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Chemical and pharmacological studies on the novel antiallergic drugs from medicinal plants

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1. Introduction

Histamine is a naturally occurring mediator of inflammation and is abundantly present in the secretory granules of mast cells and basophils which reside in the respiratory tract. The tissue effects of histamine are presumably mediated by cell membrane receptors. Three histamine receptor subtypes (H_1, H_2 and H_3) are known. Histamine H_1 receptor antagonists have been developed and used in the therapy of many allergic diseases. In peripheral tissues, the histamine H_1 receptor mediates the contraction of smooth muscles, increase in capillary permeability due to contraction of terminal venules, and catecholamine release from adrenal medulla. On the other hand, 5-Hydroxytryptamine (5-HT) is a neurotransmitter and its role has been associated with many central nervous system-related activities. 5-HT receptors in the aorta are characterized as the 5-HT_{2A} subtype by examining the relationships between structure and activity of tryptamine analogs. Platelet aggregation produced by ADP is amplified by 5-HT through the 5-HT_{2A} receptor subtype.

In the course of our survery on pharmacologically active substances in medicinal plants including *Garcinia mangostana* L. and *Nandina domestica* Thunberg, much attention has been given to the occurrence of natural products possessing histamine or 5-HT antagonistic activities.

Materials and methods

2.1. Tissue preparation

The procedure of preparing the tissues and technique for measurement of contractions were performed as described by Furukawa (1996). Male albino rabbits weighing 2-3 kg and male, Dunkin-Hartley, guinea-pigs (250-300 g) were used for this study. The strips

were suspended in a 20 ml organ bath containing modified Krebs-Ringer-bicarbonate solution of the following composition (mM) : NaCl, 120; KCl, 6.0; $CaCl_2$, 1.2; $MgSO_4$, 1.3; $NaHCO_3$, 25.2; glucose 5.8; pH 7.4. The solution was gassed with 95% O_2 and 5% CO_2 and temperature was maintained at 37□. Isometric contractions were measured by the transducer and recorded on a polygraph (Nihon Kohden, Tokyo, Japan). After equilibration, the strips were precontracted with 60 mM KCl (10 min) and two or three contractile responses to 60 mM KCl were obtained until the response remained constant.

2.2. Cell cultures

Vascular smooth muscle cells were isolated from aortas of Wister rat by enzymatic dispersion as described by Chamley et al. (1977). The resulting cells were seeded in 18-mm culture dishes for measurements of ligand binding. Cells were cultured for 5-6 days in Dulbecco's modified Eagle's media (DMEM) supplemented with 10% heat-inactivated fetal calf serum, 10 U/ml penicillin, and 100 μg/ml streptomycin.

2.3. Receptor binding experiments

Confluent vascular smooth muscle cells ($2x10^5$ cells/ml) in culture dishes were incubated for 30 min at 37□ with [^{3}H]mepyramine or [^{3}H]spiperone in 250 μl balanced salt solution (BSS) containing (mM): NaCl 146; KCl 4; $MgCl_2$ 2; $CaCl_2$ 0.5; glucose 10; bovine serum alblumin 0.1% and HEPES 10 (adjusted to pH 7.4 with Tris base) in the absence or presence of drugs. Specific binding of ligand to cells was estimated by subtracting the nonspecific component from total binding.

2.4. Data analysis

Three or four preparations were used for each experiments. Data are presented as means□S.E.M. Statistical analyses were done by means of Stucent's *t*-test.

2. Results

3.1. Isolation of receptor blocking substances

In the present study, we have found that a methanolic extract of the fruit hull of *G. mangostana* almost completely inhibited the histamine (10 μM) and 5-HT (1μM) induced contractions of isolated rabbit aorta but has no effect on contraction induced by KCl

(60mM) or phenylephrine (1μM). To isolate the active substances, the methanolic extract of the fruit hull was fractionated accompanied by pharmacological test methods using isolated rabbit aorta. Silica gel chromatography of the ethyl acetate soluble portions of the methanolic extract gave a histaminergic receptor blocking substance (α-mangostin, 2g) and a serotonergic receptor blocking substance (γ-mangostin, 500mg). Their physicochemical properties (i.e. melting point, specific optical rotation, UV absorption, ^{1}H-, ^{13}C-NMR, and mass spectrum) agreed with those of α- and γ-mangostin, which were previosuly isolated as major xanthones from the same material (Fig.1).

A methanolic extract of the fruit of *N. domestica* almost completely inhibited the serotonin-induced contractions of isolated rabbit aorta and had no effect on contractions induced by KCl or histamine. To isolate the active substance, fractionation of the methanolic extract of the fruits were performed, accompanied by a bioassay using isolated rabbit aorta. Silica gel chromatography of the *n*-butyl alcohol-soluble portion of the methanolic extract afforded the active substance as colorless crystals (8.0 g, 0.2 % dry weight of the fruit). The physicochemical properties of this compound (i.e., melting point, specific optical rotation, UV absorption, and mass spectrum) agreed with those of nantenine(1,5,6,11,13),which was previously isolated as a major alkaloid of the same material. (Fig.1).

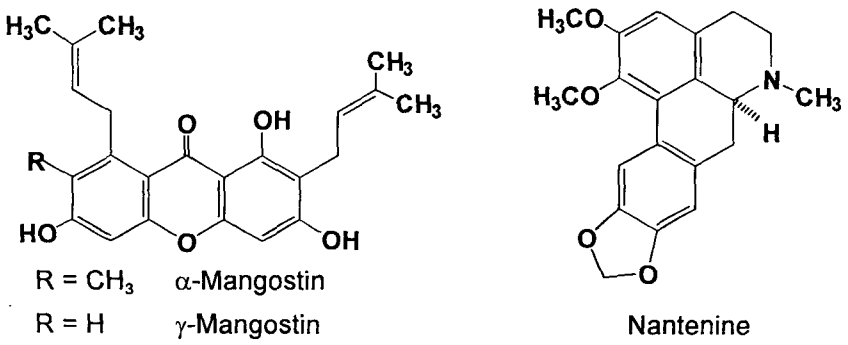

Fig.1. Chemical structures of α-mangostin, γ-mangostin and nantenine.

3.2. Mechanical response of rabbit thoracic aorta

The contractile response to histamine was antagonized in a concentration-dependent manner by α-mangostin without depression of the maximal response. The Schild plot of the data revealed the pA_2 value to be 5.78 with a slope of the regression line (1.16) not being significantly different from unity. Conversely, the concentration-response curves for KCl (7.5 to 120 mM, pD_2 value of 1.71) and phenylephrine ($3x10^{-8}$ to $1x10^{-5}$ M, pD_2 value of 6.28) were unaffected by α-mangostin ($1.5x10^{-5}$ M).

To investigate the histamine receptor type involved in the inhibitory effect of α-mangostin, the experiments were done in both histamine H_1 and H_2 receptors. After

blocking histamine H_1 receptor by using chlorpheniramine ($1x10^{-5}$ M), histamine ($3x10^{-7}$ to $5x10^{-4}$ M) induced the relaxation of aorta mediated by histamine H_2 receptor with a pD_2 value of 4.32. α-Mangostin ($1.5x10^{-5}$ M) did not affect the concentration-response curve of histamine-induced relaxation in rabbit aorta.

3.3 Mechanical response of guinea-pig trachea

Pretreatment of trachea for 10 min with α-mangostin ($1x10^{-6}$ M to $1.5x10^{-5}$ M) resulted in a concentration-related rightward shift of the histamine-induced contractile-response curve. No significant changes in the maximal response were observed. The pA_2 value for α-mangostin was calculated to be 5.80 and slope of Schild plot is 0.95. In contrast, α-mangostin ($1.5x10^{-5}$ M) had no effect on the contractile responses to KCl (7.5 to 120 mM, pD_2 value of 1.65) or carbachol ($1x10^{-8}$ to $3x10^{-6}$ M, pD_2 value of 6.87).

Dimaprit, a specific histamine H_2 agonist caused the concentration-dependent relaxation of trachea (pD_2 value of 3.92) which was completely inhibited by cimetidine. α-Mangostin ($1.5x10^{-5}$ M) did not inhibit the concentration-relaxation curve of trachea induced by dimaprit.

3.4. Receptor binding

α-Mangostin competitively inhibited histamine-induced contraction of both aorta and trachea. Then, receptor binding analysis was carried out to clarify whether α-mangostin is a histamine H_1 receptor antagonist. α-Mangostin inhibited the [^{3}H]mepyramine binding to rat aortic smooth muscle cells with an IC_{50} value of 2.27 μM. α-Mangostin increased the K_d value (11.7 nM) to 38.02 nM without affecting the B_{max} value 276.0 fmol/mg. These results clearly indicate that α-mangostin is a specific histamine H_1 receptor antagonist.

Receptor-binding analysis was carried out to investigate the interaction of γ-mangostin with 5-HT_{2A} receptors. γ-Mangostin also inhibited the binding with the IC_{50} value of 3.5 nM. γ-Mangostin (3 nM) increased the K_d value (11.7 nM) to 27.4 nM without affecting the B_{max} value (447 fmol/mg). These results clearly indicate that γ-mangostin inhibited [^{3}H]spiperone binding to 5-HT_{2A} receptor in a competitive manner.

3. Discussion

In isolated rabbit thoracic aorta and guinea-pig trachea, α-mangostin inhibited histamine-induced contractions in a concentration-dependent manner in the presence or absence of cimetidine, a histamine H_2 receptor antagonist. But KCl- , phenylephrine- or carbachol-induced contractions were not affected by α-mangostin. The concentration-contractile response curve for histamine was shifted to the right in a parallel manner by α-mangostin.

In the presence of chlorpheniramine, a histamine H_1 receptor antagonist, α-mangostin did not affect the relaxation of rabbit aorta induced by histamine. In guinea-pig trachea, α-mangostin had no effect on the relaxation induced by dimaprit, histamine H_2 receptor agonist. α-Mangostin caused a concentration-dependent inhibition of the binding of [^{3}H]mepyramine, a specific histamine H_1 receptor antagonist to rat aortic smooth muscle cells. Kinetic analysis of [^{3}H]mepyramine binding indicated the competitive inhibition by α-mangostin. These results suggest that α-mangostin is a novel competitive histamine H_1 receptor antagonist in smooth muscle cells.

γ-Mangostin purified from a fruit hull of the medicinal plant *Garcinia mangostana* caused a parallel rightward shift of the concentration-contractile response curve for 5-HT (5-HT_2) in the rabbit aorta (pA_2 = 8.2) without affecting the contractile responses to KCl, phenylephrine (α_1) or histamine (H_1). 5-HT amplified, ADP-induced aggregation of rabbit platelets (5-HT_{2A}) was inhibited by γ-mangostin, whereas that induced by thrombin was not affected by it [13]. γ-Mangostin did not affect 5-HT-induced contraction of the guinea-pig ileum (5-HT_3) in the presence of 5-HT_1, 5-HT_2 and 5-HT_4 antagonists. γ-Mangostin caused an inhibition of the [^{3}H]spiperone binding to cultured rat aortic myocytes. K_d value of the [^{3}H]spiperone binding was increased by γ-mangostin without affecting the B_{max} value. These results suggest that γ-mangostin is a novel competitive antagonist free from a nitrogen atom for the 5-HT_{2A} receptors in vascular smooth muscles and platelets.

Nantenine caused a concentration-dependent (1 mg/kg ~ 10 mg/kg p.o.) antihypertensive effect in spontaneously hypertensive rats without affecting heart rates (unpublished data). Nantenine brough about a marked parallel, rifhtward shift of concentration-contractile response curve for 5-HT in the rabbit aorta without effect on contractions induced by KCl or histamine, indicating, a selective and competitive antagonist for 5-HT receptor [11]. The concentration-contractile response curve for norepinephrine was shifted slightly to the right in the presence of nantenine in the aorta. Nantenine even at 3×10^{-5} M had no effect on the inotropic response of isolated guinea-pig left atria to isoproterenol (unpublished data). These results suggest that antihypertensive activity of nantenine is related more to 5-HT_2 vascular receptor blocking properties than to its ability to block α- or β-adrenergic receptor. It is also suggested that antihypertensive effects of nantenine may be due to 5-HT_2 receptor blocking activity.

Numerous receptor antagonists for histamine or 5-HT having nitrogen atom in the molecules have been synthesized, suggesting that nitrogen atom is essential for the 5-HT receptor blocking activity. It is an important finding that α- and γ-mangostin free from a nitrogen atom possesses a remarkable histamine H_1 and 5-HT_{2A} receptor blocking activity, respectively. We have succeeded in finding α- and γ-mangostin as a lead for the development of histamine H_1 and 5-HT_{2A} receptor antagonists lacking a nitrogen atom.