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Effect of γ-mangostin through the inhibition of 5-hydroxytryptamine$_{2A}$ receptors in 5-fluoro-α-methyltryptamine-induced head-twitch responses of mice

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1 Intracerebronventricular (i.c.v.) injection of γ-mangostin (10–40 nmol/mouse), a major compound of the fruit hull of *Garcinia mangostana* Lin., like ketanserin (10, 20 nmol/mouse, i.c.v.) inhibited 5-fluoro-α-methyltryptamine (5-FMT) (45 mg kg^{-1}, i.p.)-induced head-twitch response in mice in the presence or absence of citalopram (a 5-hydroxytryptamine (5-HT)-uptake inhibitor).

2 Neither the 5-FMT- nor the 8-hydroxy-2-(di-n-propylamino)tetralin (5-HT$_{1A}$-agonist)-induced 5-HT syndrome (head weaving and hindlimb abduction) was affected by γ-mangostin or ketanserin.

3 The locomotor activity stimulated by 5-FMT through the activation of α_1-adrenoceptors did not alter in the presence of γ-mangostin.

4 5-HT-induced inositol phosphates accumulation in mouse brain slices was abolished by ketanserin. γ-Mangostin caused a concentration-dependent inhibition of the inositol phosphates accumulation.

5 γ-Mangostin caused a concentration-dependent inhibition of the binding of [^{3}H]-spiperone, a specific 5-HT$_{2A}$ receptor antagonist, to mouse brain membranes.

6 Kinetic analysis of the [^{3}H]-spiperone binding revealed that γ-mangostin increased the K_d value without affecting the B_{max} value, indicating the mode of the competitive nature of the inhibition by γ-mangostin.

7 These results suggest that γ-mangostin inhibits 5-FMT-induced head-twitch response in mice by blocking 5-HT$_{2A}$ receptors not by blocking the release of 5-HT from the central neurone. γ-Mangostin is a promising 5-HT$_{2A}$ receptor antagonist in the central nervous system.

Keywords: γ-Mangostin; 5-HT$_2$ receptor antagonist; behavioural pharmacology; head-twitch response; 5-FMT; mouse brain

Introduction

5-Hydroxytryptamine (5-HT) is a neurotransmitter which plays an important role in numerous behavioural systems. The recent identification of various 5-HT receptor subtypes (Gozlan *et al*., 1983; Peroutka, 1988; Middlemiss & Tricklebank, 1992; Humphrey *et al*., 1993; Hoyer *et al*., 1994), and the increasing availability of specific agonists and antagonists for some of these receptors have made it possible to investigate the role of specific 5-HT receptors in various behaviours (Glennon *et al*., 1991; Murphy *et al*., 1991; Lucki, 1992). Activation of 5-HT$_2$ receptors in the brain results in hyperactivity syndromes such as head-twitch response (HTR), resting tremor, head weaving and hypertonicity. In these behavioural features, HTR and the symptoms of the 5-HT syndrome have been studied extensively as behavioural models for the activation of 5-HT receptors in the central nervous system (Corne *et al*., 1963; Grahame-Smith, 1971a,b). The 5-HT syndrome consists of a series of complex behavioural symptoms that usually include repetitive treading of the forepaws, abduction of the hindlimbs, Straub's tail and resting tremor (Grahame-Smith, 1971a). The HTR and 5-HT syndrome can be produced in mice and rats by the administration of the 5-HT precursors, tryptophan or 5-hydroxytryptophan (5-HTP) (Corne *et al*., 1963; Grahame-Smith, 1971a), by the administration of 5-HT receptor agonists that directly activate 5-HT receptors such as 5-methoxy-N,N-dimethyltryptamine or D-lysergic acid diethylamide (LSD) (Anden *et al*., 1968; Grahame-Smith, 1971b; Trulson & Jacobs, 1976) and by drugs that release 5-HT like fenfluramine (Trulson & Jacobs, 1976; Mathews & Smith, 1980). It has been found that the two behavioural models of 5-HT receptor activation are associated with different types of 5-HT receptor. The HTR in mice is closely associated with the function of 5-HT$_2$ receptors on postsynaptic neurones (Green *et al*., 1983; Goodwin & Green, 1985; Godfrey *et al*., 1988), whereas the 5-HT syndrome is associated with postsynaptic 5-HT$_1$ receptors (Lucki *et al*., 1984). The 5-HT$_2$ receptors consists of three different subtypes (5-HT$_{2A}$, 5-HT$_{2B}$ and 5-HT$_{2C}$). The HTR in mice or rats is considered as a specific behavioural model for the activation of 5-HT$_{2A}$ receptors (Tadano *et al*., 1995; Schreiber *et al*., 1995). It has been shown that intraperitoneal administration of 5-fluoro-α-methyltryptamine (5-FMT) induces the head-twitches by the activation of central 5-HT neurones (Kim *et al*., 1991; Tadano *et al*., 1995). Monoamine oxidase-A inhibition by 5-FMT may cause an increase in intracellular levels of 5-HT followed by an increase in 5-HT release. This probably results in increased stimulation of postsynaptic 5-HT$_2$ receptors (Tadano *et al*., 1995). These findings indicate that 5-FMT-treated animals can be used to test for effects of 5-HT$_2$ receptor antagonists acting on the postsynaptic 5-HT$_2$ receptors.

In order to discover new types of 5-hydroxytryptamine (5-HT) antagonists, we have devoted our attention to investigating naturally occurring compounds having anti-5-HT activity *in vitro*. Recently, γ-mangostin [1,3,6,7-tetrahydroxy-2,8-bis(3-methyl-2-butenyl)-9*H*-xanthen-9-one] (Figure 1) from the fruit hull of *Garcinia mangostana* L. has been shown to be a selective antagonist for 5-HT$_{2A}$ receptors in smooth muscle and

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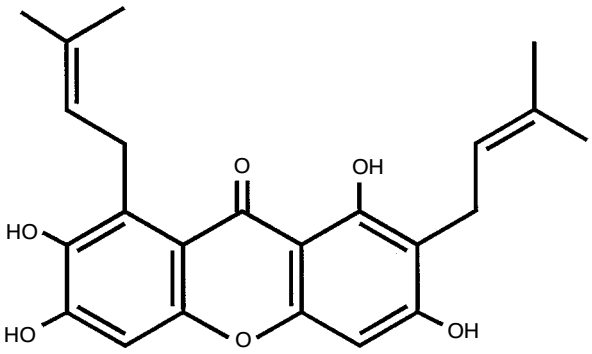

Figure 1 Chemical structure of γ-mangostin.

platelets (Chairungsrilerd *et al*., 1996). It is of interest that γ-mangostin, which does not have a nitrogen atom, possesses marked 5-HT_{2A} receptor blocking activity. The present study was undertaken to investigate the effects of γ-mangostin on central 5-HT receptors by using animal behavioural models, 5-HT-induced inositol phosphates accumulation in mouse brain slices and reduced [^{3}H]-spiperone binding to brain membranes. This is the first study to show an inhibitory action of γ-mangostin on the 5-HT_{2A} receptors in central nervous system

Methods

Animals

Adult male ddY mice weighing 22–25 g were used. They were housed in plastic cages with free access to food and water, except during observation of the HTR and 5-HT syndrome. The mice were observed in individual plastic cages ($2.5 \times 18 \times 13$ cm) immediately after intraperitoneal (i.p.) injection of 5-FMT, after pretreatment with either vehicle, γ-mangostin or ketanserin. The temperature of the room in which the animals were housed, treated and the HTR or 5-HT syndrome observed was maintained at 23 ± 1°C with constant humidity (55 ± 5%). The room was illuminated from 09 h 00 min to 21 h 00 min. All behavioural studies were performed between 10 h 00 min and 17 h 00 min.

Measurements of HTR and 5-HT syndrome

In the experiments on effects of a single administration of the test compounds, γ-mangostin, ketanserin or vehicle was injected intracerebroventricularly (i.c.v., 5 μl/mouse) 15 min before the administration of 5-FMT (25 mg kg^{-1}, i.p.). The technique employed for i.c.v. administration was the same as that originally described by Brittain and Handley (1967). The number of HTR or the 5-HT syndrome (head-weaving; side to side movements of the head, hindlimb abduction; a dramatic splaying out of the hindlimbs) was counted for 2 min at 10 min intervals from 10 to 90 min after the i.p. injection. The total HTR number was counted for 90 min after i.p. injection. γ-Mangostin was dissolved in Ringer solution (NaCl 147 mM, KCl 4.4 mM, $CaCl_2$ 2.7 mM) containing 30% DMSO, ketanserin was dissolved in Ringer solution and 5-FMT was dissolved in saline.

In the experiment in which 5-FMT was combined with citalopram (5-HT reuptake blocker), the mice were first administered 5-FMT (45 mg kg^{-1}, i.p.), and 15 min later citalopram (10 mg kg^{-1}, i.p.) was injected. Test compounds or vehicle were injected i.c.v. 15 min after citalopram. The number of HTR was counted for 2 min at 10 min intervals from 10 to 90 min after the injection of test compounds or vehicle.

*Measurement of head-weaving induced by 8-hydroxy-2-(di-*n*-propylamino)tetralin (8-OHDPAT)*

Each mouse was allowed 5 min in an observation cage before the i.c.v. injection of test compounds or vehicle. Fifteen min after i.c.v. injection of drugs, 8-OHDPAT (in saline) was injected subcutaneously (s.c., 2.5 mg kg^{-1}). The number of individual weaves was counted for 2 min at 10 min intervals from 10 to 90 min after injection of 8-OHDPAT.

Measurement of locomotor activity

Locomotor activity of each mouse was measured by Animex Auto MK-110 (Muromachi Kikai Co., Ltd). Mice were individually placed in the activity cages and the cases were put on Animex activity meters. The mice were allowed to adapt 15 min before the injection of the test compound. For testing compounds, mice were injected i.c.v. with vehicle or drugs (γ-mangostin or ketanserin), and the activity of mice was measured immediately after injection. The number of the activity counts was recorded every 15 min in a 90 min period.

Inositol phosphate accumulation

Slices (350×350 μm) from the frontal cortex of adult male ddY mice (22–25 g) were made by chopping the cortex in perpendicular directions as described previously (Bristow *et al*., 1993). Slices were washed three times by incubation at 37°C in oxygenated Krebs-Ringer buffer solution (composition in mM NaCl 120, KCl 4.7, $CaCl_2$ 1.3, KH_2PO_4 1.2, $MgSO_4$ 1.2, $NaHCO_3$ 25 and glucose 11.7, equilibrated to pH 7.4 with 95% O_2/5% CO_2) for 10 min in a shaking water bath. Inositol phospholipids were labelled by incubation with 3.2 μM *myo*-[^{3}H]-inositol (15 Ci mmol^{-1}) in oxygenated Krebs-Ringer buffer solution for 90 min with continuous shaking. Following incubation, excess *myo*-[^{3}H]-inositol was removed by washing slices three times with 5 ml of Krebs-Ringer solution.

To measure 5-HT stimulated accumulation of [^{3}H]-inositol phosphates, 50 μl of slices (approximately 0.2 mg) was added to 200 μl of Krebs-Ringer buffer solution containing 5-HT (5 μM). After 15 min of incubation at 37°C, stimulation of slices was terminated by the addition of 1 ml of 5% trichloroacetic acid (TCA). It was centrifuged at $2{,}000 \times g$ for 5 min. The supernatant was applied to 1 ml of bed volume of AG 1X-8 (100–200 mesh, formate form) after removal of TCA by washing with 5 ml of diethylether three times. Inositol 1,4,5-phosphate (IP_1) inositol 1,4,5-diphosphate (IP_2) and inositol 1,4,5-trisphosphate (IP_3) were eluted by 0.2, 0.45 and 1 M ammonium formate in 100 mM formic acid, respectively. TCA precipitate was washed with 5% TCA and water, and it was dissolved in 1 ml of 1 N NaOH. After neutralization by 1 ml HCl, the radioactivity of the TCA precipitate was counted as total phosphoinositides labelled. The accumulations of inositol phosphates were expressed as % of the radioactivity of total phosphoinositides or in disintergrations min^{-1} 0.1 mg^{-1} protein.

Measurement of [^{3}H]-spiperone binding

The frontal cortex of adult male ddY mice (22–25 g) was homogenized in buffer A containing 0.32 M sucrose, 10 mM

2-[4-(2-hydroxyethyl)-1-piperazinyl]ethanesulphonic acid (HEPES)/Tris (pH 7.6), 10 mM EGTA and protease inhibitors (100 μM *p*-aminophenylmethylsulphonylfluoride, 0.5 mg ml^{-1} aprotinin, 0.5 mg ml^{-1} leupeptin and 0.83 mM benzamidine). Homogenates were centrifuged at $60 \times g$ for 5 min and supernatants were centrifuged at $100{,}000 \times g$ for 60 min. Pellets were resuspended in buffer A and frozen with liquid nitrogen and stored at −80°C. These procedures were carried out below 4°C.

Binding assays with [^{3}H]-spiperone were done in 500 μl of Tris-HCl buffer (20 mM Tris-HCl (pH 7.4), 120 mM NaCl, 2 mM $MgCl_2$) containing 0.6 mg ml^{-1} membrane protein. Brain membranes were incubated with 0.1–30 nM [^{3}H]-spiperone in the absence or presence of γ-mangostin. Triplicate incubations were carried out at 30°C for 60 min and were terminated by the addition of 4 ml of ice-cold Tris-HCl buffer and rapidly filtered through glass fibre filters (Wattman GF-B, 2.5 cm in diameter) under reduced pressure. The filters were immediately washed 3 times with 4 ml of ice-cold Tris-HCl buffer, placed in a scintillation vial with Tritosol-scintillation cocktail and then membrane-bound radioactivity was measured by a liquid scintillation spectrometer. Non-specific binding of [^{3}H]-spiperone was defined as the total binding measured in the presence of 20 μM ketanserin. Specific binding of the ligand to membranes was estimated by subtracting the non-specific component from the total binding in the absence of ketanserin.

Protein determination

Protein concentration was determined by the protein-dye method of Bradford (1976), with bovine serum albumin as a standard.

Isolation and purification of γ-mangostin

γ-Mangostin was isolated from the fruit hull of *G. mangostana* as described previously (Chairungsrilerd *et al*., 1996). Briefly, 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.

Drugs and chemicals

5-FMT (5-fluoro-α-methyl-tryptamine) hydrochloride (Sigma), citalopram hydrobromide (H. Lundbeck), ketanserin tartrate and 8-OHDPAT (8-hydroxy-2-(di-*n*-propyl-amino)tetralin (Research Biochemicals), [^{3}H]-spiperone and *myo*-[^{3}H]-inositol (15 Ci $mmol^{-1}$) (Du Pont New England Nuclear). γ-Mangostin was dissolved in 30% dimethyl sulphoxide (DMSO) in Ringer solution, ketanserin in Ringer solution, citalopram and 5-FMT were dissolved in saline. Thirty percent DMSO (Ringer solution) was used as the control for γ-mangostin and Ringer solution for ketanserin,

Statistical analysis

Experimental data are expressed as mean ± s.e.mean. The data were analysed by analysis of variance (ANOVA), and the statistical significance of the results was calculated according to Dunnett's tests. In locomotor activity experiments, 2-way split-plot ANOVA was used to estimate the effects of γ-mangostin at each 15 min time point separately. When significantly main effects were found, comparison of the group means at each time point was performed by Dunnett's test.

In receptor binding experiments curve fitting were performed by means of non-linear regression analysis.

Results

Effects of γ-mangostin and ketanserin on 5-FMT-induced HTR

Mice were injected i.c.v. with vehicle or drugs (γ-mangostin or ketanserin) and the HTR to i.p. injection of 5-FMT (45 mg kg^{-1}) was determined, as detailed in the Methods section. Figure 2(a and b) shows that γ-mangostin (10–40 nmol kg^{-1}, i.c.v.) reduced the total number of head-twitches induced by 5-FMT (45 mg kg^{-1}, i.p.) in a dose-dependent manner. Even in the presence of citalopram, a relatively selective 5-HT reuptake inhibitor, γ-mangostin reduced markedly the total number of head-twitches induced by 5-FMT for 90 min (Figure 2c). The inhibitory action of γ-mangostin on 5-FMT-induced head-twitches was the same as ketanserin, a selective 5-HT_{2A} antagonist (10 nmol kg^{-1}) which also blocked the head twitches in the presence or absence of citalopram (Figure 3).

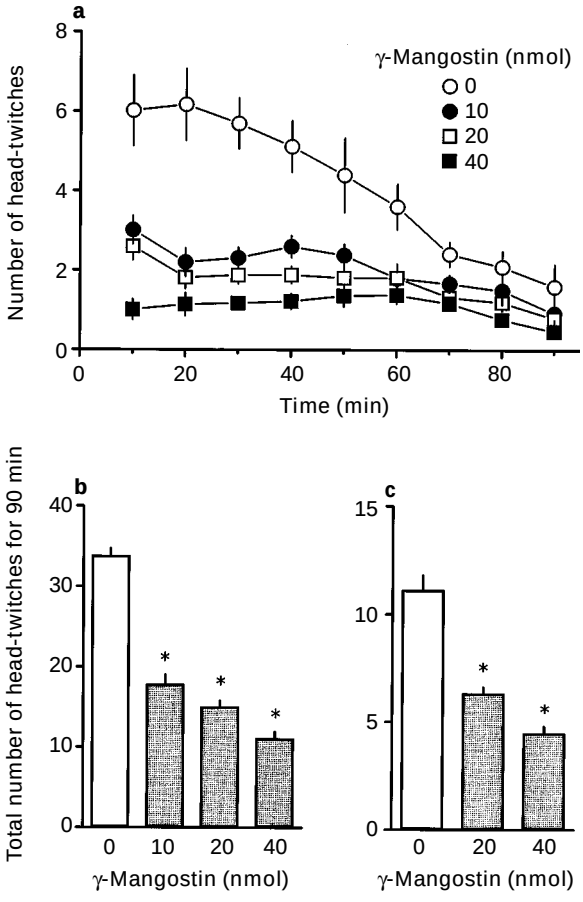

Figure 2 Effect of i.c.v. injection of γ-mangostin on 5-FMT-induced HTR in mice in the absence of (a,b) or presence (c) of citalopram. (a) Each point represents the mean number of head-twitches for 2 min, pretreated with γ-mangostin (10, 20 and 40 nmol). (b) Each column shows the total number of head-twitches induced by 5-FMT counted for 90 min, pretreated with γ-mangostin (10, 20 and 40 nmol). (c) Each column shows the total number of head-twitches induced by 5-FMT in the presence of citalopram (10 mg kg^{-1}, i.p.) counted for 90 min, pretreated with γ-mangostin (20 and 40 nmol). Vertical lines show the s.e.mean ($n = 10$). *$P < 0.01$, different from control (b and c).

Effects of γ-mangostin and ketanserin on 5-FMT-induced 5-HT syndrome and HTR

5-FMT (45 mg kg^{-1}) produced the 5-HT syndrome characterized by head weaving and hindlimb abduction as well as any other agonist or releaser of 5-HT in mice. γ-Mangostin (20 and 40 nmol kg^{-1}, i.c.v.) and ketanserin (10 nmol kg^{-1}, i.c.v.) were examined for their ability to block the appearance of the 5-HT syndrome induced by 5-FMT. As shown in Figures 4 (head-weaving) and 5 (hindlimb abduction), in contrast to head-twitches, 5-FMT-induced 5-HT syndrome was not affected by γ-mangostin and ketanserin up to doses as high as 40 and 10 nmol kg^{-}, respectively.

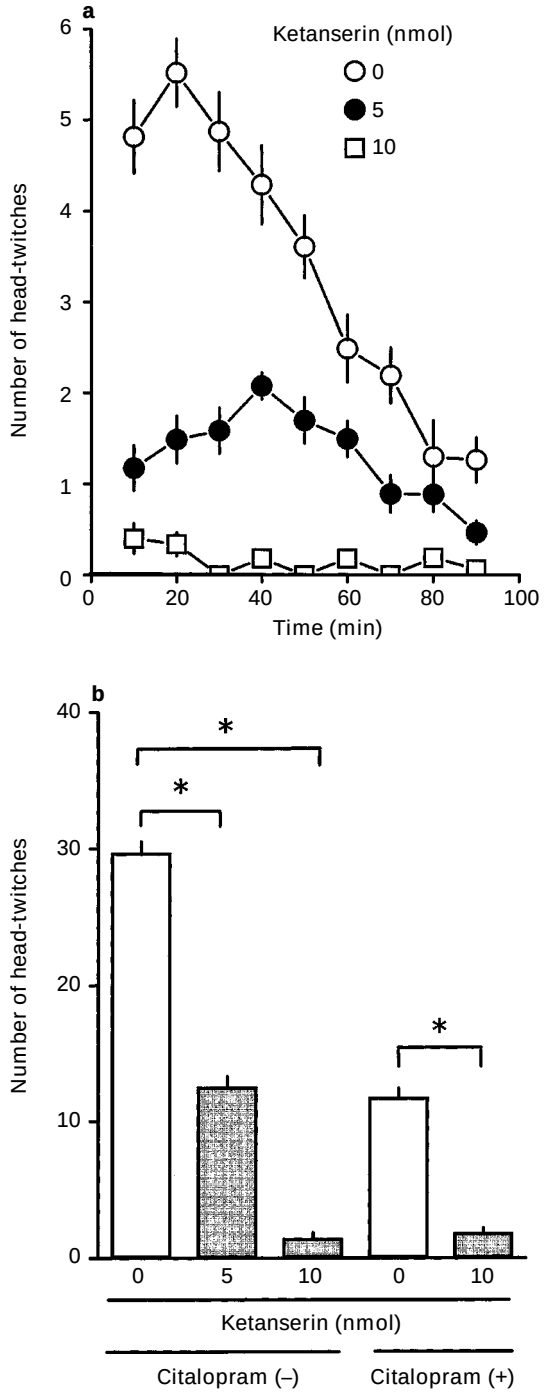

Figure 3 Effect of i.c.v. injection of ketanserin on 5-FMT-induced HTR in mice in the absence and presence of citalopram. (a) Each point represents the mean number of head-twitches for 2 min, pretreated with ketanserin (5 and 10 nmol). (b) Each column shows the total number of head-twitches induced by 5-FMT in the absence and presence of citalopram (10 mg kg^{-1}, i.p.) counted for 90 min, pretreated with ketanserin (5 and 10 nmol). Vertical lines show s.e.mean ($n=10$). *$P<0.01$, different from control.

Effects of γ-mangostin and ketanserin on 8-OHDPAT-induced head weaving

To study the effects of γ-mangostin and ketanserin on 5-HT_1 receptor-mediated head weaving, drugs or vehicle was injected i.c.v. to mice 15 min before the subcutaneous injection of 8-OHDPAT (2.5 mg kg^{-1}). γ-Mangostin and ketanserin had no effect on 8-OHDPAT-induced head weaving in mice (Figure 6).

Effects of γ-mangostin and ketanserin on locomotor activity

The effects of γ-mangostin and ketanserin on locomotor activity were examined and the data obtained were analysed by 2-way split-plot ANOVA test (Figure 7). γ-Mangostin at doses of 10 and 20 nmol did not affect the locomotor activity after administration of γ-mangostin to mice except that at 90 min. Ketanserin (10 nmol) showed the same effect on the locomotor activity.

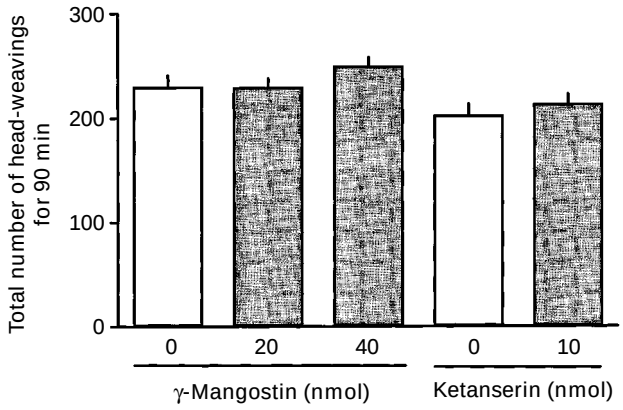

Figure 4 Effect of i.c.v. injection of γ-mangostin and ketanserin on 5-FMT-induced head weaving in mice. Each column shows the total number of head-weavings induced by 5-FMT counted for 90 min, pretreatment with γ-mangostin (20 and 40 nmol) or ketanserin (10 nmol). Vertical lines show s.e.mean ($n=10$).

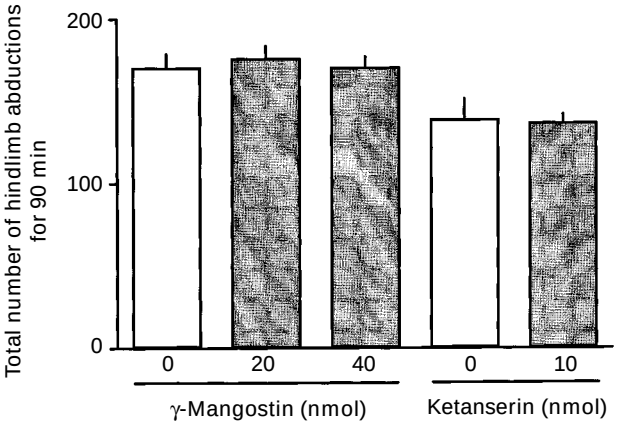

Figure 5 Effect of i.c.v. injection of γ-mangostin and ketanserin on 5-FMT-induced hindlimb abduction in mice. Each column shows the total number of hindlimb abductions induced by 5-FMT counted for 90 min, pretreated with γ-mangostin (20 and 40 nmol) or ketanserin (10 nmol). Vertical lines show s.e.mean ($n=10$).

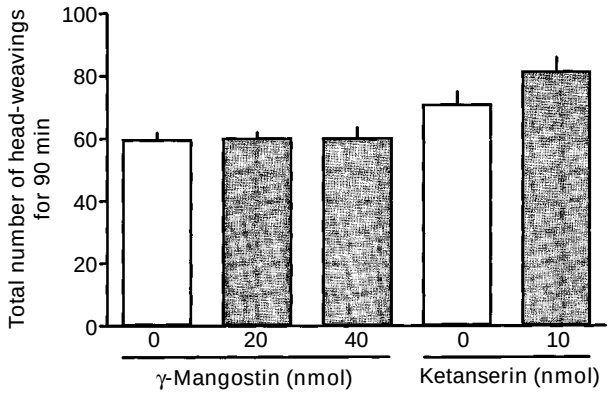

Figure 6 Effect of i.c.v. injection of γ-mangostin and ketanserin on 8-OHDPAT-induced head weaving in mice. Each column shows the total number of head-weavings induced by 8-OHDPAT counted for 90 min, pretreated with γ-mangostin (20 and 40 nmol) or ketanserin (10 nmol). Vertical lines show s.e.mean ($n=10$).

Effect of γ-mangostin on inositol phosphates accumulation in mouse brain slices

In mouse brain slices, 5-HT (5 μM) significantly increased the amounts of IPs (Table 1). 5-HT-induced accumulation of IPs was abolished in the presence of ketanserin (10 nM), indicating that it might be due to the stimulation of 5-HT_{2A} receptors by 5-HT. γ-Mangostin attenuated the increase in the amount of IPs in a concentration-dependent manner.

Effects of γ-mangostin on [^{3}H]-spiperone binding to mouse brain membrane

Figure 8 illustrates the inhibition of specific [^{3}H]-spiperone binding to mouse brain membrane by γ-mangostin. γ-Mangostin inhibited the [^{3}H]-spiperone binding in a concentration-dependent manner. Non-linear regression analysis revealed that the concentration of γ-mangostin which displaced 50% of the binding in the absence of γ-mangostin was 2.98 ± 0.49 nM. The dependence of [^{3}H]-spiperone binding on free [^{3}H]-spiperone concentration in the presence or absence of γ-mangostin (3 nM) is illustrated in Figure 9. Specific binding of [^{3}H]-spiperone to mouse brain membrane preparation was saturable and the curve was moved rightward in the presence of γ-mangostin. Non-linear regression analysis of the binding revealed that γ-mangostin increased the value of the equilibrium dissociation constant (from 0.539 ± 0.05 to 1.63 ± 0.10 nM) without affecting the maximum number of binding sites (from 146.2 ± 1.63 to 141.9 ± 2.68 fmol mg^{-1} protein), suggesting a competitive manner of inhibition. The K_i value of γ-mangostin was estimated to be 0.454 nM. However, the binding of [^{3}H]-prazosin (a specific α_1-adrenoceptor antagonist) was not affected by γ-mangostin up to 100 nM (data not shown).

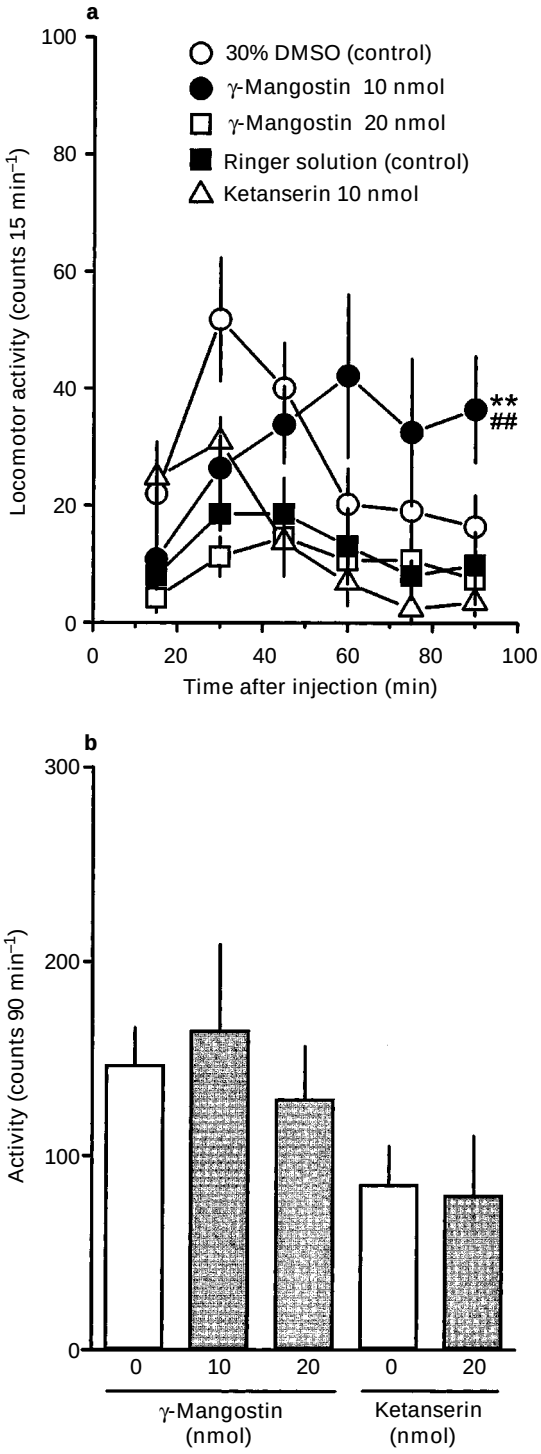

Figure 7 Effects of a single injection of γ-mangostin and ketanserin on mouse locomotor activity. (a) Locomotor activity (counts 15 min^{-1}) during the 90 min period after i.c.v. injection of γ-mangostin (10 and 20 nmol) or ketanserin (10 nmol). (b) Each column shows the total locomotor activity (counts 90 min^{-1}) after injection with γ-mangostin (10 and 20 nmol) or ketanserin (10 nmol). Vertical lines show s.e.mean ($n=20$). $^{**}P<0.01$ vs DMSO and 10 nmol γ-mangostin. $^{\#\#}P<0.01$ vs 10 nmol γ-mangostin and ketanserin.

Discussion

The focus of our research was to clarify the mechanism of action of γ-mangostin on central 5-hydroxytryptaminergic neurones by using animal behavioural models and biochemical assays. The actions of γ-mangostin were examined on two types of behavioural responses, the HTR and 5-HT syndrome, that have been associated with 5-HT receptor activation in mice. 5-FMT, a 5-HT releasing agent, induced both HTR and the 5-HT syndrome in mice. It has been shown that 5-FMT induces a release of 5-HT with a concomitant increased interaction with the central postsynaptic 5-HT_2 receptor subtype (Lucki *et al*., 1984; Tadano *et al*., 1995). 5-HT reuptake inhibitors would reduce 5-HT-induced behaviour. Indeed, examination of Figures 2(b, c) and 3b suggests that

Table 1 Effects of γ-mangostin and ketanserin on IP_1, IP_2 and IP_3 accumulations

Addition	*Concentration* (μM)	*IP1*	*IP2* (d.p.m. 0.1 mg^{-1} protein)	*IP3*
Control		973±29	516±5.1	308±9.1
5-HT	5	1776±61*	844±8.1*	534±5.6*
Ketanserin	0.01	960±44**	546±9.2**	326±5.8**
γ-Mangostin	0.01	1522±47**	754±6.7**	440±9.3**
	0.1	1004±41**	608±9.2**	342±2.2**

Brain slices were labelled with 20 μCi ml^{-1} [^{3}H]-inositol for 90 min and then incubated with 5-HT (5 μM) for 15 min. γ-Mangostin (10 or 100 nM) and ketanserin (10 nM) were added 15 min before the addition of 5-HT. Accumulations of IP_1, IP_2 and IP_3 were measured as described in Methods. Values are means±s.e.means of three observations. *Significantly different from the control ($P<0.05$); **significantly different from the values stimulated by 5-HT in the absence of ketanserin and γ-mangostin ($P<0.05$).

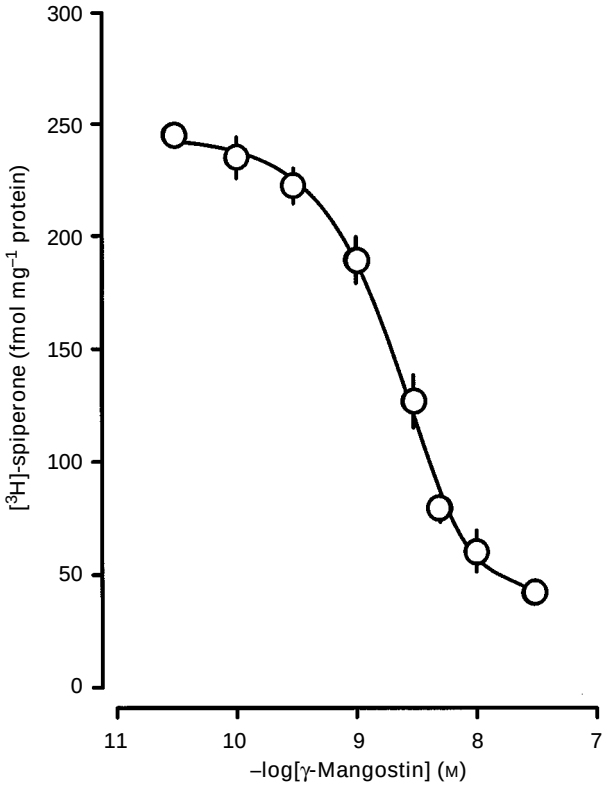

Figure 8 Concentration-inhibition curve for γ-mangostin in [^{3}H]-spiperone binding to mouse brain membrane preparation. Mouse brain membrane preparation was incubated with 3 nM [^{3}H]-spiperone and the indicated concentration of γ-mangostin at 30°C for 60 min. Non-specific binding in the presence of 10 μM ketanserin was subtracted from the results. Each point is the mean of at least three experiments and vertical lines show s.e.mean. Curve fit by nonlinear regression with Origin (Microcal Software, Northampton, MA, U.S.A.). Correlation coefficient = 0.995.

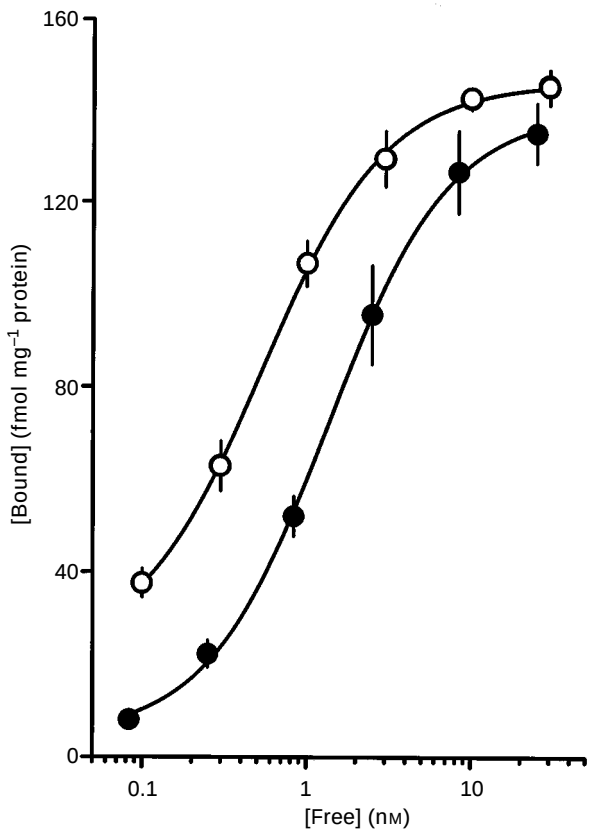

Figure 9 Binding of [^{3}H]-spiperone to mouse brain preparation in the absence and presence of γ-mangostin. Mouse brain membrane preparation was incubated with [^{3}H]-spiperone (0.1 to 30 nM) in the absence (○) and presence (●) of γ-mangostin (3 nM) at 30°C for 60 min. [^{3}H]-spiperone binding (bound) was measured and expressed versus the free [^{3}H]-spiperone concentration (free). Non specific binding in the presence of 10 μM ketanserin was subtracted from the results. Each point is the mean of at least three experiments and vertical lines show s.e.mean. Curve fit by nonlinear regression with Origin (Microcal Software, Northampton, MA, U.S.A.). Correlation coefficient values for both plots were 0.992.

this may be the case for citalopram, a representative 5-HT uptake inhibitor. Pretreatment with γ-mangostin (10–40 nmol kg^{-1}, i.c.v.) reduced the 5-FMT-induced HTR in mice in a dose-dependent manner. The magnitude of HTR induced by 5-FMT in the presence of γ-mangostin was significantly ($P<0.01$) less than the response in the vehicle-treated control. γ-Mangostin inhibited 5-FMT-induced HTR even in the presence of citalopram. Furthermore, γ-mangostin did not affect 5-FMT-induced head-weaving which was due to 5-HT release. It seems unlikely that the inhibitory effect of γ-mangostin relates to the inhibition of the 5-HT release from the central neurones. These results suggest that γ-mangostin reduces 5-FMT-induced HTR by blocking the 5-HT receptor not by blocking the release of 5-HT from the central neurone. However, it would be better to carry out confirmatory experiments with a selective 5-HT_2 receptor agonist, such as (2, 5-dimethoxy-4-iodophenyl)-2-aminopropane HCl.

It has been shown that γ-mangostin is a competitive antagonist for the 5-HT_{2A} receptors in vascular smooth muscles and platelets (Chairungsrilerd *et al*., 1996). γ-Mangostin attenuated 5-FMT-induced HTR which was abolished by ketanserin, a selective 5-HT_{2A} receptor antagonist. It has been found that phosphoinositide turnover in the rat frontal cortex *in vivo* is stimulated by 5-HT_{2A} receptor activation (Hide *et al*., 1989). Inositol phosphates accumulations in brain slices were reduced markedly by both γ-

mangostin and ketanserin. The specific binding of [^{3}H]-spiperone (selective radioligand for 5-HT_{2A} receptors) to mouse brain membranes was competitively inhibited by γ-mangostin. The 5-HT syndrome (head-weaving, hindlimb abduction) is caused by stimulation of the 5-HT_1 receptor subtype (Lucki *et al*., 1984). Neither γ-mangostin nor ketanserin affected the 5-HT syndrome produced by 5-FMT. 8-OHDPAT produces the 5-HT syndrome by stimulating 5-HT_{1A} receptors (Tricklebank, 1984; Eide, 1992; Czyrak *et al*., 1994). γ-Mangostin did not reduce the total number of head-weaving (5-HT syndrome) induced by 8-OHDPAT. Mianserin, on the other hand, is considered to be an antagonist for the 5-HT_2 receptor and α_1-receptor. It causes not only a reduction in the number of head-twitches (Van Riezen, 1972) but also decreases the locomotor activity by blocking α_1-adrenoceptors (Clineschmidt *et al*., 1979). However, γ-mangostin or ketanserin did not affect the locomotor activity. Furthermore, the binding of [^{3}H]-prazosin to mouse brain membrane was not affected by γ-mangostin. On the basis of these results, it is suggested that the inhibitory action of γ-mangostin on HTR is not related to an effect on adrenergic systems and that γ-mangostin acts as a competitive 5-HT_{2A} receptor antagonist in the central nervous system.

Generally, potent 5-HT-receptor antagonists possess at least one tertiary nitrogen group in the molecules, suggesting that nitrogen atom is essential for the 5-HT receptor blocking activity (Hoyer *et al*., 1994). It is of interest that γ-mangostin which does not have a nitrogen atom, possesses 5-HT_{2A} receptor blocking activity. With γ-mangostin we have succeeded in finding a novel type compound with 5-HT_{2A} receptor antagonist activity which does not contain a nitrogen atom.

In conclusion, γ-mangostin inhibits the 5-FMT-induced HTR in mice by blocking 5-HT_{2A} receptors not by blocking the release of 5-HT from the central neurone. It is also suggested that γ-mangostin is a promising 5-HT_{2A} receptor antagonist in the central nervous system.

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