitch response. As shown in Fig. 3A, the administration of

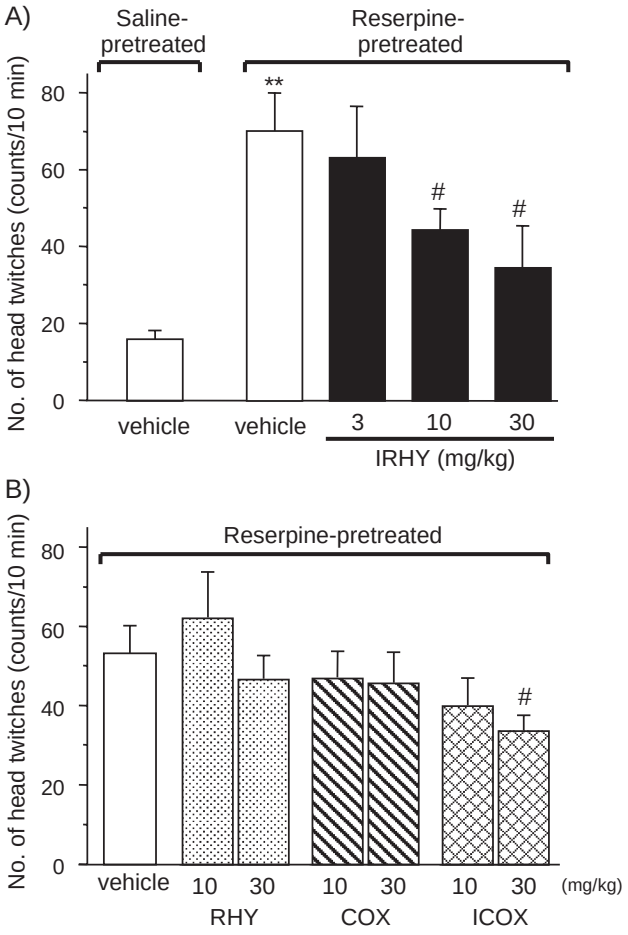


Fig. 3. Effect of isorhynchophylline, rhynchophylline, corynoxeine and isocorynoxeine on the 5-MeO-DMT-induced head-weaving response in reserpinized mice. A) Mice were pretreated with reserpine (5 mg/kg, i.p.) or saline 3 h before the start of the experiments. Isorhynchophylline (IRHY) was injected i.p. 30 min before the 5-MeO-DMT injection. Each datum represents the mean±S.E.M. ($n=10–11$). **$P<0.01$ vs. the saline-pretreated group. #$P<0.05$ vs. the reserpine-pretreated vehicle control group. B) Mice were pretreated with reserpine (5 mg/kg, i.p.) 3 h before the start of the experiments. Rhynchophylline (RHY), corynoxeine (COX), isocorynoxeine (ICOX) or vehicle was injected i.p. 30 min before 5-MeO-DMT. Each datum represents the mean±S.E.M. #$P<0.05$ vs. the reserpine-pretreated vehicle control group ($n=10$).

isorhynchophylline also caused a dose-dependent decrease in 5-MeO-DMT-induced head-twitching in reserpinized mice [$H=11.556$, $P<0.01$]. We also tested the effects of rhynchophylline, corynoxeine, and isocorynoxeine, isorhynchophylline-related alkaloids present in *Uncaria* species, on 5-MeO-DMT-induced head-twitch behaviour in reserpinized mice. Neither rhynchophylline [$H=1.369$, $P=0.504$] nor corynoxeine [$H=0.242$, $P=0.886$] affected the behaviour, while isocorynoxeine significantly attenuated it at 30 mg/kg (i.p.) [$H=7.582$, $P<0.01$] (Fig. 3B).

3.2. Effects of isorhynchophylline on mCPP-induced hypolocomotion in mice

The ability of mCPP to produce hypolocomotion via activation of the 5-HT_{2C} receptor is well documented (Gleason et al., 2001; Gleason and Shannon, 1998; Lucki et al., 1989). Thus, we tested if

isorhynchophylline has the ability to modulate 5-HT_{2C} receptor function in the brain using mCPP-induced hypolocomotion as a behavioural index in mice. When administered at 0.3–10 mg/kg, i.p., mCPP produced a dose-dependent decrease of ambulatory and rearing activities during a 30-min observation period (data not shown). As shown in Fig. 4, pretreatment with mianserin, a mixed 5-$HT_{2A/2C}$ antagonist with a higher affinity for 5-HT_{2C} (Hartman and Northup, 1996; Labrecque et al., 1995), significantly reversed the mCPP (3 mg/kg, i.p.)-induced decrease of motor activity [A1: $F_{ambulation}(2,25)=7.073$, $P<0.01$ and $F_{rearing}(2,25)=7.140$, $P<0.01$], whereas ketanserin, a mixed 5-$HT_{2A/2C}$ antagonist, did not have a reversing effect at the dose which antagonized the 5-MeO-DMT-induced head-twitch response [A2: $F_{ambulation}(2,25)=7.199$, $P<0.01$ and $F_{rearing}(2,25)=15.618$, $P<0.01$]. Isorhynchophylline, rhynchophylline, corynoxeine, or isocorynoxeine (30 mg/kg, i.p.) did not influenced spontaneous motor activity in normal mice [B1: $F_{ambulation}(2,25)=0.135$, $P=0.875$ and $F_{rearing}(2,25)=3.159$, $P=0.06$; B2: $F_{ambulation}(2,27)=0.677$, $P=0.516$ and $F_{rearing}(2,27)=1.960$, $P=0.160$]. When examined the effect of these alkaloids on the mCPP-induced hypolocomotion, there were significant differences in the mean values among the treatment groups [C1: $F_{ambulation}(3,33)=4.773$, $P<0.01$ and $F_{rearing}(3,33)=8.155$, $P<0.001$; C2: $F_{ambulation}(3,40)=4.239$, $P<0.01$ and $F_{rearing}(3,40)=14.040$, $P<0.001$] (Fig. 4C1 and

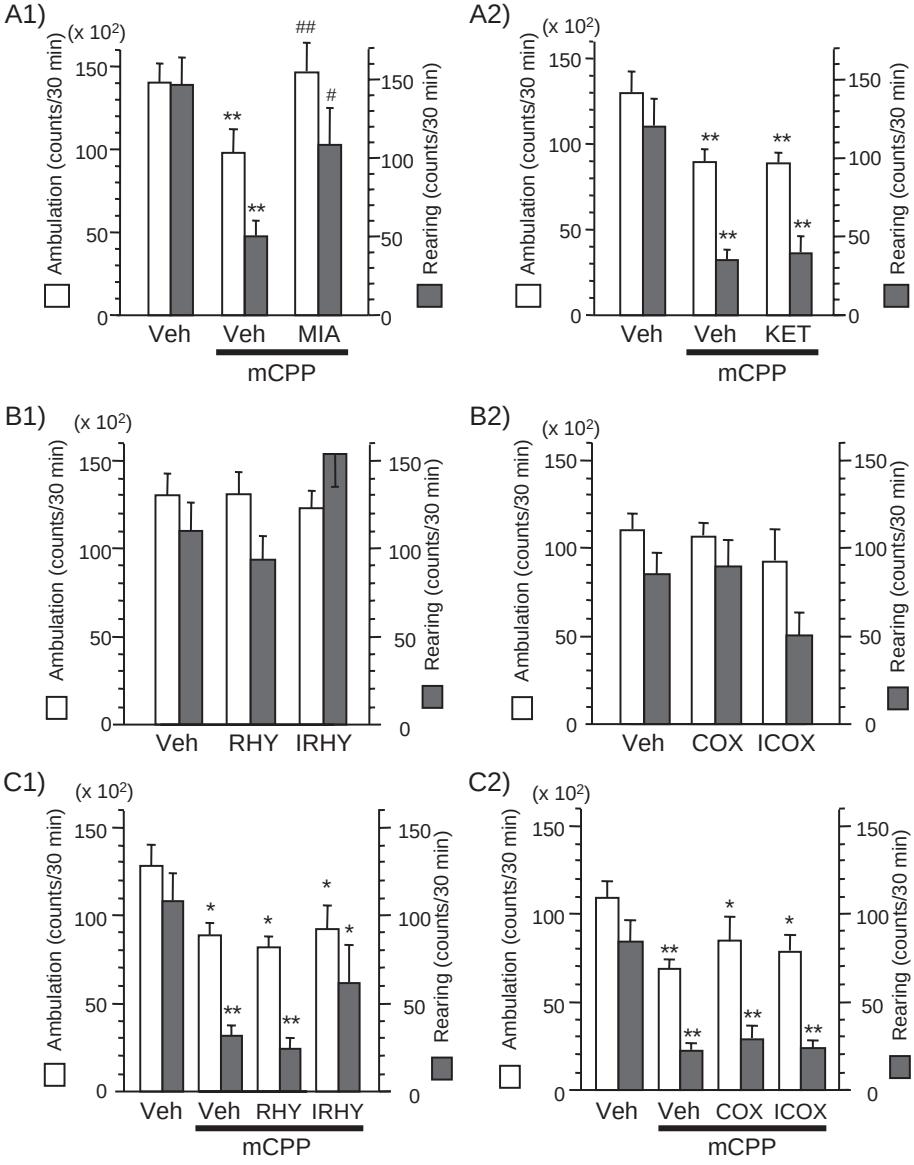


Fig. 4. Effects of oxindole alkaloids on 5-HT_{2C}-receptor mediated hypolocomotion in mice. A1 and A2: effects of mianserin (MIA, 3 mg/kg, i.p.), a mixed 5-$HT_{2A/2C}$ antagonist with a higher affinity for 5-HT_{2C}, and ketanserin (KET, 0.5 mg/kg, i.p.), a mixed 5-$HT_{2A/2C}$ antagonist, on meta-chlorophenylpiperazine (mCPP)-induced hypolocomotion. B1 and B2: effects of isorhynchophylline (IRHY), rhynchophylline (RHY), isocorynoxeine (ICOX), and corynoxeine (COX), on motor activity in normal mice. C1 and C2: effects of IRHY, RHY, ICOX, and COX (30 mg/kg, i.p.) on mCPP-induced hypolocomotion. Each test drug was injected 30 min before the start of the experiments. Immediately after the i.p. injection of vehicle (Veh) or the 5-HT_{2C} receptor agonist mCPP (3 mg/kg), the changes in motor activity (ambulation and rearing) were measured during a 30-min period. Each datum represents the mean±S.E.M. ($n=9–10$). *$P<0.05$, **$P<0.01$ vs. vehicle alone. #$P<0.05$, ##$P<0.01$ vs. mCPP-treated vehicle control.

C2). Post hoc analyses, however, revealed no significant difference between mCPP treatment and mCPP plus alkaloid treatment.

3.3. Interaction of isorhynchophylline with 5-HT2A and 5-HT2C receptors expressed on Xenopus oocyte membrane

We tested if isorhynchophylline and related oxindole alkaloids are able to directly interact with 5-HT_{2A} and 5-HT_{2C} receptors using a receptor expression model in *Xenopus* oocytes. Stimulation of 5-HT_{2A} and 5-HT_{2C} receptor subtypes with bath-applied 5-HT induced Ca^{2+}-activated Cl^- currents in a concentration-dependent manner with ED_{50} values of 48.3 nM and 6.1 nM, respectively, and a Hill coefficient of 1.69±0.52 and 0.85±0.54, respectively. Therefore, the effects of oxindole alkaloids on 5-HT_{2A} and 5-HT_{2C} receptor-mediated current responses were subsequently examined using 50 and 5 nM 5-HT, respectively.

As shown in Fig. 5A, when applied alone, neither oxindole alkaloid elicited any measurable membrane current in *Xenopus* oocytes expressing 5-HT_{2A} receptors. However, when applied to such oocytes with 50 nM 5-HT, isorhynchophylline inhibited 5-HT-evoked currents in a dose-dependent manner with an IC_{50} of 52.5 μM (Fig. 5A and B). Isocorynoxeine, as well as isorhynchophylline, exhibited a dose-dependent inhibition of 5-HT_{2A} receptor-mediated current response with an IC_{50} of 72.4 μM, whereas rhynchophylline and corynoxeine showed a less potent suppression of 5-HT-evoked currents (IC_{50} > 100 μM) than their respective stereoisomers, isorhynchophylline and isocorynoxeine. To clarify the properties of the isorhynchophylline-and isocorynoxeine-induced inhibition of 5-HT_{2A} receptor function, we analysed the concentration–response curve of 5-HT-evoked currents in the presence and absence of 50 μM isorhynchophylline or isocorynoxeine in oocytes expressing 5-HT_{2A} receptors. As depicted in Fig. 6, neither alkaloid had an effect on the maximal current responses of 5-HT_{2A} receptors. However, the ED_{50} of 5-HT was 48.3 [31.1 to 75.1] nM (mean [95% confidence interval (CI)]) with a Hill coefficient of 1.69±0.52 (mean±S.E.M.) in the control medium, while ED_{50} values in the presence of isorhynchophylline and isocorynoxeine were 120.8 [66.8 to 218.4] nM (mean [95% CI]) and 91.2 [63.4 to 131.4] nM (mean [95% CI]) with Hill coefficients of 1.38±0.60 and 1.57±0.43 (mean±S.E.M.), respectively.

As shown in Fig. 7, these alkaloids produced a slight suppression of the 5-HT (5 nM)-induced currents in oocytes expressing 5-HT_{2C} receptors with respective IC_{50} values of > 100 μM.

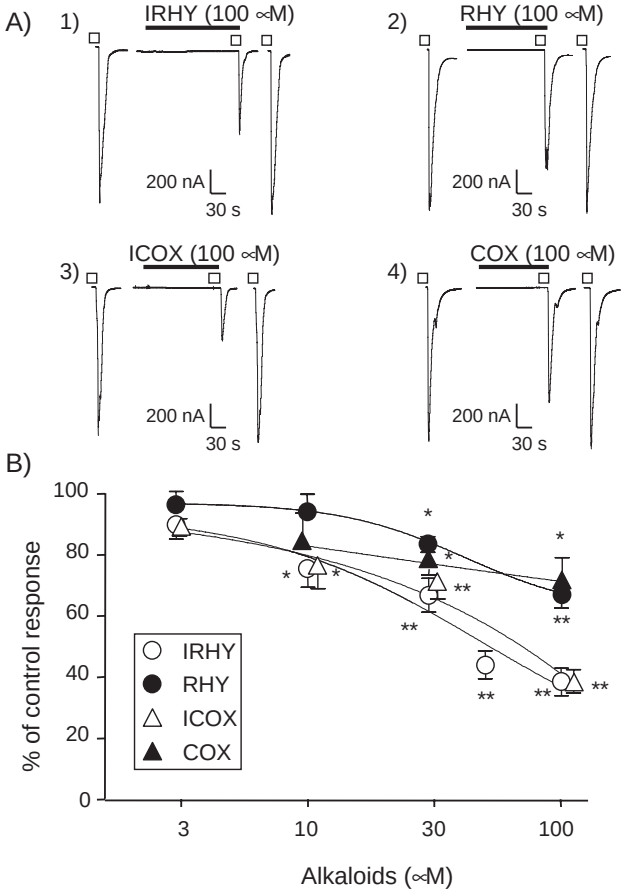


Fig. 5. The effects of tetracyclic oxindole alkaloids on 5-HT_{2A} receptor-mediated current responses in oocytes. A) Typical current responses elicited by 5-HT in the presence and absence of test drugs. 5-HT (□: 50 nM) was applied for 15 s. The left-and right-most traces are the current responses before application and after washing out of the alkaloids, respectively. Horizontal bars above the middle of the traces represent a bath application of 100 μM isorhynchophylline (IRHY), rhynchophylline (RHY), isocorynoxeine (ICOX) or corynoxeine (COX). B) Summary of the effects of IRHY (○), RHY (●), ICOX (△) and COX (▲) on 5-HT-induced currents. Each data point represents the mean±S.E.M. from 4-8 different oocytes. *P < 0.05, **P < 0.01 versus control response obtained with 50 nM 5-HT alone.

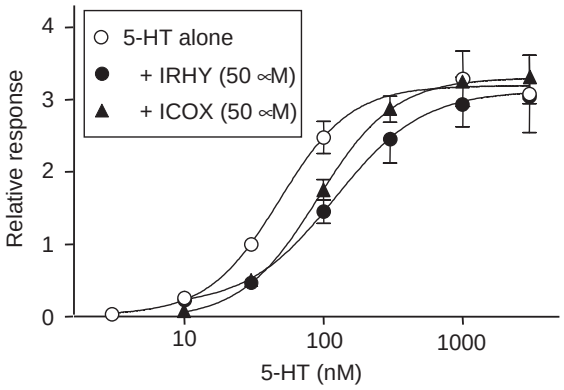


Fig. 6. Competitive inhibition of 5-HT-induced Ca^{2+}-activated Cl^- currents by isorhynchophylline and isocorynoxeine in *Xenopus* oocytes expressing 5-HT_{2A} receptor cRNA. Concentration–response curve for 5-HT in the presence and absence of the alkaloids. Various concentrations of 5-HT (3–3000 nM) were applied to *Xenopus* oocytes for 15 s. Isorhynchophylline (IRHY, 50 μM) and isocorynoxeine (ICOX, 50 μM) were applied to the oocytes for 2 min before and for 15 s simultaneously with 50 nM 5-HT. The EC_{50} and Hill coefficient values for 5-HT were 48.3 [31.1 to 75.1] nM (mean [95% CI]) and 1.69±0.52 (mean±S.E.M.) in the control medium, 120.8 [66.8 to 218.4] nM and 1.38±0.60 in the presence of 50 μM IRHY and 91.2 [63.4 to 131.4] nM and 1.57±0.43 in the presence of 50 μM ICOX, respectively. Each data point represents the mean±S.E.M. from 4 to 7 different oocytes.

4. Discussion

The present study demonstrated that isorhynchophylline, a major alkaloid of *Uncaria* plants, exhibits a suppressive effect on 5-HT_{2A} receptor-mediated head-twitch behaviour

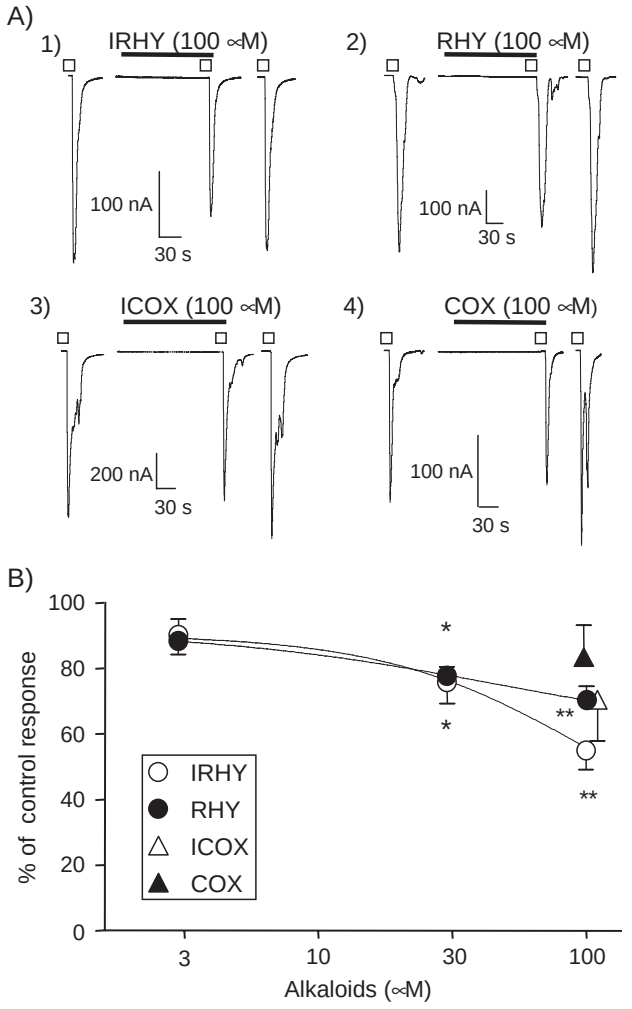


Fig. 7. Effects of tetracyclic oxindole alkaloids on 5-HT_{2C} receptor-mediated current responses in *Xenopus* oocytes. A) Typical current responses elicited by 5-HT in the presence and absence of test drugs. 5-HT (□: 5 nM) was applied for 15 s. The left-and right-most traces are the current responses before application and after washing out of the alkaloids, respectively. Horizontal bars above the middle of the traces represent a bath application of 100 μM isorhynchophylline (IRHY), rhynchophylline (RHY), isocorynoxeine (ICOX) or corynoxeine (COX). B) Summary of the effects of IRHY (○), RHY (●), ICOX (△) and COX (▲) on 5-HT-induced currents. Each data point represents the mean ± S.E.M. from 4 to 10 different oocytes. $^{**}P<0.01$ versus control response obtained with 5 nM 5-HT alone.

in mice and suggested that a competitive antagonism at 5-HT_{2A} receptor sites is involved in the effect of this alkaloid.

The 5-HT receptor agonist-induced head-twitch and head-weaving responses are primarily mediated by stimulation of the 5-HT_{2A} and 5-HT_{1A} receptor subtype, respectively and these behavioural changes provide experimental models with which to study receptor function in vivo and to elucidate if drugs are capable of interacting with each subtype. In this study, we used the 5-HT receptor agonist 5-MeO-DMT because it allowed us to elucidate the effect of drugs on both behavioural indices in the same animals (Matsumoto et al., 1997a,b). The results of preliminary experiments using reference drugs revealed that 5-MeO-DMT-induced head-weaving and head-twitch responses were selectively antagonised by the 5-HT_{1A} antagonist p-MPPI and the mixed 5-$HT_{2A/2C}$ antagonist ketanserin, respectively, supporting the aforementioned notion. In this study, isorhynchophylline, as well as ketanserin, dose-dependently attenuated the head-twitch responses to 5-MeO-DMT without affecting the 5-HT_{1A} receptor-mediated head-weaving behaviour. Thus, it is likely that isorhynchophylline can selectively modulate the 5-HT_{2A} receptor-mediated behavioural response.

The 5-HT_{2A} receptor-mediated head-twitch response can be modulated by several neurotransmitter systems. Previous studies from this and other laboratories have demonstrated that depletion of endogenous noradrenaline by the monoamine-depleting agent reserpine enhances the 5-HT_{2A} receptor-mediated head-twitch behaviour and that the α_2-adrenoceptor agonist clonidine inhibits it, while the α_2-adrenoceptor antagonist idazoxan, enhances the behaviour, indicating that the stimulation of postsynaptic α_2-adrenoceptors by endogenous noradrenaline negatively regulates the appearance of the 5-HT_{2A} receptor-mediated head-twitching (Darmani et al., 1991; Heal et al., 1986; Matsumoto et al., 1997b). It is therefore likely that endogenous noradrenaline is involved in the inhibition by isorhynchophylline of the head-twitch response in mice. In order to test this possibility, we examined the effect of isorhynchophylline on head-twitch behaviour in mice pretreated with reserpine. Consistent with previous reports (Matsumoto et al., 1997a,b), reserpine treatment markedly enhanced head-twitch responses to 5-MeO-DMT but had no effect on the suppression by isorhynchophylline. Thus, it seems unlikely that the suppression of head-twitch responses by isorhynchophylline is mediated by the stimulation of α_2-adrenoceptors in the brain by endogenous noradrenaline. However, we cannot exclude the possibility that isorhynchophylline and/or its metabolites stimulate postsynaptic α_2-adrenoceptors and thereby inhibit 5-MeO-DMT-induced head-twitch behaviour.

We previously demonstrated using a receptor expression model in *Xenopus* oocytes that both isorhynchophylline and rhynchophylline had a non-competitive antagonistic effect on *N*-Methyl-D-aspartate (NMDA)-type glutamate receptor-mediated cation current responses without affecting the current responses mediated by ionotropic kainic acid-type and (±)-α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA)-type glutamate receptors or by the metabotropic glutamate $receptor_{1\ and\ 5}$ (Kang et al., 2002c). On the other hand, there are a number of in vivo and in vitro studies indicating interactions between 5-HT_{2A} and NMDA receptor function (Higgins et al., 2003; Pei et al., 2004; Regina et al., 2004). Thus, the antagonistic property of isorhynchophylline toward the NMDA receptor may indirectly influence 5MeO-DMT-induced head-twitch behaviour. Moreover, considering the report by Kim et al. (2000) that blockade of the NMDA type glutamate receptor enhances

head-twitch behaviour caused by stimulation of the 5-HT_{2A} receptor, it is likely that the suppressive effect of isorhynchophylline on the head-twitch responses has been underestimated by the isorhynchophylline-induced blockade of NMDA receptor function.

Interestingly, elucidation of the pharmacological properties of other oxindole alkaloids, rhynchophylline, isocorynoxeine, and corynoxeine, isolated from *Uncaria* plants revealed that only isocorynoxeine exhibited a dose-dependent suppression of 5-MeO-DMT-induced head-twitch behaviour in reserpinized mice. Isorhynchophylline and isocorynoxeine are epimers of rhynchophylline and corynoxeine at the C-7 position of the oxindole alkaloid structure (see Fig. 1). Moreover, the configuration of the oxindole moiety at C-7 is the same in isocorynoxeine and isorhynchophylline. Considering the stereo-structural properties of the oxindole alkaloids tested in this study, the configuration of the oxindole moiety at position C-7 is likely to play an important role in the suppressive effect of isorhynchophylline.

Recently, Gleason et al. (Gleason et al., 2001; Gleason and Shannon, 1998) have demonstrated that stimulation of the 5-HT_{2C} receptor in the brain induces hypolocomotion in rodents. We used this behavioural model to test if isorhynchophylline can interact with 5-HT_{2C} receptors as well as with 5-HT_{2A} receptors. Consistent with the findings of Gleason et al., the systemic administration of mCPP reduced spontaneous motor activity in mice and the effect was reversed by mianserin but not by ketanserin, a mixed 5-$HT_{2A/2C}$ antagonist. Interestingly, isorhynchophylline and the other oxindole alkaloids at a dose of 30 mg/kg failed to affect spontaneous motor activity or the hypolocomotion induced by the 5-HT_{2C} receptor agonist mCPP. These results suggest that isorhynchophylline and isocorynoxeine prefer to interact with 5-HT_{2A} receptors rather than with 5-HT_{2C} receptors in the brain.

Based on the data from the behavioural experiments, we performed electrophysiological experiments using *Xenopus* oocytes expressing 5-HT_{2A} or 5-HT_{2C} receptors to test the possibility that isorhynchophylline directly and selectively modifies the function of 5-HT_{2A} receptors. The dose response data summarized in Fig. 5 clearly reveal that isorhynchophylline and isocorynoxeine inhibit 5-HT_{2A} receptor-mediated 5-HT currents more potently than do rhynchophylline and corynoxeine, while no marked difference in inhibitory potency was observed between isorhynchophylline and isocorynoxeine. These results indicate that the configuration of the oxindole moiety of isorhynchophylline and isocorynoxeine plays a critical role in their inhibitory effects on the 5-HT_{2A} receptor-mediated current response. This stereospecific property of isorhynchophylline and isocorynoxeine found in vitro is consistent with that found in the present behavioural experiments. Moreover, our results demonstrated a rightward shift of the 5-HT dose response relationship caused by isorhynchophylline and isocorynoxeine in the oocytes with 5-HT_{2A} receptors, indicating that these alkaloids exhibit competitive antagonistic activity toward the 5-HT_{2A} receptor. Considered together with the in vivo effect of these alkaloids, the present findings suggest that competitive inhibition of the 5-HT_{2A} receptor by isorhynchophylline and isocorynoxeine is closely associated with the suppression of 5-HT_{2A} receptor-mediated head-twitch responses by these alkaloids.

Interestingly, isorhynchophylline as well as isocorynoxeine exhibited less potent inhibitory activity (with IC_{50} values of >100 μM) against the 5-HT_{2C} receptor-mediated response than the 5-HT_{2A} receptor-mediated response in oocytes. These findings are consistent with the results of the behavioural study, and support the idea that isorhynchophylline and isocorynoxeine are capable of interacting with the 5-HT_{2A} receptor subtype in the brain.

In conclusion, the present in vivo study demonstrated a suppressive effect of isorhynchophylline and isocorynoxeine, oxindole alkaloids present in *Uncaria* species used for medicinal purposes, on 5-HT_{2A} receptor-mediated head-twitch responses in mice. The in vitro study suggests that the effects of these alkaloids are at least partly due to a competitive antagonism at the 5-HT_{2A} receptor. These pharmacological properties of isorhynchophylline and isocorynoxeine may partly account for the beneficial effect of Choto-san, a formula containing *Uncaria* species as a main herbal component, on neuropsychiatric symptoms.

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