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BISCOCLAURINE ALKALOIDS INHIBIT RECEPTOR-MEDIATED PHOSPHOLIPASE A_2 ACTIVATION PROBABLY THROUGH UNCOUPLING OF A GTP-BINDING PROTEIN FROM THE ENZYME IN RAT PERITONEAL MAST CELLS

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Abstract—The mechanism underlying the inhibitory effect of biscoclaurine (bisbenzylisoquinoline) alkaloids on phospholipase A_2 activation in the signalling system of stimulated rat peritoneal mast cells was studied. Cepharanthine, berbamine and isotetrandrine inhibited antigen- and compound 48/80-induced arachidonic acid liberation, but not diacylglycerol formation or histamine release. They had no effect on A23187-induced arachidonic acid liberation, which was prevented by *p*-bromophenacyl bromide, a known phospholipase A_2 inhibitor, and also did not affect phospholipase A_2 activity in a cell-free system including an exogenous phospholipid substrate. Each alkaloid also inhibited arachidonic acid liberation induced by guanosine 5′-*O*-(3-thiotriphosphate) in saponin-permeabilized mast cells, and by mastoparan or NaF plus $AlCl_3$ in intact cells. Furthermore, each alkaloid abolished the inhibitory effect of islet-activating protein on the compound 48/80-induced arachidonic acid liberation. These data suggest that these alkaloids suppress the receptor-mediated phospholipase A_2 activation through, at least in part, uncoupling of a GTP-binding protein from the enzyme, rather than by affecting the enzyme directly.

The biscolaurine (bisbenzylisoquinoline) alkaloids, including cepharanthine, berbamine, tetrandrine and isotetrandrine, have been reported to affect the biological responses of several cell types through membrane modification. We and other investigators have shown that these alkaloids inhibit platelet activation [1–4], histamine release from rat mast cells [5], superoxide generation by polymorphonuclear leukocytes [6] and lipid peroxidation in some biomembranes [7]. Our recent study involving rabbit platelets demonstrated that these alkaloids, except tetrandrine, can suppress thrombin-induced arachidonic acid liberation, without affecting diacylglycerol formation, the increase in the cytoplasmic Ca^{2+} concentration or aggregation, indicating that these alkaloids selectively inhibit phospholipase A_2 activation in the cells [8]. Furthermore, we have also shown, using saponin-permeabilized platelets, that these alkaloids prevent arachidonic acid liberation induced by an addition of guanosine 5′-*O*-(3-thiotriphosphate) in the presence of Ca^{2+}, but not by a high concentration of Ca^{2+} alone. Therefore, we suggested that the inhibitory effect of these alkaloids may be due to suppression of the interaction of a GTP-binding protein with phospholipase A_2 [8].

Phospholipase A_2 activation in mast cells stimulated by an antigen–antibody interaction on the plasma membrane results in arachidonic acid liberation, leading to the production of chemical mediators, such as prostanoids and leukotrienes, thus playing a role in anaphylactic reactions. Since the enzyme activity was suggested to be regulated by a GTP-binding protein [9, 10], it is possible that the alkaloids may also exert an anti-allergic effect by suppressing phospholipase A_2 activation through a mechanism similar to that in platelets. In the present work, therefore, we examined the suppressive effects of cepharanthine, berbamine and isotetrandrine (Fig. 1) on arachidonic acid liberation in rat peritoneal mast cells stimulated with one of two types of stimulators, i.e. ones which act through a ligand–receptor interaction, such as an antigen and compound 48/80, and ones which bypass it, such as Ca^{2+}-ionophore and GTP-binding protein activator.

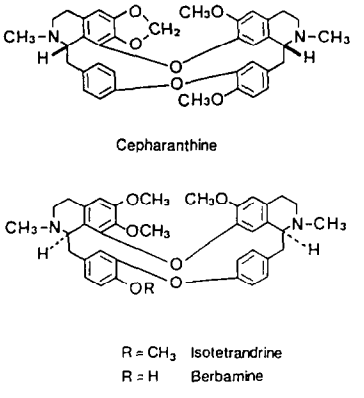

Fig. 1. Structures of the biscoclaurine alkaloids tested.

MATERIALS AND METHODS

Chemicals. Compound 48/80 and bovine serum

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albumin (BSA*, fraction V) were obtained from the Sigma Chemical Co. (St Louis, MO, U.S.A.). Monoclonal anti-2,4-dinitrophenyl mouse immunoglobulin E (anti-DNP IgE) was from BioMakor (Israel), A23187 from Calbiochem (La Jolla, CA, U.S.A.), guanosine 5'-*O*-(3-thiotriphosphate) (GTPγS) from Boehringer Mannheim GmbH (Mannheim, F.R.G.), mastoparan from Peptide Institute Inc. (Osaka, Japan), lysophosphatidylserine from Avanti Polar Lipids Inc. (Alabaster, AL, U.S.A.) and islet-activating protein (pertussis toxin) from Kaken Pharmaceutical Co. Ltd (Kyoto, Japan). [^{3}H]Arachidonic acid (76.0 Ci/mmol) and 1-palmitoyl-2-[^{14}C]arachidonoyl glycero phospho ethanolamine (52.0 mCi/mmol) were from New England Nuclear (Boston, MA, U.S.A.). Cepharanthine, berbamine and isotetrandrine were donated by Kaken Shoyaku Co. Ltd (Tokyo, Japan). The antigen used was a protein conjugate with an average of seven 2,4-dinitrophenyl groups per molecule of BSA (DNP-BSA). Other reagents were obtained from commercial sources.

Preparation of rat peritoneal mast cells. Mast cells were harvested from peritoneal fluid taken from male Wistar rats of 8 weeks of age. The cells were purified essentially according to the method of Sullivan *et al.* [11]. Briefly, the peritoneal cells suspended in a medium, composed of 150 mM NaCl, 3.7 mM KCl, 3 mM Na_2HPO_4, 3.5 mM KH_2PO_4, 6 mM glucose, 1 mg/mL BSA, 1 mg/mL geltatin and 10 U/mL heparin (pH 6.8), were centrifuged at 50 *g* for 6 min at 4°, and then the pellet obtained was suspended in the medium. The suspension was layered over a 31.5% BSA–saline solution, followed by centrifugation at 300 *g* for 10 min at 4°. The pellet was washed twice and then the cells were resuspended at 5×10^6 cells/mL in the medium. For sensitization and/or [^{3}H]arachidonic acid labeling of the cells, anti-DNP IgE (1 μg/mL) and/or [^{3}H]arachidonic acid (10 μCi/mL) were added, and the cell suspension was incubated at 37° for 1.5 hr. After being washed twice, the cell number was adjusted to 5×10^5 cells/mL in the same medium without heparin.

Measurement of lipid metabolism. [^{3}H]Arachidonic acid-labeled mast cells (5×10^5 cells/mL), sensitized with or without anti-DNP IgE, were incubated with BW755C (80 μM; 3-amino-1-[*m*-(trifluoromethyl)-phenyl]-2-pyrazoline, a lipoxygenase and cyclooxygenase inhibitor) at 37° for 2 min in the presence of $CaCl_2$ (1.3 mM), and then treated with various concentrations of the alkaloids for 1 min. The mast cells were stimulated with DNP-BSA (10 μg/mL) for 10 min in the presence of lysophosphatidylserine (5 μg/mL), or with A23187 (20 nM), compound 48/80 (1 μg/mL) or mastoparan (10 μM) for 5 min. In the experiments involving a cell-free system, a cell lysate, prepared from non-labeled mast cells (2.5×10^6 cells/mL) by sonication, was incubated with 1-palmitoyl-2-[^{14}C]arachidonoyl glycerophosphoethanolamine (1 μM), $CaCl_2$ (5 mM) and sodium deoxycholate (2 mM) at 37° for 30 min in the presence or absence of each alkaloid (20 μM). In the case of stimulation with GTPγS, the labeled, BW755C-pretreated mast cells were treated with saponin (15 μg/mL) at 37° for 5 min, and then incubated with the alkaloids for 1 min. The mast cells were further incubated with GTPγS (10 μM) for 10 min in the presence of Ca^{2+} (10 μM), the concentration of which was adjusted with Ca^{2+}-EGTA (1 mM) buffer. The reactions were terminated by the addition of ice-cold chloroform/methanol/HCl (200:200:1, by vol.). After lipid extraction, each lipid was separated by TLC on a Silica Gel G plate (Merck) with a developing solvent system of petroleum ether/diethyl ether/acetic acid (80:80:1.5, by vol.) or the combination of chloroform/methanol/7 M NH_4OH (90:54:11, by vol.), for the first dimension, and chloroform/methanol/acetic acid/water (30:15:4:2, by vol.), for the second dimension. Each free fatty acid, diacylglycerol and phosphatidic acid fraction, identified on the basis of comigration with authentic standards, was scraped off and its radioactivity was determined by liquid scintillation counting [12].

Measurement of histamine release. Sensitized or non-sensitized mast cells were incubated with $CaCl_2$ (1.3 mM) at 37° for 2 min, treated with the alkaloids, and then stimulated with agonists, as described above. The reactions were terminated by adding a 3-fold volume of ice-cold saline containing EGTA (1 mM). The supernatant obtained from the suspension by centrifugation, at 600 *g* for 10 min at 4°, was used for determination of histamine released according to the method of May *et al.* [13], using the fluorometric method of Shore *et al.* [14].

Islet-activating protein treatment. After 30 min labeling of mast cells with [^{3}H]arachidonic acid, the cells (1×10^6 cells/mL) were incubated with islet-activating protein (1 ng/mL) at 37° for 2 hr in the presence or absence of the alkaloids (20 μM). After being washed twice, the cells (5×10^5 cells/mL) were pretreated with BW755C (80 μM) at 37° for 2 min in the presence of $CaCl_2$ (1.3 mM) and then stimulated with compound 48/80 (1 μg/mL) for 5 min. After lipid extraction, [^{3}H]arachidonic acid liberated was determined as described above.

RESULTS

Effects of the alkaloids on DNP-BSA- and compound 48/80-induced lipid metabolism and histamine release

Figure 2A illustrates the effects of cepharanthine, berbamine and isotetrandrine on arachidonic acid liberation induced by antigen (DNP-BSA) in rat peritoneal mast cells previously sensitized with an anti-DNP IgE and labeled with [^{3}H]arachidonic acid. Stimulation of the mast cells with DNP-BSA (10 μg/mL) in the presence of lysophosphatidylserine (5 μg/mL) caused significant [^{3}H]arachidonic acid liberation, the amount liberated being 2.7-fold that in the case of unstimulated mast cells (Fig. 2A). Pretreatment of the cells with each alkaloid inhibited the DNP-BSA-induced liberation in a dose-dependent manner, the inhibition reaching about 75% with 20 μM of each alkaloid. Each alkaloid

* Abbreviations: BSA, bovine serum albumin; anti-DNP IgE, monoclonal anti-2,4-dinitrophenyl mouse immunoglobulin E; DNP-BSA, 2,4-dinitrophenyl group-conjugated BSA; GTYγS, guanosine 5'-*O*-(3-thiotriphosphate); EGTA, [ethylenebis(oxyethylenenitrilo)]tetraacetic acid.

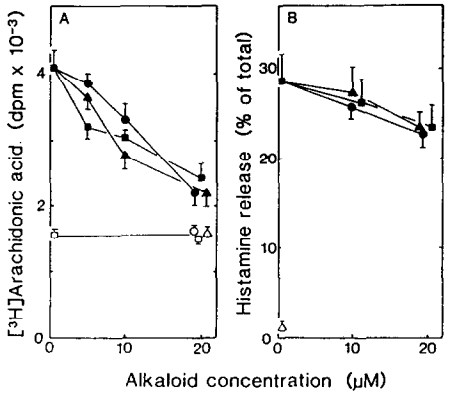

Fig. 2. Effects of each alkaloid on arachidonic acid liberation and histamine release induced by DNP-BSA. Rat mast cells were sensitized with anti-DNP IgE, and labeled with [^{3}H]arachidonic acid in (A), as described in Materials and Methods. The mast cells were incubated with (A) or without (B) BW755C (80 μM) at 37° for 2 min in the presence of $CaCl_2$ (1.3 mM), treated with various concentrations of cepharanthine (●), berbamine (▲) or isotetrandrine (■) for 1 min, and then stimulated with DNP-BSA (10 μg/mL) for 10 min in the presence of lysophosphatidylserine (5 μg/mL). [^{3}H]Arachidonic acid liberated (A) and histamine released (B) were determined as described in Materials and Methods. Each point represents the mean ± SD of three separate experiments performed in duplicate. (○, △, □) Without stimulation.

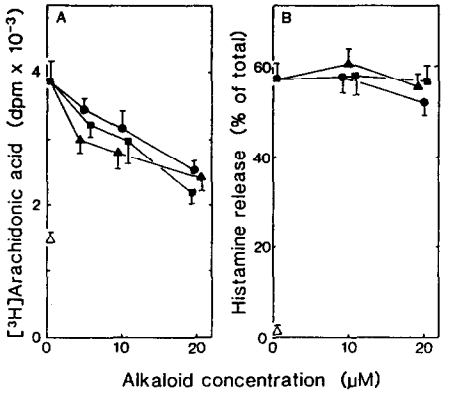

Fig. 3. Effects of each alkaloid on arachidonic acid liberation and histamine release induced by compound 48/80. [^{3}H]Arachidonic acid-labeled or non-labeled mast cells were incubated with (A) or without (B) BW755C (80 μM) at 37° for 2 min in the presence of $CaCl_2$ (1.3 mM), treated with various concentrations of cepharanthine (●), berbamine (▲) or isotetrandrine (■) for 1 min, and then stimulated with compound 48/80 (1 μg/mL) for 5 min. [^{3}H]Arachidonic acid liberated (A) and histamine released (B) were determined as described in Materials and Methods. Each point represents the mean ± SD of three separate experiments performed in duplicate. (△) Without stimulation.

alone had no effect on the level of [^{3}H]arachidonic acid in the concentration range used. Following stimulation with DNP-BSA, the formation of [^{3}H]-diacylglycerol increased to 2-fold (1468 ± 160 dpm versus 701 ± 61 dpm, N = 3), but none of the alkaloids (20 μM) had a suppressive effect on the formation (cepharanthine, 1407 ± 157 dpm; berbamine, 1449 ± 128 dpm, isotetrandrine, 1584 ± 192 dpm). Under the same conditions, DNP-BSA-induced [^{3}H]phosphatidic acid formation, which was increased to about 2.5-fold that in unstimulated cells, was also not affected by the alkaloids (20 μM, data not shown). Since, in antigen-stimulated mast cells, phosphatidic acid was shown to be formed through the direct action of phospholipase D other than the sequential actions of phospholipase C and diacyglycerol kinase [15], our results seem to indicate that the inhibition of arachidonic acid liberation by the alkaloids may not be due to inhibition of phospholipase C and/or D activation. Furthermore, as shown in Fig. 2B, DNP-BSA-caused histamine release, which amounted to 28% of the total content, was not inhibited by pretreatment with these alkaloids.

As shown in Fig. 3A, stimulation of [^{3}H]-arachidonic acid-labeled mast cells with compound 48/80 (1 μg/mL) caused a 2.6-fold increase in the [^{3}H]arachidonic acid liberation, as compared with that in unstimulated mast cells. When the mast cells had been pretreated with one of the alkaloids and then exposed to the stimulant, the increase was dose-dependently prevented, the inhibition being about 70% with 20 μM of each alkaloid. On the contrary, the increase in [^{3}H]diacylglycerol formation caused by compound 48/80 (from 705 ± 84 dpm to 1355 ± 121 dpm, N = 3) was not affected by pretreatment with each alkaloid (cepharanthine, 1339 ± 148 dpm; berbamine, 1318 ± 165 dpm; isotetrandrine, 1227 ± 104 dpm). Furthermore, these alkaloids did not affect compound 48/80-induced histamine release in the same concentration ranges (Fig. 3B).

The results described above appear to indicate that the alkaloids tested selectively inhibit phospholipase A_2 activation induced through receptor stimulation.

Direct effects of the alkaloids on phospholipase A_2 enzyme activity

To determine whether or not the alkaloids influence phospholipase A_2 molecules directly, we examined their effects on A23187-induced arachidonic acid liberation. As shown in Fig. 4, each alkaloid had no effect on A23187 (20 nM)-induced [^{3}H]arachidonic acid liberation from labeled mast cells. However, when the cells had been pretreated with *p*-bromophenacyl bromide, a phospholipase A_2 inhibitor, as a positive control, the A23187-induced liberation was significantly prevented. We further examined the effects of the alkaloids on phospholipase A_2 activity in a cell-free system, involving a mast cell lysate prepared by sonication, as an enzyme source, and an exogenous substrate. When a lysate prepared from mast cells (2.5×10^6 cells/mL, pH 7.4) had been incubated with 1-palmitoyl-2-[^{14}C]arachidonoyl glycerophosphoethanolamine (1 μM), $CaCl_2$ (5 mM) and sodium deoxycholate (2 mM) at 37° for 30 min in the presence or absence of each alkaloid (20 μM), the amounts of free [^{14}C]arachidonic acid liberated were similar to the

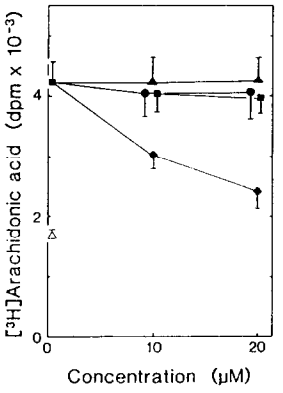

Fig. 4. Effects of each alkaloid or *p*-bromophenacyl bromide on arachidonic acid liberation induced by A23187. [^{3}H]Arachidonic acid-labeled mast cells were incubated with BW755C (80 μM) at 37° for 2 min in the presence of $CaCl_2$ (1.3 mM), treated with various concentrations of cepharanthine (●), berbamine (▲) or isotetrandrine (■) for 1 min, or *p*-bromophenacyl bromide (◆) for 10 min, and then stimulated with A23187 (20 nM) for 5 min. [^{3}H]Arachidonic acid liberated was determined as described in Materials and Methods. Each point represents the mean ± SD of three separate experiments performed in duplicate. (△) Without stimulation.

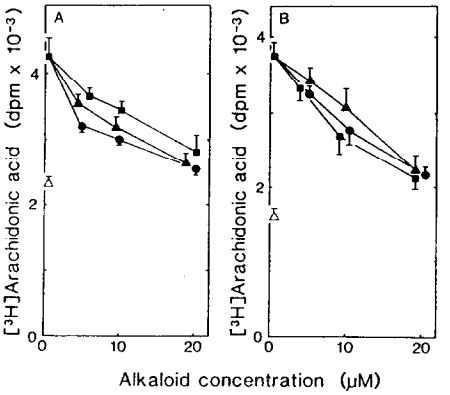

Fig. 5. Effects of each alkaloid on arachidonic acid liberation induced by GTPγS (A) or mastoparan (B). (A) [^{3}H]Arachidonic acid-labeled mast cells were incubated with BW755C (80 μM) at 37° for 2 min, and further with saponin (15 μg/mL) for 5 min. The mast cells were treated with various concentrations of cepharanthine (●), berbamine (▲) or isotetrandrine (■) for 1 min, and then incubated with GTPγS (10 μM) and Ca^{2+} (10 μM) for 10 min. (△) Without GTPγS. (B) [^{3}H]Arachidonic acid-labeled mast cells were incubated with BW755C (80 μM) at 37° for 2 min, treated with various concentrations of each alkaloid for 1 min, and then stimulated with mastoparan (10 μM) for 5 min. The symbols are the same as in (A). (△) Without stimulation. In both experiments, [^{3}H]arachidonic acid liberated was determined as described in Materials and Methods, and each point represents the mean ± SD of three separate experiments performed in duplicate.

control level (control, 1485 ± 156 dpm; cepharanthine, 1594 ± 165 dpm; berbamine, 1496 ± 173 dpm; isotetrandrine, 1413 ± 102 dpm; mean ± SD in triplicate experiments). These results showing that the alkaloids inhibited arachidonic acid liberation induced by the antigen and compound 48/80, but not that by A23187 or in a cell-free system, led us to assume that the inhibition of phospholipase A_2 may be due to impairment of receptor-mediated signaling, rather than direct suppression of the enzyme activity.

Effects of the alkaloids on GTP-binding protein-mediated phospholipase A_2 activation

It has been reported that compound 48/80-elicited phospholipase A_2 activation may be regulated by a GTP-binding protein [9], and that the stimulation of mast cells with a GTP-binding protein activator such as GTPγS or mastoparan causes arachidonic acid liberation [10, 16]. Based on these ideas, the results obtained here suggest the possibility that the inhibition of arachidonic acid liberation by the alkaloids may result from their interference with the association of a GTP-binding protein with phospholipase A_2. Therefore, we examined whether or not the alkaloids actually affect GTP-binding protein-mediated phospholipase A_2 activation, using GTP-binding protein activators. As shown in Fig. 5A, when saponin (15 μg/mL)-permeabilized mast cells had been pretreated with each alkaloid, and stimulated with GTPγS (10 μM) and Ca^{2+} (10 μM), GTPγS-induced [^{3}H]arachidonic acid liberation was inhibited dose-dependently. In the case of stimulation of intact mast cells with mastoparan (10 μM), which can directly activate a GTP-binding protein [17], these alkaloids exhibited similar inhibitory effects on the [^{3}H]arachidonic acid liberation (Fig. 5B). Furthermore, the increase in the liberation caused by NaF (20 mM) plus $AlCl_3$ (10 μM) (from 1486 ± 117 dpm to 4501 ± 380 dpm, N = 3) was also suppressed by about 50% by each of these alkaloids. However, the alkaloids tested had no effect on GTPγS-, mastoparan- or NaF plus $AlCl_3$-induced [^{3}H]diacylglycerol formation, or histamine release (data not shown). These results suggest that the alkaloids may influence the association of the GTP-binding protein with the phospholipase A_2 molecule.

Effects of the alkaloids on the inhibition of compound 48/80-induced arachidonic acid liberation by islet-activating protein (pertussis toxin)

Islet-activating protein is known to catalyse the ADP-ribosylation of the α-subunit of a GTP-binding protein of mast cells, and to inhibit compound 48/80-induced arachidonic acid liberation as well as histamine release [9]. To further examine whether or not the alkaloids tested directly affect the GTP-binding protein itself, the effects of the alkaloids on the inhibition of compound 48/80-induced arachidonic acid liberation by islet-activating protein were studied. As shown in Table 1, preincubation of [^{3}H]arachidonic acid-labeled mast cells with islet-activating protein (1 ng/mL) inhibited compound 48/80 (1 μg/mL)-induced [^{3}H]arachidonic acid liberation by about 65%. When the mast cells were

Table 1. Effects of each alkaloid on the inhibition of compound 48/80-induced arachidonic acid liberation by islet-activating protein

Treatment	[^{3}H]Arachidonic acid (dpm)	
	− compound 48/80	+ compound 48/80
Control	1573 ± 270	4481 ± 723
+ IAP	1609 ± 417	2571 ± 246
+ IAP + CP, then washed	1747 ± 157	4006 ± 608
+ IAP + BR, then washed	1669 ± 459	4365 ± 765
+ IAP + IT, then washed	1576 ± 383	4406 ± 726

[^{3}H]Arachidonic acid-labeled mast cells were pretreated with or without islet-activating protein (IAP, 1 ng/mL) at 37° for 2 hr in the presence or absence of 20 μM of cepharanthine (CP), berbamine (BR) or isotetrandrine (IT), and then washed twice. The mast cells were treated with BW755C (80 μM) for 2 min in the presence of $CaCl_2$ (1.3 mM), and then stimulated with compound 48/80 (1 μg/mL) for 5 min. [^{3}H]Arachidonic acid liberated was determined as described in Materials and Methods. Each value represents the mean ± SD of three separate experiments performed in duplicate.

pretreated with islet-activating protein in the presence of each alkaloid (20 μM), washed with a medium to remove the alkaloid, and then stimulated with compound 48/80, this inhibitory effect of islet-activating protein could be almost completely abolished.

DISCUSSION

Activation of mast cells causes the release of some chemical mediators such as histamine, prostanoids and leukotrienes, to induce an allergic reaction. The formation of eicosanoids results from arachidonic acid liberation, which is caused by the catalytic action of phospholipase A_2 on membrane phospholipids. In a recent publication, we presented evidence that some of the biscoclaurine alkaloids have the ability to selectively inhibit phospholipase A_2 activation through modification of membrane properties of platelets [8]. Therefore, the present study was undertaken to detect an inhibitory effect of these alkaloids on receptor-mediated phospholipase A_2 activation in rat peritoneal mast cells, to analyse the underlying mechanism, and to assess their potential use as anti-allergic drugs.

As shown in the present work, cepharanthine, berbamine and isotetrandrine inhibited antigen (DNP-BSA)- and compound 48/80-induced arachidonic acid liberation, whereas they did not affect diacylglycerol formation or histamine release in response to the same agonists. Since, on stimulation with an antigen or compound 48/80, arachidonic acid is mainly liberated through the action of phospholipase A_2 from membrane phospholipids [9, 18], our results appear to indicate that these alkaloids selectively suppress phospholipase A_2 activation, and further suggest that the suppressed activation may not be secondary to suppression of phospholipase C and/or D activation or to interference with the ligand–receptor interaction. This possibility is also supported by our recent finding in rabbit platelets that these alkaloids selectively inhibit thrombin-induced phospholipase A_2 activation at a certain concentration that does not influence phospholipase C activation or aggregation [8].

The results obtained here showed that the alkaloids tested had no effect on A23187-induced arachidonic acid liberation, which could be prevented by *p*-bromophenacyl bromide, a phospholipase A_2 inhibitor, despite the fact that they inhibited the liberation caused by receptor-mediated stimulation. Furthermore, in the experiment involving a cell lysate and an exogenous substrate, the alkaloids did not affect phospholipase A_2 activity under the conditions used. These observations indicate the possibility that these alkaloids may block a signal transduction step prior to phospholipase A_2 activation rather than the enzyme activity directly. In rat mast cells, a GTP-binding protein is suggested to be involved in the process leading to phospholipase A_2 activation on stimulation with compound 48/80 [9]. We showed that the alkaloids inhibited arachidonic acid liberation induced by GTPγS, mastoparan or NaF plus $AlCl_3$, which can directly activate a GTP-binding protein. Therefore, it is conceivable that the inhibition of phospholipase A_2 activation by the alkaloids may result from interference with GTP-binding protein-mediated signaling, probably through changes in membrane properties surrounding this protein. This idea is supported by our further observation that the alkaloids eliminated the inhibitory effect of islet-activating protein, which is known to abolish the function of a GTP-binding protein through ADP-ribosylation, on compound 48/80-induced arachidonic acid liberation. Although the mechanism by which the alkaloids prevent the effect of the islet-activating protein is unclear, the results might be interpreted as suggesting that they interfere with ADP-ribosylation of a GTP-binding protein. A

similar effect of non-steroidal anti-inflammatory drugs was recently reported in human neutrophils [19]. Thus, it is likely that the alkaloids tested may induce uncoupling of a GTP-binding protein from phospholipase A_2, and as a result interfere with the receptor-mediated activation of the enzyme.

It has been shown that antigen- and compound 48/80-induced histamine release is prevented by phospholipase A_2 inhibitor [9, 20], suggesting that phospholipase A_2 activation may be involved in the histamine release reaction. However, despite the inhibition of arachidonic acid liberation by each of the alkaloids tested, antigen- or compound 48/80-induced histamine release was not affected by any of them in our study. Although we have no explanation for this discrepancy, it was also shown that the phospholipase A_2 activation does not contribute to the histamine release in the case of stimulation with GTPγS and Ca^{2+} [21].

Although tetrandrine, one of the biscoclaurine alkaloids, was previously shown to inhibit antigen-induced histamine release [5], the alkaloids used in this work did not suppress the release in the concentration ranges employed. It is reasonable to conclude that since tetrandrine exhibits strong ability to act on membranes, the reported suppression of histamine release might be due to extensive changes in membrane properties induced by its non-specific membrane action, as suggested in our previous paper [8].

In conclusion, the present study showed that cepharanthine, berbamine and isotetrandrine selectively inhibit phospholipase A_2 activation in response to receptor-mediated stimulation. We propose that the inhibition of the enzyme by these alkaloids might result from blockading of the receptor-mediated signal transduction via a GTP-binding protein rather than from their direct effect on the enzyme molecule or the ligand–receptor interaction, and that this may be due, at least in part, to uncoupling of the GTP-binding protein from the enzyme, through alkaloid-induced changes in membrane properties. Thus, these alkaloids seem to have an anti-allergic effect through the suppression of the formation of arachidonic acid metabolites such as leukotrienes.

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