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Pteropodine and isopteropodine positively modulate the function of rat muscarinic M_1 and 5-HT_2 receptors expressed in *Xenopus* oocyte

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Abstract

Pteropodine and isopteropodine are heteroyohimbine-type oxindole alkaloid components of *Uncaria tomentosa* (Willd.) DC, a Peruvian medicinal plant known as cat's claw. In this study, the effects of these alkaloids on the function of Ca^{2+}-activated Cl^- currents evoked by stimulation of G protein-coupled muscarinic M_1 acetylcholine and 5-HT_2 receptors were studied in *Xenopus* oocytes in which rat cortex total RNA was translated. Pteropodine and isopteropodine (1–30 μM) failed to induce membrane current by themselves. However, these alkaloids markedly enhanced the current responses evoked by both acetylcholine and 5-hydroxyhyptamine (5-HT) in a concentration-dependent and reversible manner with the maximal effects at 30 μM. Pteropodine and isopteropodine produced 2.7- and 3.3-fold increases in the acetylcholine response with EC_{50} values of 9.52 and 9.92 μM, respectively, and 2.4- and 2.5-fold increases in the 5-HT response with EC_{50} values of 13.5 and 14.5 μM, respectively. In contrast, in oocytes injected with total RNA from the rat cerebellum or spinal cord, neither alkaloid had an effect on the metabotropic current responses mediated by glutamate $receptor_{1\ and\ 5}$ ($mGlu_{1/5}$) receptors or ionotropic responses mediated by *N*-methyl-D-aspartate, kainic acid or glycine. Pteropodine and isopteropodine (10 μM) significantly reduced the EC_{50} values of acetylcholine and 5-HT that elicited current responses, but had no effect on the maximal current responses elicited by acetylcholine and 5-HT. On the other hand, mitraphylline, a stereoisomer of pteropodine, failed to modulate acetylcholine- and 5-HT-induced responses. These results suggest that pteropodine and isopteropodine act as positive modulators of muscarinic M_1 and 5-HT_2 receptors.

Keywords: Pteropodine; Isopteropodine; Heteroyohimbine-type oxindole alkaloid; Muscarinic M_1 receptor; 5-HT_2 receptor; *Xenopus* oocyte

1. Introduction

Uncaria tomentosa (Willd.) DC, a medicinal plant known as uña de gato or cat's claw, has been used as a folk medicine by native people of the Peruvian rain forest to treat some conditions such as arthritis, digestive complaints and inflammatory disorders (Laus et al., 1997; Reinhard, 1999). Many alkaloids have been isolated and identified from this plant, and have been alleged to be responsible for the medicinal actions of this plant (Laus et al., 1997; Senatore et al., 1989; Wagner et al., 1985).

In a previous study which aimed to clarify the neuropharmacological profiles of *U. tomentosa* extract (Abdel-Fattah Mohamed et al., 2000), it was found that the total alkaloid prepared from this plant produced an ameliorative effect on the memory disruption induced by dysfunction of central cholinergic systems in the step-down test in mice. Moreover, the effect of total alkaloid was shown to be partly due to major heteroyohimbine-type alkaloids such as pteropodine, isopteropodine and mitraphylline (Abdel-Fattah Mohamed et al., 2000). However, the molecular mechanism underling the action of these alkaloids remained to be clarified.

A number of lines of evidence have demonstrated that the muscarinic acetylcholine receptor, particularly the muscarinic M_1 subtype (which is coupled with a G-protein-phospholipase C mechanism), is abundant in forebrain areas such as the cerebral cortex and hippocampus (Wei et al., 1994) and plays a key role in higher cognitive processes such as memory and learning (Bartus et al., 1982; Coyle et al., 1983; Fibiger, 1991; Hagan et al., 1987; Roldan et al., 1997). Moreover, it has been suggested that serotonergic systems in the central nervous system also affect cognitive

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behavior directly or through interactions with central cholinergic systems (see Buhot, 1997; Cassel and Jeltsch, 1995 for review). In fact, systemic administration of agonists for G-protein and phospholipase C-linked 5-$HT_{2A/2C}$ receptors enhanced learning in conditioned avoidance response tests in rats (Harvey, 1996), while 5-HT_{2C} receptor mutant mice exhibited cognitive impairments (Tecott et al., 1998).

In this study, to clarify the possible mechanisms of actions of the *U. tomentosa* alkaloids pteropodine, isopteropodine and mitraphylline, we investigated the effects of these alkaloids on the function of muscarinic M_1 and 5-HT_2 receptors using *Xenopus* oocytes expressing receptors encoded by rat cortex total RNA. The present report presents evidence that pteropodine and isopteropodine fail to activate muscarinic M_1 and 5-HT_2 receptors but exert potent modulatory effects on these receptors.

2. Materials and methods

2.1. Isolation of total RNA

Male Wistar rats (12–14 weeks old, Japan SLC, Shizuoka, Japan) were used for the experiments. Immediately after the rats were decapitated, the cerebral cortex, cerebellum and spinal cord were dissected and frozen in liquid nitrogen. The frozen tissues were homogenized in Sepasol-RNA I Super® (Nacalai Tesque, Kyoto, Japan) using a glass homogenizer with a Teflon pestle. The total RNA was extracted according to the protocol provided by the manufacturer, and was stored at −80 °C until use.

2.2. Oocytes preparation and injection

Xenopus oocytes were prepared by a slight modification of the method described in previous reports (Leewanich et al., 1998a,b). Briefly, oocytes were isolated from *Xenopus laevis* (Hamamatsu Seibutsu, Shizuoka, Japan) that had been anesthetized in ice water and were defolliculated with 1.0 mg/ml collagenase type I (Wako Pure Chemical, Osaka, Japan) in Ca^{2+}-free Modified Barth's Solution (MBS). Oocytes at stages V or VI were injected with 47 nl of 5 mg/ml total RNA prepared from the cerebral cortex, cerebellum or spinal cord, and were incubated in MBS containing 88 mM NaCl, 1 mM KCl, 0.41 mM $CaCl_2$, 0.33 mM $Ca(NO_3)_2$, 0.82 mM $MgSO_4$, 2.4 mM $NaHCO_3$, 2.5 mM sodium pyruvate, 2.5 unit/ml penicillin, 2.5 μg/ml streptomycin and 5 mM HEPES at 18 °C for 2 days before electrophysiological recordings. The MBS was replaced daily. In the experiments, oocytes expressing total RNA from the cerebral cortex were used to examine acetylcholine-, serotonin (5-HT)-, *N*-methyl-D-aspartate (NMDA)- and kainic acid-induced current responses. To induce expression of metabotropic glutamate$_{1 \text{ and } 5}$ ($mGlu_{1/5}$) receptors and ionotropic glycine receptors, total RNA from the cerebellum or spinal cord, respectively, was injected into oocytes.

2.3. Electrophysiological recordings

An oocyte was placed in a 50-μl volume chamber and continuously perfused with MBS at 1.5 ml/min at room temperature (22–25 °C) except in some specific cases noted below. The membrane currents were recorded from oocytes at a holding potential of −60 mV using a two-electrode voltage clamp method (Gene Clamp 500, Axon Instruments, Foster City, CA). Electrodes were filled with 3 M KCl and had a resistance of 1–3 MΩ. Only oocytes with resting membrane potential more negative than −30 mV were used for the experiments. The current responses mediated by G protein-linked receptors in *Xenopus* oocytes were induced by applying MBS containing acetylcholine, 5-HT or quisqualic acid for 30 s using the ValveLink System (AutoMate Scientific, Oakland, CA), while the current responses mediated by ionotropic receptors were elicited by applying the same medium containing kainic acid or glycine. To examine NMDA receptor-mediated currents, oocytes were perfused with Mg^{2+}-free MBS and current responses were evoked by applying NMDA in the absence or presence of test drugs. For these tests, NMDA and test drugs were dissolved in 3 μM glycine-containing Mg^{2+}-free MBS.

Test drugs (*U. tomentosa* alkaloids, pirenzepine or ketanserin) were applied to the oocytes for 3 min before and for 30 s simultaneously with each receptor agonist described above. Considering the expected desensitization of current responses evoked by repeated application of each receptor agonist, the currents measured before and after test drug treatment were averaged as control responses. Reversal potentials of acetylcholine- and 5-HT-induced current were measured by inducing the responses at different holding potentials. Acetylcholine (1 μM)- and 5-HT (100 nM)-induced currents at an external Cl^- concentration of 89.8 mM were reversed at around −20 to −30 mV, indicating that the current was carried mainly by Cl^- (Sugiyama et al., 1985).

In agreement with other reports (Lin et al., 1993; Sanna et al., 1994), the first currents evoked by metabotropic receptor stimulation were substantially larger than the subsequent current responses even after a long washout period of 1–2 h, but these subsequent responses were relatively stable and reproducible in replicate experiments. Moreover, a gradual decrease of baseline responses was sometimes observed during the course of the recordings. Therefore, in this study, to avoid rundown and to obtain reproducible current responses, the first acetylcholine-, 5-HT- and quisqualic acid-induced responses were excluded from the data analysis, and at least 20–30 min was allowed for washout between successive drug applications.

2.4. Drugs

Pteropodine, isopteropodine and mitraphylline, heteroyohimbine type oxindole alkaloids (Fig. 1), were isolated from *U. tomentosa* (Willd.) DC as previously reported (Abdel-Fattah Mohamed et al., 2000). These alkaloids were dissolved

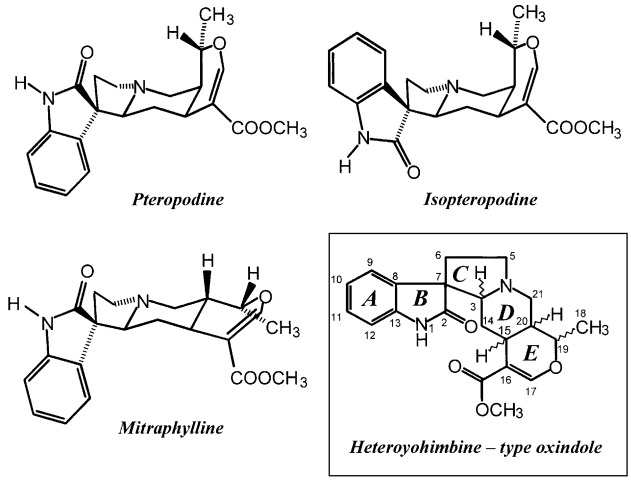

Fig. 1. Stereo-chemical structures of pteropodine, isopteropodine and mitraphylline, *U. tomentosa* alkaloids used in this study. These alkaloids are all heteroyohimbine-type pentacyclic oxindoles containing (containing A, B, C, D, and E rings), the basic plane skeleton of which is depicted as a square. The Arabic numericals indicate the respective carbon position according to general numbering system.

in 100% dimethylsulfoxide (DMSO), and then diluted with MBS to a final concentration of $\leq 0.1\%$ for an electrophysiological study. This concentration of DMSO was tested in oocytes and found not to induce any observable current. The following drugs were used: acetylcholine chloride (Wako Pure Chemical), serotonin tartrate, pirenzepine, ketanserin, quisqualic acid, DMSO and *N*-methyl-D-aspartic acid (Sigma, St. Louis, MO) and glycine and kainic acid (Nacalai Tesque).

2.5. Data analysis

The control responses were measured before and after each drug application to take into account possible shifts in the control currents as the recording proceeded. The results are presented as percentages of control responses in order to compensate for variability of the level of receptor in different oocytes. The *n* values are the number of different oocytes studied. All values are expressed as the mean ± S.E.M. Each experiment was carried out with oocytes from at least two different frogs. Statistical analyses were performed with the *t*-test or paired *t*-test. Curve fitting and estimation of EC_{50} values from concentration–response curves were performed using PRISM® (GraphPAD Software, San Diego, CA).

3. Results

3.1. Effects of pteropodine, isopteropodine and mitraphylline on Ca^{2+}-activated Cl^- currents evoked by acetylcholine and 5-HT in Xenopus oocytes injected with rat cortex total RNA

First, the physiological and pharmacological properties of the expressed receptors were characterized. In agreement with previous studies (Lubbert et al., 1987; Sanna et al., 1994; Sugiyama et al., 1985), bath application of acetylcholine or 5-HT to oocytes injected with rat cortex RNA elicited an inward current at a clamp potential of − 60 mV (Fig. 2A). The current amplitude was dependent on the acetylcholine and 5-HT concentrations; acetylcholine and 5-HT induced maximal currents at 10 and 1 μM, respectively (Fig. 2A). Consistent with previous reports (Lin et al., 1993; Sanna et al., 1994), the selective M_1 receptor antagonist pirenzepine significantly inhibited 1 μM acetylcholine-induced current by 80.1 ± 6% ($n = 5$ oocytes) at a concentrations close to the

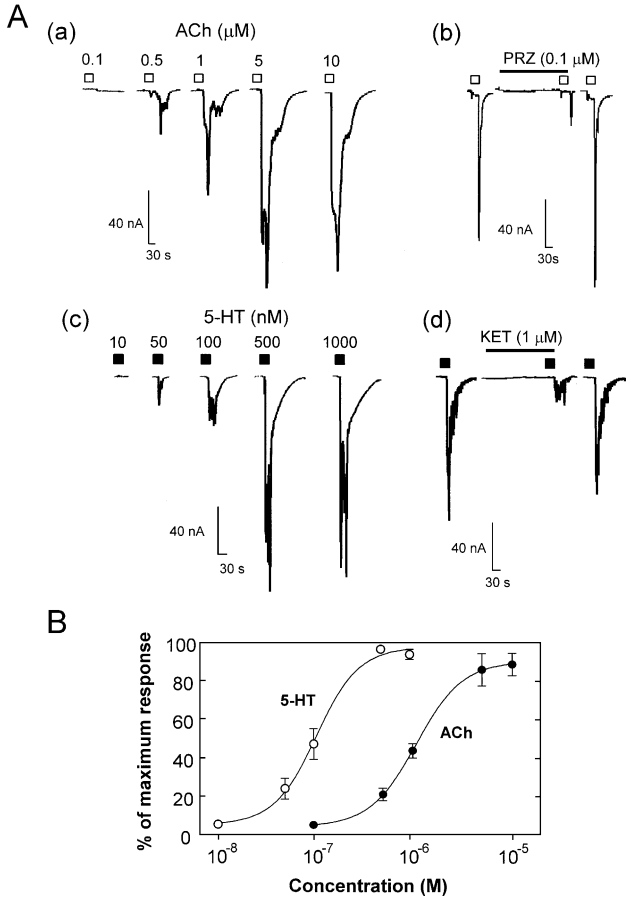

Fig. 2. Pharmacological properties of acetylcholine and 5-HT receptors in *Xenopus* oocytes injected with rat cerebral cortex total RNA. (A–a) and (A–c): Typical concentration-dependent inward currents caused by bath application of 0.1–10 μM acetylcholine (□) and 5-HT (■) for 30 s are seen as downward deflections of the trace. (A–b) and (A–d): Effects of the selective muscarinic M_1 receptor antagonist pirenzepine and the selective 5-HT_2 receptor antagonist ketanserin on acetylcholine- and 5-HT-induced currents, respectively. Acetylcholine (□, 1 μM) or 5-HT (■, 100 nM) was applied to oocytes in the absence or presence of 0.1 μM pirenzepine (PRZ) or 1 μM ketanserin (KET), respectively, for 30 s. Horizontal bars above the middle of the traces indicate bath application of pirenzepine (b) or ketanserin (d). The left- and right-most traces are the current responses before the application of antagonists and after washing out the antagonists, respectively. (B) Concentration–response curves for acetylcholine (●) and 5-HT (○). Each point represents the mean ± S.E.M. from 7 to 10 different oocytes. The EC_{50} values for acetylcholine and 5-HT were 1.28 and 0.1 μM, respectively.

IC_{50} (89 nM) for the M_1 receptor subtype (Buckley et al., 1989). It was considered that acetylcholine receptors in *Xenopus* oocytes expressing rat cortex RNA were primarily of the M_1 subtype because the other muscarinic receptor subtypes have much lower affinity (561–1540 nM) for pirenzepine (Buckley et al., 1989). Moreover, the 5-HT-induced currents were markedly suppressed by a selective 5-HT_2 antagonist, ketanserin (Leysen et al., 1982), indicating that the actions of 5-HT are mediated by interactions with $5\text{-HT}_{2(2A/2C)}$ receptors in oocytes in which rat cortex total RNA was translated (Lin et al., 1993; Lubbert et al., 1987; Snutch, 1988). Analysis of the concentration–response curves for acetylcholine and 5-HT indicated that the EC_{50} values for acetylcholine and 5-HT were 1.28 μM and 0.1 μM, respectively (Fig. 2B). Therefore, the effects of the *U. tomentosa* alkaloids on acetylcholine- and 5-HT-induced currents were subsequently examined using 1 μM acetylcholine and 100 nM 5-HT, respectively.

As shown in Fig. 3A, when applied in the bath alone, pteropodine and isopteropodine (an epimer of pteropodine at the spiro C-7 position) failed to elicit any measurable membrane current. However, when applied with 1 μM acetylcholine, pteropodine and isopteropodine increased acetylcholine-evoked current responses by 53 ± 12.7% and 41 ± 16.2%, respectively, at 3 μM, and by 171 ± 64.2% and 226 ± 79.8%, respectively, at 30 μM, indicating a concentration-dependent potentiation of the acetylcholine receptor function. The increases of the acetylcholine responses in the presence of pteropodine and isopteropodine disappeared following washing out of these alkaloids. Analysis of the concentration–response curves for pteropodine and isopteropodine showed that EC_{50} values of these alkaloids were 9.52 and 9.92 μM, respectively (Fig. 3B). In contrast, mitraphylline (3–30 μM), a stereoisomer at the C20 position of pteropodine, did not elicit any membrane current or modulate the current responses elicited by 1 μM acetylcholine (Fig. 3).

Similar results were obtained regarding the effects of *U. tomentosa* alkaloids on currents evoked by 100 nM 5-HT (Fig. 3A). Pteropodine and isopteropodine enhanced 5-HT-evoked currents by 30 ± 10.2% ($p < 0.05$, paired *t*-test) and 35 ± 13.2% ($p < 0.5$, paired *t*-test), respectively, at 3 μM, and 138.5 ± 45.3% ($p < 0.05$) and 147.8 ± 43.3%, respectively, at 30 μM. The enhancing effects of pteropodine and isopteropodine were also reversible and completely abolished by washing out of these alkaloids. The EC_{50} values for enhancement of 100 nM 5-HT-induced currents by pteropodine and isopteropodine were 13.5 and 14.5 μM, respectively (Fig. 3B).

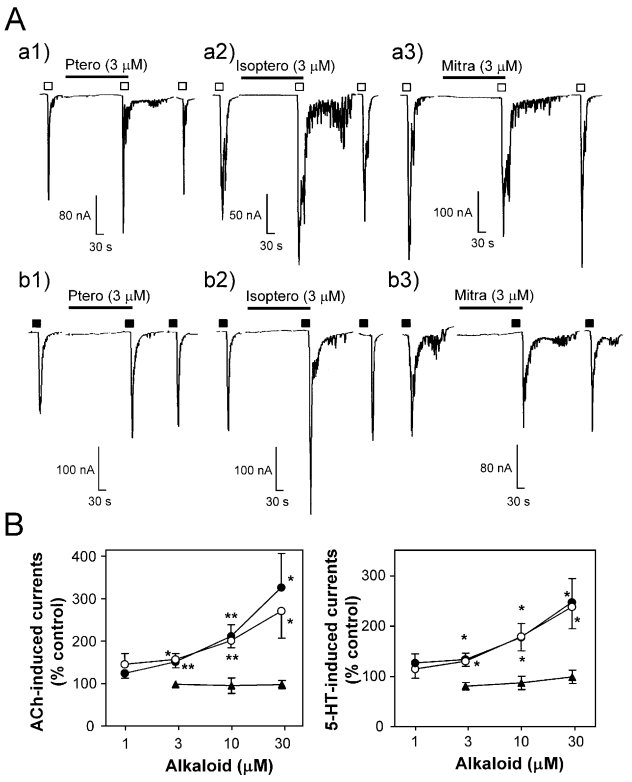

Fig. 3. Pteropodine and isopteropodine but not mitraphylline potentiate muscarinic M_1 and 5-HT_2 receptor-mediated current responses in oocytes in which rat cortex total RNA is translated. (A) Traces represent Ca^{2+}-activated Cl^- currents elicited by acetylcholine (a1–a3) or 5-HT (b1–b3). acetylcholine (□: 1 μM) and 5-HT (■: 100 nM) were applied for 30 s. Horizontal bars above the middle of the traces represent bath application of pteropodine (Ptero, a1 and b1), isopteropodine (Isoptero, a2 and b2), or mitraphylline (Mitra, a3 and b3). The left- and right-most traces are the current responses before alkaloid application and after washing out the alkaloids, respectively. (B) Summary of the effects of pteropodine (○), isopteropodine (●) and mitraphylline (▲) on acetylcholine- and 5-HT-induced currents. Each data point represents the mean ± S.E.M. from five to seven different oocytes. $^*p < 0.05$, $^{**}p < 0.01$ versus control response obtained with 1 μM acetylcholine or 100 nM 5-HT alone (paired *t*-test).

3.2. Effects of pteropodine and isopteropodine on metabotropic glutamate receptor-mediated Ca^{2+}-activated Cl^- currents and ionotropic receptor-mediated currents in Xenopus oocytes

In order to investigate the receptor specificity of pteropodine and isopteropodine, we examined the effects of these alkaloids on the current responses elicited by stimulation of metabotropic and ionotropic glutamate receptors or glycine receptors in *Xenopus* oocytes. Current responses were evoked by applying specific agonists for each receptor subtype at concentrations that produced about 50% of the maximal current responses. As summarized in Fig. 4, pteropodine and isopteropodine at 30 μM, a concentration that caused a significant increase in current responses elicited by acetylcholine and 5-HT, had no effect on Ca^{2+}-activated Cl^- currents caused by quisqualic acid, a selective metabotropic $mGlu_{1/5}$ receptor agonist (Abe et al., 1992; Aramori and Nakanishi, 1992; Masu et al., 1991). These alkaloids did not affect ionotropic currents evoked by 100 μM kainic acid or 200 μM glycine, and slightly suppressed the currents evoked by 100 μM NMDA ($IC_{50} > 30$ μM; Fig. 4).

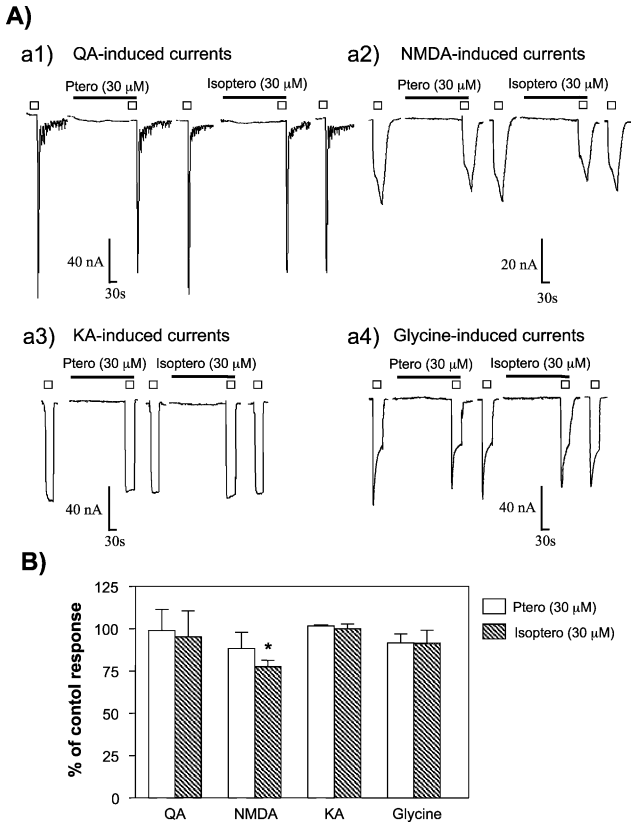

Fig. 4. Effects of pteropodine and isopteropodine on metabotropic and ionotropic glutamate receptor- and glycine receptor-mediated current responses in *Xenopus* oocytes. (A) Typical current responses caused by 1 μM quisqualic acid (a1), 100 μM NMDA (a2), 100 μM kainic acid (a3) and 200 μM glycine (a4). Open squares in traces represent the application of each receptor agonist for 30 s. Horizontal bars above the middle of the traces represent bath application of pteropodine (Ptero) or isopteropodine (Isoptero). The left- and right-most traces are the current responses before alkaloid application and after washing out the alkaloids, respectively. (B) Summary of the effects of pteropodine and isopteropodine on quisqualic acid-, kainic acid-, NMDA- and glycine-induced currents. Each data point represents the mean ± S.E.M. from five to eight different oocytes. * $P < 0.05$ compared to control response (paired *t* test).

3.3. Pteropodine and isopteropodine alteration of the concentration–response curves for acetylcholine and 5-HT-induced currents

To characterize further the effects of the alkaloids on the acetylcholine and 5-HT responses, we analyzed the concentration–response curves for acetylcholine and 5-HT in the absence and presence of constant concentrations of pteropodine and isopteropodine (Fig. 5). Application at concentrations ranging from 100 nM to 10 μM revealed that the EC_{50} value and Hill coefficient of acetylcholine was 1.21 [0.93–1.56] μM (mean [95% confidence interval (CI)]) and 1.524 ± 0.15, respectively. In the presence of 10 μM pteropodine or 10 μM isopteropodine, the concentration–response curve for acetylcholine was shifted to the left (Fig. 5A). The half-maximal concentration (EC_{50}) values of acetylcholine-induced current in the presence of pteropodine and isopteropodine were 0.62 [0.52–0.73] μM (mean [95% CI], $p < 0.01$, *t*-test) and 0.55 [0.48–0.63] μM (mean [95% CI], $p < 0.01$, *t*-test) with Hill coefficients of 2.85 ± 0.45 and 3.39 ± 0.56, respectively. In contrast, neither 10 μM pteropodine nor 10 μM isopteropodine had any effect on the E_{max} of the current response induced by acetylcholine in *Xenopus* oocytes ($P = 0.605$ and $P = 0.156$, respectively, *t*-test). Similar results were obtained with 5-HT (Fig. 5B). Perfusion with 5-HT concentrations between 10 nM and 1 μM induced increasing inward currents, with an EC_{50} value of 118 [87.3–158.1] nM (mean [95% CI]).

The presence of 10 μM pteropodine or isopteropodine caused a significant leftward shift of the 5-HT concentration–response curve. EC_{50} values for 5-HT in the presence of 10 μM pteropodine and 10 μM isopteropodine were 64.4 [36–115.1] (mean [95% CI], $p < 0.05$, *t*-test) and 61.2 [48.3–77.5] nM (mean [95% CI], $p < 0.01$, *t*-test), respectively. Hill coefficients for 5-HT in the presence of 10 μM pteropodine and 10 μM isopteropodine were 1.79 ± 0.36 and 1.36 ± 0.09, respectively. However, the E_{max} values for the 5-HT response were not significantly affected by 10 μM pteropodine or isopteropodine (pteropodine: $P = 0.36$ and isopteropodine: $P = 0.72$; *t*-test). The potentiation of the 5-

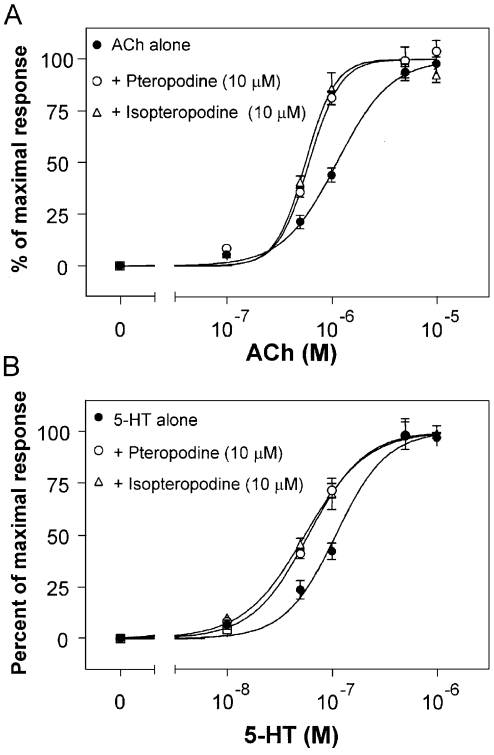

Fig. 5. Pteropodine- and isopteropodine-induced alteration of the concentration–response curve for acetylcholine- and 5-HT-induced Ca^{2+}-activated Cl^- currents. Increasing concentrations of acetylcholine (A: 0.1–10 μM) and 5-HT (B: 0.01–1 μM) were applied in the absence or presence of 10 μM pteropodine and isopteropodine. Each point represents the mean ± S.E.M. from 10 to 15 different oocytes.

HT response by pteropodine and isopteropodine at 10 μM was slightly less than that of the acetylcholine response.

4. Discussion

In this study, we investigated the effects of pteropodine, isopteropodine and mitraphylline, alkaloids isolated from *U. tomentosa*, on muscarinic M_1 receptor- and 5-HT_2 receptors expressed in *Xenopus* oocytes. Our results clearly demonstrated that pteropodine and isopteropodine fail to activate muscarinic M_1 and 5-HT_2 receptors but are able to exert potent positive modulatory effects on these receptors.

Considering the present finding that pteropodine and isopteropodine had either no effects or a slight suppressive effect on responses elicited by stimulation of other receptors such as the metabotropic $mGlu_{1/5}$, kainite, the glycine and the NMDA receptors, it is likely that the enhancing effects of these alkaloids are specific to muscarinic M_1 and 5-HT_2 receptors. Moreover, the present study revealed that the potentiation of acetylcholine- and 5-HT-induced currents by pteropodine and isopteropodine was due to an increase in the potencies (EC_{50}) but not the muscarinic M_1 receptor and the 5-HT_2 receptor efficacies. This finding indicates that pteropodine and isopteropodine increase the affinity of agonists for each receptor and may act as positive modulators of the muscarinic M_1 and 5-HT_2 receptor functions.

In a previous study (Abdel-Fattah Mohamed et al., 2000), pteropodine and isopteropodine produced an ameliorative effect on anti-cholinergic agent-induced memory disruption in the passive avoidance test. A body of evidence indicates the important role of muscarinic acetylcholine receptors, particularly the M_1 subtype, in higher cognitive processes (Hagan et al., 1987; Roldan et al., 1997). All together, these facts indicate that positive modulation of the muscarinic M_1 receptor by pteropodine and isopteropodine contributes to the ameliorative effects of these alkaloids on memory disruption caused by the dysfunction of central cholinergic systems. Moreover, it is also possible that the potentiation of 5-$HT_{2A/2C}$ responses is involved in the beneficial effects of pteropodine and isopteropodine on memory impairment in rodents, since previous reports demonstrated that systemic administration of agonists for 5-$HT_{2A/2C}$ receptors enhanced learning in conditioned avoidance response tests in rats (Harvey, 1996) and that the activation of 5-$HT_{2A/2C}$ receptors had a facilitatory influence on cholinergic release in the cortex in rodents (Hirano et al., 1995). In view of the potent modulation of muscarinic M_1 and 5-HT_2 receptors by pteropodine and isopteropodine, these alkaloids may serve as useful drugs for the treatment of cognitive disorders with decreased acetylcholine and 5-HT signaling, such as Alzheimer's disease (Engelborghs and De Deyn, 1997; Meltzer et al., 1998).

A previous preliminary study demonstrated that mitraphylline also ameliorated the memory disruption induced by scopolamine, a muscarinic receptor antagonist (Abdel-Fattah Mohamed et al., 2000). In the present study, however, mitraphylline had no effect on muscarinic M_1 receptor- or 5-HT_2 receptor-mediated current responses. Thus, the mechanism underlying the ameliorative effect of mitraphylline on memory disruption may be different from those of the effects of pteropodine and isopteropodine observed here.

The mechanism by which pteropodine and isopteropodine positively modulate muscarinic M_1 and 5-HT_2 receptor functions remains unclear. However, several known facts may help to explain the actions of these alkaloids in *Xenopus* oocytes expressing receptors encoded by rat cortex total RNA. It is conceivable that pteropodine and isopteropodine may inhibit intracellular kinases and thereby inhibit the desensitization process of muscarinic M_1 and 5-HT_2 receptor functions in *Xenopus* oocytes. Lines of evidence indicate that the muscarinic M_1 and 5-HT_2 receptors display desensitization (Lin et al., 1993; Sanna et al., 1994) via receptor phosphorylation by intracellular kinases such as G protein-coupled receptor kinases and protein kinase C (Hausdorff et al., 1990). However, the possibility that the effects of these alkaloids involve protein kinase C inhibition seems little, if any, since in this study acute treatment with pteropodine or isopteropodine had no effect on the current responses mediated by the $mGlu_{1/5}$ receptor which has the same intracellular signaling systems as the muscarinic M_1 and 5-HT_2 receptors in *Xenopus* oocytes. Another possibility is that pteropodine and isopteropodine may enhance receptor-G protein coupling or may act as positive allosteric modulators of the muscarinic M_1 and 5-HT_2 receptors. Nevertheless, further studies are necessary to elucidate the exact mechanism by which pteropodine and isopteropodine positively modulate muscarinic M_1 and 5-HT_2 receptor functions.

It is of interest to note that in contrast to pteropodine, mitraphylline failed to modulate acetylcholine- and 5-HT-induced current responses in *Xenopus* oocytes, while isopteropodine exhibited pharmacological properties quite similar to those of pteropodine. According to previous chemical studies (Seki et al., 1993; Shamma et al., 1967), both pteropodine and isopteropodine are classified as allo-type based on the configuration of the D and E rings of heteroyohimbine-type oxindole alkaloids. In addition, pteropodine and isopteropodine have a stereoisomer relationship at the spiro C7-position of their structures, and their D and E rings are in the *cis*-configuration (Shamma et al., 1967). On the other hand, mitraphylline, a stereoisomer of pteropodine, has the *trans* configuration of the D/E rings and the β configuration of C20-H (Seki et al., 1993; Shamma et al., 1967). Considering these stereochemical properties of these alkaloids, the present findings suggest that the allo-type configuration of the D/E rings, but not the oxindole moiety of the A/B rings, of pteropodine plays an important role in positive modulation of the muscarinic M_1 and 5-HT_2 receptor functions in *Xenopus* oocytes.

In conclusion, this is the first report demonstrating a potent facilitatory effect of pteropodine and isopteropodine on muscarinic M_1 and 5-HT_2 receptors in vitro. Pteropo-

dine- and isopteropodine-induced positive modulation of muscarinic M_1 and 5-HT_2 receptor functions may be involved in the improvement of impaired higher cognitive processes by these alkaloids.

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