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ORIGINAL ARTICLE

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γ-Mangostin, a novel type of 5-hydroxytryptamine 2A receptor antagonist

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Abstract γ-Mangostin, purified from the fruit hull of the medicinal plant *Garcinia mangostana* caused a parallel rightwards shift of the concentration/response curve for the contraction elicited by 5-hydroxytryptamine (5-HT) in the rabbit aorta (pA_2 = 8.2) without affecting the contractile responses to KCl, phenylephrine (α_1) or histamine (H_1). The perfusion pressure response of rat coronary artery to 5-HT (5-HT_{2A}) was reduced concentration dependently by γ-mangostin (IC_{50} = 0.32 μM). 5-HT amplified, ADP-induced aggregation of rabbit platelets (5-HT_{2A}) was inhibited by γ-mangostin (IC_{50} = 0.29 μM), whereas that induced by thrombin was not affected, nor did γ-mangostin 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 receptor antagonists. Furthermore, 5-HT-induced contraction of the rat fundus (5-HT_{2B}) and 5-HT-induced relaxation of the rabbit aorta in the presence of ketanserin (5-HT_1) and carbachol-induced contraction of the guinea-pig ileum (muscarinic M_3) were not affected by γ-mangostin (5 μM). γ-Mangostin inhibited [^{3}H]spiperone binding to cultured rat aortic myocytes (IC_{50} = 3.5 nM). The K_d for [^{3}H]spiperone binding was increased by γ-mangostin (3 nM) from 11.7 to 27.4 nM without affecting B_{max}. 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.

Key words γ-Mangostin · 5-Hydroxytryptamine · 5-Hydroxytryptamine 2A receptor antagonist · Rabbit aorta · Rat coronary · Rabbit platelet

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Introduction

5-Hydroxytryptamine (5-HT) is a neurotransmitter believed to be associated with many central nervous system-related activities (Zifa and Fillion 1992; Hoyer et al. 1994; Fuller 1996; Lucas and Hen 1996). 5-HT_2 receptor agonists act as peripheral vasoconstrictors to increase arterial blood pressure (Alper 1990). 5-HT receptors in the aorta have been identified as the 5-HT_{2A} subtype by examining the relationships between structure and activity of tryptamine analogues (Feniuk et al. 1985; Clancy and Maayani 1985; Ullmer et al. 1995). Platelet aggregation produced by ADP is amplified by 5-HT through the 5-HT_{2A} receptor (Cohen et al. 1981, 1983; Leysen et al. 1983; Pletscher and Affolter 1983; Pine et al. 1996). Many 5-HT_2 receptor antagonists containing a nitrogen atom have been synthesized and used as therapeutic agents for the treatment of various nervous system disorders (Rinaldi-Carmona et al. 1992; Doble et al. 1992). Since acute administration of ketanserin, a representative 5-HT_{2A} receptor antagonist, reduces phenylephrine pressor response through the combination of α_1-adrenergic and 5-HT_{2A} receptor blockade (Balasubramaniam et al. 1993), more selective antagonists have been sought.

Since 5-HT receptor antagonists are theoretically and clinically important, in the course of our survey on the novel type of active substances from natural sources much attention was paid to the occurrence of substances having 5-HT receptor blocking activity. Recently, we found that the crude extract of the fruit hull of the medicinal plant *Garcinia mangostana* inhibited the contractions of rat aorta induced by histamine and 5-HT. α- and γ-mangostins have been isolated from the extract as blockers for histamine and 5-HT receptors respectively (Chairungsrilerd et al. 1996a). α-Mangostin has been shown to be a specific histamine H_1 receptor antagonist (Chairungsrilerd et al. 1996b). On the other hand, the detailed pharmacological properties of γ-mangostin, a related compound of α-mangostin, have not been studied yet. In the present study, we characterize γ-mangostin as a novel and specific 5-HT_{2A} receptor antagonist free from a nitrogen atom.

Methods

Contractile responses of aorta, fundus and ileum.

The thoracic aorta was prepared from male albino rabbits (2–3 kg). The endothelium was removed by gently rubbing the endothelial surface with cotton pellets. The aorta was cut into helical strips, approximately 4 mm wide and 20 mm long. The strips were mounted and suspended in a 20 ml organ bath containing modified Krebs' Ringer bicarbonate solution (37° C) (for composition, see Furukawa et al. 1996). Resting force was adjusted to 1 g at the beginning of each experiment. The tissues were allowed to equilibrate for 60–90 min. Isometric contractions were recorded. 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 became constant. The control concentration/contractile response curves for the agonists (5-HT, KCl or phenylephrine) were obtained cumulatively and the contractions expressed as a percentage of the maximal contraction produced by each agonist [5-HT (0.5 mM), KCl (30 mM) or phenylephrine (3 μM)]. γ-Mangostin (0.03–1 μM) was added to the bath 10 to 20 min before the addition of agonists in the concentration previously used as control. The concentration/response curves for the agonists were then obtained in the presence of γ-mangostin. The time interval between two consecutive curves was usually set at 60 min.

To investigate the effect of γ-mangostin on the 5-HT_1 receptor, the rabbit aortic strips were treated with ketanserin (3 μM) for 10 min to block the 5-HT_{2A} receptor followed by precontraction with prostaglandin $F_{2\alpha}$ (10 μM). After contraction had stabilized, control 5-HT-induced, concentration-dependent relaxation was obtained. The relaxation responses to 5-HT of the aortic strips were expressed as percentage of the contraction induced by prostaglandin $F_{2\alpha}$ (10 μM). To test the antagonism, γ-mangostin (5 μM) was added to the organ bath 10 min before the addition of ketanserin.

The fundi were excised from male Wistar rats (150–200 g) anesthetized with sodium pentobarbital (30 mg/kg body weight) and longitudinal sections were used as previously described by Cohen and Wittenauer (1987). Four strips were obtained from one rat fundus. Tissues were placed under optimum resting force (4 g) and were allowed to equilibrate for approximately 60–90 min. Non-cumulative contractile concentration/response curves for 5-HT were obtained by stepwise increases in concentration after washing out the preceding concentrations every 15 to 20 min. The tissue was exposed to each 5-HT concentration for approximately 2 min before washout and maximum response to 5-HT concentration was measured. After control responses were obtained, tissues were incubated with γ-mangostin (0.1–5 μM) for 30 min. Responses to 5-HT were then repeated in the presence of γ-mangostin in the concentration previously used as control. In each tissue, only one antagonist concentration was examined. The contractions were expressed as a percentage of the maximal contraction produced by 1 μM 5-HT.

Small intestine was removed proximally to the ileo-caecal valve of male guinea-pig (250–300 g). Strips of longitudinal muscle with adhering myenteric plexus, 2 cm long and proximal to the ileocaecal junction, were prepared as previously described by Buchheit et al. (1985) and mounted isometrically under a tension of approximately 500 mg in organ baths filled with modified Krebs' Ringer bicarbonate solution (37° C). In all experiments, 5-methoxytryptamine (10 μM) was present for desensitization of 5-HT_4 receptors and methysergide (1 μM) to block 5-HT_1 and 5-HT_2 receptors (Craig et al. 1990). After a stabilization period of 30 min the strips were stimulated three times with 5-HT (3 μM) during a period of 60 min to establish tissue viability. Two non-cumulative concentration/effect curves were constructed at an interval of 60 min on each strip by adding increasing concentrations of 5-HT to the organ bath (0.3–100 μM). Each concentration was left in contact with the strip for 1 min followed by washout with modified Krebs' Ringer bicarbonate solution. The next higher concentration of 5-HT was administered 15 min later. γ-Mangostin (5 μM) was added 10 min before the second concentration/effect curve and remained in the bath fluid during construction of the second curve. The contractions were expressed as a percentage of the maximal contraction produced by 100 μM 5-HT.

Whole segments of ileum (1.5 cm long) from guinea-pigs were mounted in organ baths containing modified Krebs' Ringer bicarbonate solution (37° C). After a stabilization period of 30 min, the strips were stimulated 3 times with carbachol (1 μM) over a period of 45 min to establish a constant response. Non-cumulative concentration/response curves for carbachol were established by adding increasing concentrations of carbachol (30 nM–30 μM) to the organ bath at intervals of 20 min. Each concentration was left in contact with the tissue for 1 min. γ-Mangostin (5 μM) was added 10 min before the second concentration/effect curve and was retained in the bath fluid during construction of the second curve. The contractions were expressed as a percentage of the maximal contraction produced by 30 μM carbachol.

Perfusion pressure response of coronary artery, Langendorff method. Hearts were rapidly excised from male Wistar rats (150–200 g) anaesthetized with pentobarbitone sodium (30 mg/kg body weight) and immediately arrested in ice-cold and oxygenated Krebs' Ringer bicarbonate solution as described above. Isolated hearts were quickly mounted on the non-working, non-recirculating Langendorff perfusion apparatus and were perfused retrogradely via the aorta with the Krebs' Ringer bicarbonate solution at a constant 2 ml/min (37° C) (Brandes et al. 1993). After 30 min preequilibration, the perfusion pressure response was monitored through a branch of the aortic cannula by means of a pressure transducer. Treatment of the perfused hearts with 5-HT, ketanserin or γ-mangostin was carried out by changing from normal Krebs' Ringer solution to one containing each drug.

Platelet aggregation. The preparation of platelet-rich plasma was performed as described by Hsieh et al. (1994). Fresh blood was obtained from rabbits (male albino rabbits weighing about 2–3 kg), collected into plastic tubes containing trisodium citrate glucose solution (1/10 volume of blood) composed of trisodium citrate (3.2%), and glucose (2%) at pH 7.4, subsequently centrifuged at $250 \times g$ for 10 min to yield platelet-rich plasma (PRP). A portion of PRP was centrifuged at $2{,}000 \times g$ for 10 min at room temperature (20–25° C) to obtain platelet-poor plasma (PPP). PPP was used to adjust PRP to a final platelet concentration of 5×10^8 cells/ml.

Platelet aggregation was determined by a standard turbidometric method (Born 1962; Hsieh et al. 1994) in aggregometer (PAM-6C, Merbanix, Tokyo, Japan). Platelet aggregation was expressed as an increase in light transmission. The levels of light transmission were calibrated as 0% for PRP and 100% for PPP. PRP (0.3 ml) in the aggregometer cuvette was preincubated for at least 5 min at 37° C under continuous stirring at 1,000 r.p.m. and then $CaCl_2$ added to a final concentration of 1 mM. After 1 min, γ-mangostin or vehicle (3 μl) was added 5 min before addition of 5-HT. 5-HT solutions were added to make final concentration 1 μM followed 1 min later by addition of a subthreshold concentration of ADP (1 μM). Thrombin (0.1 U/ml) was added 5 min after addition of γ-mangostin as a control experiment. Data are expressed as the area under the curve for the aggregation occurring during 3 min.

γ-Mangostin was dissolved in ethanol and addition of 3 μl of this solution to PRP did not alter the pH or platelet aggregation of the PRP. ADP solutions were prepared by reconstituting the ADP reagent with deionized water to yield a solution containing 1 μM ADP. 5-HT solutions were prepared with PBS (phosphate buffered saline) solution.

Receptor binding. Vascular smooth muscle cells (VSMC) were isolated from the thoracic aortae of Wistar rats by enzymatic dispersion (Chamley et al. 1977). The resulting cells were seeded in 18-mm culture dishes and incubated in a 95% air, 5% CO_2 humidified atmosphere at 37° C. 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 mg/ml streptomycin. Culture medium was then removed every other day and VSMC subcultured by treatment with 0.05% trypsin,

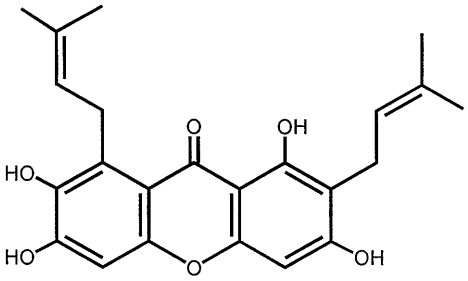

Fig. 1 The chemical structure of γ-mangostin

0.02% EDTA. For binding experiments, VSMC were seeded on 24-well cluster plates (2×10^4 cells/well) and grown to confluence (6–8 days after plating; $2–3 \times 10^5$ cells/well). The culture medium was removed and confluent VSMC (2×10^5 cells) were washed twice with 1 ml ice-cold balanced salt solution (BSS) containing (mM): NaCl 146; KCl 4; $MgCl_2$ 2; $CaCl_2$ 0.5; glucose 10; bovine serum albumin 0.1% and HEPES 10 (adjusted to pH 7.4 with TRIS base). Incubations were carried out in a total 250-µl volume of BSS containing [^{3}H]spiperone (0–30 nM) in the absence or presence of γ-mangostin (0–10 nM). Triplicate incubations were carried out at 20° C for 60 min and were terminated by the addition of 1.7 ml ice-cold BSS. Cells were then rapidly washed 5 times with 1.7 ml ice-cold BSS followed by solubilization of the cells, whereafter cell-bound radioactivity was measured by scintillation counting. Nonspecific binding of [^{3}H]spiperone was defined as the total binding measured in the presence of 10 µM ketanserin. Protein concentration was determined by the method of Bradford (1976), using bovine serum albumin as a standard.

Chemicals. The following drugs were used; 5-hydroxytryptamine creatinine sulphate complex, phosphate buffered saline tablets, carbamylcholine chloride (carbachol) and bovine serum albumin (Sigma, St. Louis, Mo., USA), phenylephrine (Wako, Osaka, Japan), ketanserin tartrate and methysergide maleate (Research Biochemicals Incorporated, Mass., USA), DMEM (Nissui Pharmaceutical, Tokyo, Japan), streptomycin sulphate (Meiji Seika, Tokyo, Japan), penicillin (Banyu Pharmaceutical, Tokyo, Japan), fetal calf serum (JRH Biosciences Lenexa, Kansans, USA), 5-methoxytryptamine (Research Organics, Ohio, USA) and ADP disodium salt (Oriental Yeast, Tokyo, Japan). [^{3}H]Spiperone (15 Ci/mmol) was purchased from Du Pont New England Nuclear (Boston, Mass., USA). γ-Mangostin (Fig. 1) was obtained from the fruit hull of *G. mangostana* L. as previously reported (Jefferson et al. 1970). The fruit hull of *G. mangostana* L. was crushed and soaked in methanol. The methanol extract was purified by silica gel chromatography to give γ-mangostin. γ-Mangostin was dissolved in dimethyl sulphoxide of which the final concentration was kept below 1% (v/v) in all experiments. In the control experiments, dimethyl sulphoxide was added instead of the solution of γ-mangostin to minimize the effect of the vehicle solvent. Other chemicals or drugs were of reagent grade or of the highest quality available.

Data analysis. Data are presented as means ± SEM. Statistical analyses were done by means of Student's *t*-test. A *P* value of less than 0.05 was considered a significant difference.

Results

Effects of γ-mangostin on the mechanical responses of the rabbit aorta

5-HT (1–1000 µM) caused concentration-dependent contraction in the rabbit aorta with a pD_2 of 5.7 (n = 8) (Fig. 2 A). The contractile response to 5-HT was antagonized concentration dependently by γ-mangostin (0.03–0.3 µM) without depression of the maximal response. The Schild plot of the data revealed the pA_2 value to be 8.2 and the slope of the regression line (0.998) was not significantly

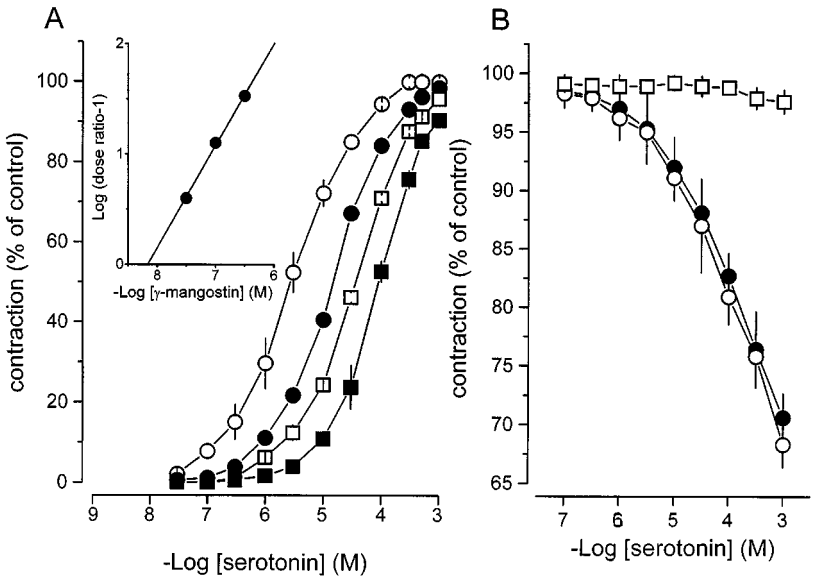

Fig. 2 A, B Effects of γ-mangostin on the mechanical responses to 5-HT in the rabbit aorta. **A** Concentration/response curves to 5-HT-induced contraction in the absence (○) and presence of γ-mangostin (● 0.03, □ 0.1, ■ 0.3 µM). γ-Mangostin was added 10 min before the stimulation by 5-HT. *Inset*, Schild plot for the antagonism of 5-HT by γ-mangostin. **B** Concentration/response curves to 5-HT-induced relaxation in the absence (○) and presence of γ-mangostin (●, 5 µM) or methysergide (□, 1 µM). Tissues were treated with ketanserin (1 µM) for 10 min and then precontracted with prostaglandin $F_{2\alpha}$ (10 µM). After contraction had stabilized, 5-HT-induced relaxation was obtained. The response to 5-HT is expressed as a percentage of the contraction induced by prostaglandin $F_{2\alpha}$ (10 µM). µ-Mangostin or methysergide was added 10 min before the addition of ketanserin. Each *point* is the mean of at least eight experiments and *vertical bars* are SEM

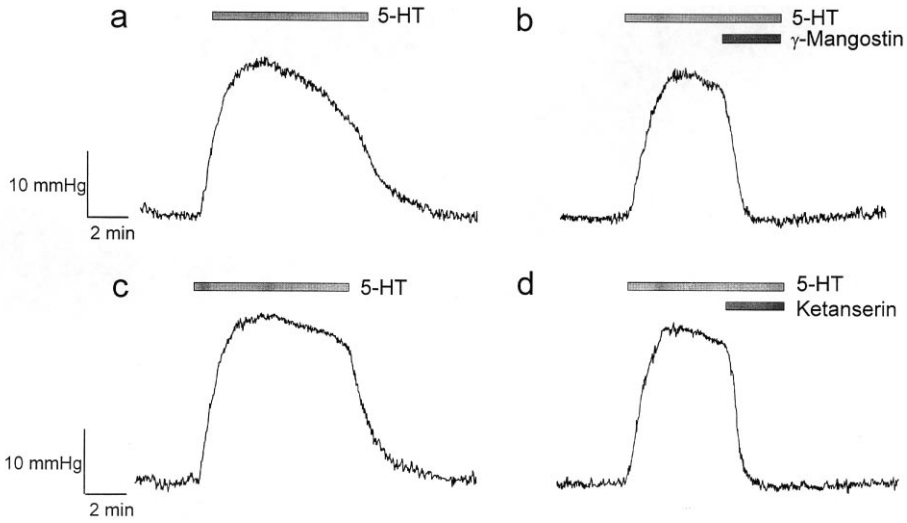

Fig. 3 a–d Typical recordings of the effects of 5-HT, γ-mangostin and ketanserin on perfusion pressure of isolated rat hearts mounted on the Langendorff perfusion apparatus. Isolated rat hearts were perfused with Krebs' Ringer bicarbonate buffer via the aorta for a 30-min preequilibration at a constant 2 ml/min. After stabilizing the perfusion pressure, 10 μM 5-HT was applied for 8 min (**a**, **c**) and then 5-HT was removed by perfusing with fresh buffer. After a 30-min-interval, 10 μM 5-HT was reapplied followed by the addition of 1 μM γ-mangostin (**b**) or 0.1 μM ketanserin (**d**)

different from unity, indicating that the affinity of γ-mangostin for the 5-HT_{2A} receptor was almost same as that of ketanserin, a potent 5-HT_{2A} receptor antagonist (pA_2 = 8.7) (Rinaldi-Carmona et al. 1992). On the other hand, 5-HT caused concentration-dependent relaxation of the aorta precontracted by prostaglandin $F_{2\alpha}$ (10 μM) in the presence of 1 μM ketanserin (Fig. 2 B). Methysergide (1 μM) which blocks the 5-HT_1 and 5-HT_2 receptors abolished the relaxation, but γ-mangostin (5 μM) did not affect it. Furthermore, the concentration/response curves for KCl (5–300 mM, a pD_2 value of 1.64), phenylephrine (10 nM–30 μM, a pD_2 value of 6.64) and histamine (0.1 μM–400 μM, a pD_2 value of 5.51) were unaffected by γ-mangostin (5 μM).

Effect of γ-mangostin on the pressure response of the coronary artery in Langendorff-perfused rat hearts

To investigate the effect of γ-mangostin on the coronary artery, isolated rat hearts mounted on the Langendorff perfusion apparatus were perfused retrogradely through the aorta at constant flow and the pressure response of coronary artery monitored (Fig. 3). A flow rate of 2 ml/min produced a perfusion pressure of 54 ± 8 mmHg (n = 4), which was monitored through a branch of the aortic cannula. Perfusion with buffer containing 5-HT (10 μM) caused an increase in the pressure up to 79 ± 13 mmHg (n = 4) (Fig. 3 A, traces a and c). The increase in pressure was abolished not only by γ-mangostin (1 μM) (trace b) but also by ketanserin (0.1 μM) (trace d). The IC_{50} value for γ-mangostin and ketanserin were 0.32 and 0.04 μM respectively (Fig. 4).

Effects of γ-mangostin on the mechanical responses of the rat stomach fundus and guinea-pig ileum

γ-Mangostin antagonized neither the contractile response produced by 5-HT in the rat stomach fundus (pD_2 value of 8.01) nor that in the guinea-pig ileum (pD_2 value of 5.82)

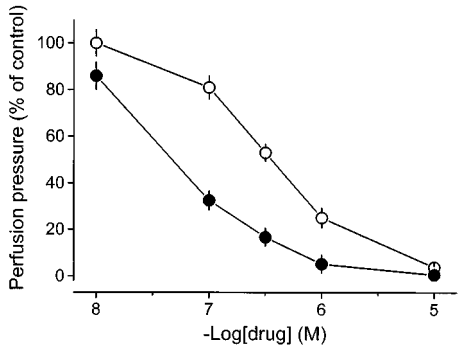

Fig. 4 Concentration-dependent effects of γ-mangostin and ketanserin on the 5-HT-induced increase in the perfusion pressure of isolated rat hearts mounted on the Langendorff perfusion apparatus. Various concentrations of γ-mangostin (○) or ketanserin (●) were applied 10 min before addition of 5-HT (10 μM). 5-HT-induced increase in the perfusion pressure in the absence of γ-mangostin was taken as 100% (control). Each *point* is the mean of at least four experiments and *vertical bars* are SEM

at a concentration as high as 5 μM. Furthermore, carbachol-induced contraction of the ileum (pD_2 value of 6.01) was not affected by 5 μM γ-mangostin.

Effect of γ-mangostin on the aggregation of rabbit platelets

5-HT can amplify the platelet aggregation response to several aggregation agents including ADP. As shown in Fig. 5, γ-mangostin induced a concentration-dependent inhibition of the platelet aggregation produced by the combination of 5-HT (1 μM) and ADP (1 μM) (IC_{50} = 0.29 μM). However, γ-mangostin (up to 1 μM) did not affect thrombin (0.1 U/ml)-induced platelet aggregation.

Effect of γ-mangostin on [^{3}H]spiperone binding to cultured rat aortic smooth muscle cells

γ-Mangostin inhibited 5-HT_{2A} receptors in both platelets and vascular smooth muscles. Receptor-binding analysis

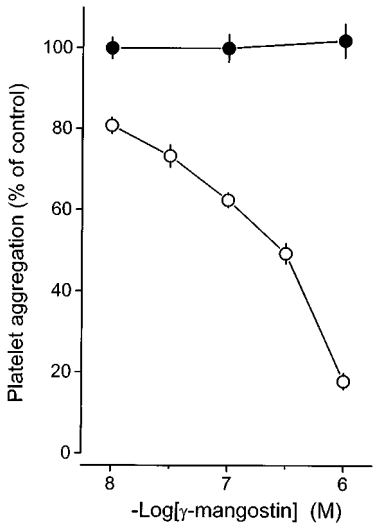

Fig. 5 Effects of γ-mangostin on the 5-HT-amplified, ADP-induced and on the thrombin-induced platelet aggregation. γ-Mangostin was added 10 min before addition of 5-HT (1 μM) or thrombin (0.1 U/ml) in the presence of 1 mM Ca^{2+}. ADP (1 μM)-induced aggregation in the presence of 5-HT (○) and thrombin-induced one (●) were determined. Platelet aggregation in the absence of γ-mangostin was taken as 100% (control). Each *point* is the mean of at least four experiments and *vertical bars* are SEM

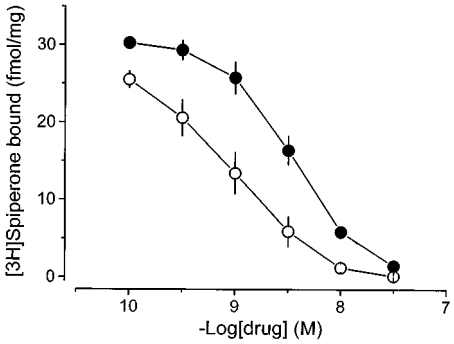

Fig. 6 Concentration/inhibition curve for γ-mangostin and ketanserin in [^{3}H]spiperone binding to rat aortic smooth muscle cells. Cultured rat aortic smooth muscle cells were incubated with 1 nM [^{3}H]spiperone for 60 min at 20° C. Total binding was measured in the presence of ketanserin (○) or γ-mangostin (●). Nonspecific binding in the presence of 10 μM ketanserin has been subtracted from the results. Specific binding in the absence of these drugs was 31.5 ± 2.1 fmol/mg protein. Each *point* is the mean of at least three experiments and *vertical bars* are SEM

was then carried out to investigate the interaction of γ-mangostin with 5-HT_{2A} receptors. Figure 6 illustrates the effects of ketanserin and γ-mangostin on the specific binding of [^{3}H]spiperone to rat aortic smooth muscle cells. Ketanserin inhibited concentration dependently the [^{3}H]spiperone binding (IC_{50} = 0.71 nM). γ-Mangostin also inhibited the binding with an IC_{50} of 3.5 nM. Although γ-mangostin is less potent than ketanserin, the slope of the binding inhibition curve for γ-mangostin seemed to be steeper than that for ketanserin. The dependence of [^{3}H]spiperone binding on free [^{3}H]spiperone concentrations in the presence or absence of γ-mangostin (3 nM) is illustrated in

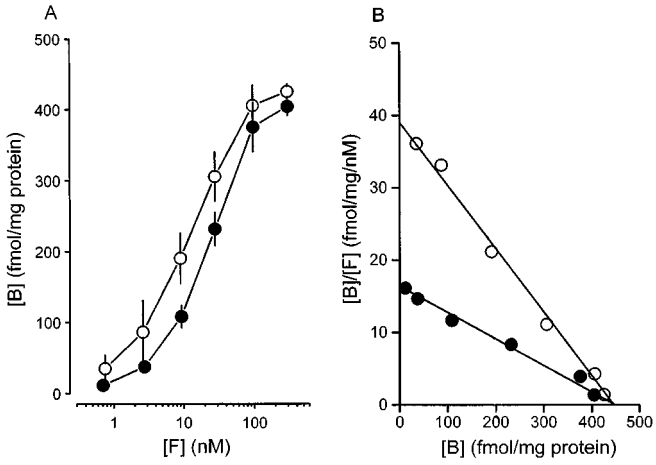

Fig. 7 A, B Binding of [^{3}H]spiperone to rat aortic smooth muscle cells in the presence or absence of γ-mangostin. Cultured rat aortic smooth muscle cells were incubated with [^{3}H]spiperone (1 to 30 nM) for 60 min at 20° C. Nonspecific binding in the presence of 10 μM ketanserin has been subtracted from the results. Each *point* is the mean of at least three experiments and vertical bars are SEM. **A** [^{3}H]Spiperone binding [*B*] was measured in the absence (○) or presence of 3 nM γ-mangostin (●) and are shown as a function of the free [^{3}H]spiperone concentration [*F*]. **B** Scatchard plot of a representative experiment of [^{3}H]Spiperone binding in the presence (●) or absence (corc) of 3 nM γ-mangostin

Fig. 7 A. Specific binding of [^{3}H]spiperone to the cells was saturable. Scatchard analysis shows that [^{3}H]spiperone bound to a receptor site with a K_d of 11.7 nM and a B_{max} of 447 fmol/mg (Fig. 7 B). γ-Mangostin (3 nM) increased the K_d to 27.4 nM without affecting B_{max}. These results clearly indicate that γ-mangostin competitively inhibited [^{3}H]spiperone binding to the 5-HT_{2A} receptor.

Discussion

Both of 5-HT-induced contraction of vascular smooth muscle such as the rabbit aorta and 5-HT-amplified, ADP-induced platelet aggregation are reportedly mediated by the activation of 5-HT_{2A} receptors (Clancy and Maayani 1985; Feniuk et al. 1985; De Clerck et al. 1984; Cook et al. 1994). The activity of γ-mangostin on 5-HT receptors was assessed by measuring its ability to block the mechanical/responses of smooth muscles. In the rabbit aorta, γ-mangostin caused concentration-dependent inhibition of 5-HT-induced contraction without affecting contractions induced by KCl, phenylephrine or histamine. γ-Mangostin had no effect on the carbachol-induced contraction of the guinea-pig ileum. In the isolated rat heart, the perfusion pressure, an indicator of the contractile state of coronary artery, was markedly increased by 5-HT and the increase was diminished in the presence of γ-mangostin or ketanserin (5-HT_{2A} antagonist). In addition to the ability of γ-mangostin to inhibit contractile response of vascular bed to 5-HT, γ-mangostin blocked the 5-HT-amplified, ADP-induced aggregation of the rabbit platelets without affecting the thrombin-induced aggregation. Furthermore, the

binding of [^{3}H]spiperone, a potent 5-HT_{2A} antagonist, to rat aortic smooth muscle cells was inhibited by γ-mangostin. These results suggest that γ-mangostin blocks the 5-HT_{2A} receptor without affecting other receptors such as histamine H_1, α_1-adrenergic and muscarinic M_3 acetylcholine receptors in smooth muscles and platelets.

To examine the selectivity of γ-mangostin in the 5-HT receptor subtypes, we investigated the effects of γ-mangostin on 5-HT-induced contraction of the rat fundus or guinea-pig ileum and the 5-HT-induced relaxation of the rabbit aorta precontracted by prostaglandin $F_{2\alpha}$. The receptor mediating the contractile response to 5-HT in the rat fundus is classified as the 5-HT_{2B} receptor (Baxter et al. 1994; Wainscott et al. 1996). The guinea-pig ileum has several subtypes of 5-HT receptors including 5-HT_3 receptors (Eglen et al. 1992; Ramirez et al. 1994). The effect of γ-mangostin on the 5-HT_3 receptor in the guinea-pig ileum was therefore investigated in the presence of blockers for 5-HT_1, 5HT_2 and 5-HT_4 receptors (5-methoxytryptamine and methysergide). 5-HT caused the relaxation of the aorta via a 5-HT_1 receptor in the presence of ketanserin (Fig. 2). γ-Mangostin affected neither the contractile response of the fundus via the 5-HT_{2B} receptor nor the ileum via the 5-HT_3 receptor nor the relaxation of the aorta via the 5-HT_1 receptor. γ-Mangostin was thus less potent at other subtypes of 5-HT receptors, apart from 5-HT_{2A}. On the basis of these results, it is suggested that γ-mangostin is a potent and selective 5-HT_{2A} receptor antagonist.

We investigated the effect of γ-mangostin on the binding of [^{3}H]spiperone to 5-HT_{2A} receptors in cultured rat aortic smooth muscle cells, because [^{3}H]spiperone has been used extensively to analyse the properties of 5-HT_{2A} receptors (Sleight et al. 1996; Abbott et al. 1996). γ-Mangostin caused concentration-dependent inhibition of the binding of [^{3}H]spiperone to the 5-HT_{2A} receptors with an IC_{50} of 3.5 nM. Scatchard plot analysis of the [^{3}H]spiperone binding showed that γ-mangostin increased the K_d value without affecting B_{max}. These results indicate a competitive mode of inhibition by γ-mangostin. However, we could not exclude the possibility of cooperativity at the γ-mangostin binding sites, because the slope of the binding inhibition curve for γ-mangostin seemed to be steeper than expected from simple competition. Detailed analysis of the mechanism of inhibition by γ-mangostin is in progress.

Many 5-HT receptor antagonists with a nitrogen atom in the molecule have been synthesized, suggesting that nitrogen atom is essential for the 5-HT receptor blocking activity (Hoyer et al. 1994). It is thus significant that γ-mangostin has no nitrogen atom but possesses remarkable 5-HT_{2A} receptor blocking activity. γ-Mangostin (pA_2 = 8.2) and ketanserin (pA_2 = 8.7) (Rinaldi-Carmona et al. 1992) have similar affinities for the 5-HT_{2A} receptor in the rabbit aorta. Whereas ketanserin not only blocks 5-HT_{2A} receptors but also α_1-adrenergic receptors (Balasubramaniam et al. 1993), γ-mangostin blocked the 5-HT_{2A} receptor without affecting the α_1-adrenergic receptor. Therefore, γ-mangostin is probably superior to ketanserin as selective 5-HT_{2A} receptor antagonist. We have succeeded in identifying γ-mangostin as a lead for the development of 5-HT_{2A} receptor antagonists lacking a nitrogen atom.

In conclusion, γ-mangostin is a promising lead compound for the development of a novel type of competitive antagonists for 5-HT_{2A} receptors in vascular smooth muscle cells and platelets.

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References

Abbott FV, Hong Y, Blier P (1996) Activation of 5-HT_{2A} receptors potentiates pain produced by inflamatory mediators. Neurophamacology 35:99–110

Alper RH (1990) Hemodynamic and renin responses to (±)-DOI, a selective 5-HT_2 receptor agonist, in conscious rats. Eur J Pharmacol 175:323–332

Balasubramaniam G, Lee HS, Mah SC (1993) Differences in the acute and chronic antihypertensive mechanism of action of ketanserin in spontaneously hypertensive rats. J Pharmacol Exp Ther 264:129

Baxter GS, Murphy OE, Blackburn TP (1994) Further characterization of 5-hydroxytryptamine receptors (putative 5-HT_{2B}) in rat stomach fundus longitudinal muscle. Br J Pharmacol 112:323–331

Born GVR (1962) Aggregation of blood platelets by adenosine diphosphate and its reversal. Nature 194:927–929

Bradford MM (1976) A rapid and sensitive method for the quantitation of microgam quantities of protein utilizing the principle of protein-dye binding. Anal Biochem 72:248–254

Brandes R, Figueredo VM, Camacho SA, Baker AJ, Weiner MW (1993) Quantitation of cytosolic [Ca^{2+}] in whole perfused rat hearts using indo-1 fluorometry. Biophys J 65:1973–1982

Buchheit K-H, Engel G, Mutschler E, Richardson B (1985) Study of the contractile effect of 5-hydroxytryptamine (5-HT), in the isolated longitudinal muscle strip from guinea-pig ileum. Evidence for two distinct release mechanisms. Naunyn-Schmiedeberg's Arch Pharmacol 329:36–41

Chairungsrilerd N, Furukawa K-I, Ohta T, Nozoe S, Ohizumi Y (1996a) Histaminergic and serotonergic receptor blocking substances from the medicinal plant Garcinia mangostana. Planta Med 62:471–472

Chairungsrilerd N, Furukawa K-I, Ohta T, Nozoe S, Ohizumi Y (1996b) Pharmacological properties of α-mangostin, a novel histamine H_1 receptor antagonist. Eur J Pharmacol 314:351–356

Chamley JH, Campbell JD, McConnell GR, Gröschel-Stewart U (1977) Comparison of vascular smooth muscle cells from adult human, monkey and rabbit in primary culture and in subculture. Cell Tissue Res 177:503–522

Clancy BM, Maayani S (1985) 5-Hydroxytryptamine receptor in isolated rabbit aorta: characterization with tryptamine analogs. J Pharmacol Exp Ther 233:761–769

Cohen ML, Wittenauer LA (1987) Serotonin receptor activation of phosphoinositide turnover in uterine, fundal, vascular, and tracheal smooth muscle. J Cardiovasc Pharmacol 10:176–181

Cohen, ML, Fuller RW, Wiley KS (1981) Evidence for 5-HT_2 receptors mediating contraction in vascular smooth muscle. J Pharmacol Exp Ther 218:421–425

Cohen ML, Mason N, Wiley KS, Fuller RW (1983) Further evidence that vascular serotonin receptors are of the 5-HT_2 type. Biochem Pharmacol 32 : 567–570

Cook EH Jr, Fletcher KE, Wainwright M, Marks N, Yan SY, Leventhal BL (1994) Primary structure of the human platelet serotonin 5-HT_{2A} receptor: identify with frontal cortex serotonin 5-HT_{2A} receptor. J Neurochem 63 : 465–469

Craig DA, Eglen RM, Walsh LKM, Perkins LA, Whiting RL, Clarke DE (1990) 5-Methoxytryptamine and 2-methyl-5-hydroxytryptamine-induced desensitization as a discriminative tool for the 5-HT_3 and putative 5-HT_4 receptors in guinea-pig ileum. Naunyn-Schmiedeberg's Arch Pharmacol 342 : 9–16

De Clerck F, Xhonneux B, Leysen J, Janssen PA (1984) Evidence for functional 5-HT_2 receptor sites on human blood platelets. Biochem Pharmacol 33 : 2807–2811

Doble A, GD, PO, Allam D, Betschart J, Boireau A, Dupuy A, Gueremy C, Menager J, Zundel JL, Blanchard JC (1992) Pharmacological characterization of RP 62203, a novel 5-hydroxytryptamine 5-HT_2 receptor antagonist. Br J Pharmacol 105 : 27–36

Eglen RM, Perkins LA, Walsh LK, Whiting RL (1992) Agonist action of indole derivatives at 5-HT_1-like, 5-HT_3, and 5-HT_4 receptors in vitro. J Auton Pharmacol 12 : 321–333

Feniuk W, Humphrey PP, Perren MJ, Watts AD (1985) A comparison of 5-hydroxytryptamine receptors mediating contraction in rabbit aorta and dog saphenous vein: evidence for different receptor types obtained by use of selective agonists and antagonists. Br J Pharmacol 86 : 697–704

Fuller RW (1996) Mechanisms and functions of serotonin neuronal systems: opportunities for neuropeptide interactions. Ann NY Acad Sci 780 : 176–184

Furukawa K-I, Komaba J, Sakai H, Ohizumi Y (1996) The mechanism of acidic pH-induced contraction in aortae from SHR and WKY rats enhanced by increasing blood pressure. Br J Pharmacol 118 : 485–492

Hoyer D, Clarke DE, Fozard JR, Hartig PR, Martin GR, Mylecharane EJ, Saxena PR, Humphrey PPA (1994) VII. International union of pharmacology classification of receptors for 5-hydroxytryptamine (serotonin). Pharmacol Rev 46 : 157–203

Hsieh CP, Sakai K, Bruns GC, Dage RC (1994) Effects of MDL 28,133A, a 5-HT_2 receptor antagonist, on platelet aggregation and coronary thrombosis in dogs. J Cardiovasc Pharmacol 24 : 761–772

Jefferson A, Quillinan J, Scheinmann F, Sim KY (1970) Studies in the xanthone series. Aust J Chem 23 : 2539–2543

Leysen J, Gommeren W, De Clerck F (1983) Demonstration of S_2-receptor binding sites on cat blood platelets using [^{3}H]ketanserin. Eur J Pharmacol 88 : 125–129

Lucas JJ, Hen R (1996) New players in the 5-HT receptor field: gene and knockouts. Trends Pharmacol Sci 16 : 246–252

DS, Wasserman GA, Coplan J, Fried JA, Huang YY, Kassir S, Greenhill L, Shaffer D, Parsons B (1996) Platelet serotonin 2A (5-HT_{2A}) receptor characteristics and parenting factors for boys at risk for delinquency. Am J Psych 153 : 538–544

Pletscher A, Affolter H (1983) The 5-hydroxytryptamine receptor of blood platelets. J Neural Transm 57 : 233–242

Ramirez MJ, Del Rio J, Cenarruzabeitia E, Lasheras B (1994) On the nature of the 5-HT receptor subtype inhibiting acetylcholine release in the guinea-pig ileum. Br J Pharmacol 113 : 77–80

Rinaldi-Carmona M, Congy C, Santucci V, Simiand J, Gautret B, Neliat G, Labeeuw B, Le Fur G, Soubrie P, Breliere JC (1992) Biochemical and pharmacological properties of SR 46349B, a new potent and selective 5-hydroxytryptamine2 receptor antagonist. J Pharmacol Exp Ther 262 : 759–768

Sleight AJ, Stam NJ, Mutel V, Vanderheyden PM (1996) Radiolabelling of the human 5-HT_{2A} receptor with an agonist, a partial agonist and an antagonist: effects on apparent agonist affinities. Biochem Pharmacol 51 : 71–6

Ullmer C, Schmuck K, Kalkman HO, Lubbert H (1995) Expression of serotonin receptor mRNAs in blood vessels. FEBS Lett 370 : 215–221

Wainscott DB, Lucaites VL, Kursar JD, Baez M, Nelson DL (1996) Pharmacological characterization of the human 5-hydroxytryptamine$_{2B}$ receptor: evidence for species differences. J Pharmacol Exp Ther 276 : 720–727

Zifa E, Fillion G (1992) 5-Hydroxytryptamine receptors. Pharmacol Rev 44 : 401–458