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Pharmacological studies of geissoschizine methyl ether, isolated from *Uncaria sinensis* Oliv., in the central nervous system

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Abstract

The pharmacological properties of geissoschizine methyl ether, isolated from *Uncaria sinensis* Oliv., were analyzed in vitro and in vivo using mice central serotonin neurons. In the in vitro experiment, geissoschizine methyl ether inhibited [^{3}H]8-hydroxy-2-(di-*n*-propylamino)tetralin) ([^{3}H]8-OH-DPAT) ($K_i = 0.8$ μM), [^{3}H]mesulergine ($K_i = 0.9$ μM) and [^{3}H]ketanserin ($K_i = 1.4$ μM), but had less affinity toward [^{3}H]prazosin ($K_i > 10$ μM) and [^{3}H]spiperone ($K_i > 15$ μM) binding to mouse brain membranes. The in vivo studies showed that geissoschizine methyl ether dose-dependently reduced 5-hydroxy-L-tryptophan (*I*-5-HTP) plus clorgyline-induced head twitch response without inhibiting the *I*-5-HTP plus clorgyline and 8-OH-DPAT-induced head weaving. On the other hand, geissoschizine methyl ether also decreased the rectal temperature of mice (hypothermic response) in a dose-dependent manner. These results suggest that geissoschizine methyl ether possesses mixed 5-HT$_{1A}$ receptor agonist/5-HT$_{2A/2C}$ receptor antagonist activities and inhibits the head twitch response by blocking the 5-HT$_{2A}$ receptors, and possibly, at least in part, by stimulating the 5-HT$_{1A}$ receptors in the central nervous system.

Keywords: Geissoschizine methyl ether; 5-HT$_{1A}$ receptor agonist; 5-HT$_{2A/2C}$ receptor antagonist; Head twitch response; Hypothermic response

1. Introduction

The 5-HT$_2$ receptors in the central nervous system (CNS) are involved in psychiatric disorders such as depression, anxiety, sleep disorders and hallucination in human (Roth, 1994; Baxter et al., 1995). The 5-HT$_{2A}$ receptors are considered to be one of the most important factors related to behavioral conditions and neuropsychiatric disorders (Hamada et al., 1998; Glennon et al., 1999); further, they appear to also be important in the treatment of schizophrenia (Adi et al., 1997). However, some findings suggest that the combination of 5-HT$_{1A}$ receptor agonist and 5-HT$_{2A/2C}$ receptor antagonist may have a more advantageous therapeutic profile (Barrett and Vanofer, 1993).

Medicinal plants have been used as traditional remedies in oriental countries over hundreds of years. In Chinese (Kampo) medicine, the dried hooks and stems of *Uncaria* plants (Rubiaceae) have been used as a spasmolytic, an analgesic and a sedative treatment for many symptoms associated with hypertension and cerebrovascular disorders. Many alkaloid compounds have been isolated from these plants and have been shown to possess various pharmacological activities (Hsieh et al., 1999; Masumiya et al., 1999). We previously reported that geissoschizine methyl ether, one of the indole alkaloids isolated from the water extracts of the hooks of *Uncaria sinensis* (Oliv.) Havil., inhibits glutamate-induced convulsion (Mimaki et al., 1997). This compound has been claimed to have partial agonistic activity on the 5-HT receptors of guinea-pig ileum (Kanatani et al., 1985) and to possess central dopamine receptor antagonist activities (Sakakibara et al., 1999).

Geissoschizine methyl ether contains β-carboline. Glennon et al (2000) have recently reported β-carboline binding with modest affinity at the 5-HT$_{2A}$ receptors in comparison with the 5-HT$_{2C}$ receptors, and it displayed little to no affinity for 5-HT$_{1A}$, dopamine D$_2$ and benzodiazepine receptors (Glennon et al., 2000). The structure of geissoschizine methyl ether also bears similarity to rau-

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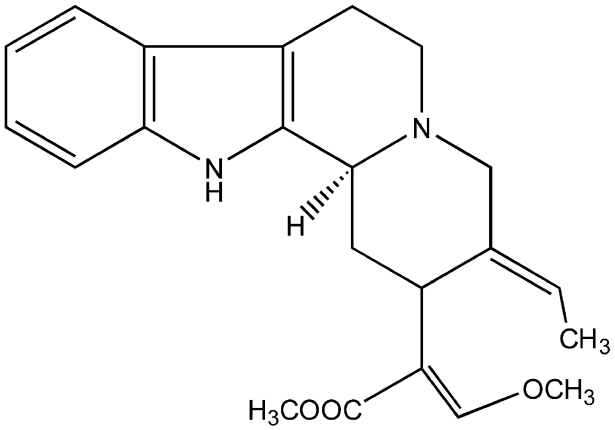

Fig. 1. Chemical structure of geissoschizine methyl ether.

wolscine, which has affinity for 5-HT_{2B} receptors (Wainscott et al., 1998). It is interesting that the affinity of β-carboline derivatives depends upon the presence of ring substituents and ring saturation (Glennon et al., 2000). In the present study, we demonstrated that geissoschizine methyl ether (Fig. 1) is a putative mixture of 5-HT_{1A} receptor agonists and 5-$HT_{2A/2C}$ receptor antagonists. This work was conducted using animal behavioral models and radioligands specific for 5-HT_{1A}, 5-HT_{2A}, 5-HT_{2C}, α_1-adrenoceptor and dopamine D_1 receptors, which bind to mouse brain membranes. This is the first study to show the action of geissoschizine methyl ether in central serotonin neurons.

2. Materials and methods

2.1. Animals

Adult male ddY mice, weighing 22–25 g, were used throughout the experiment. They were housed in plastic cages with free access to food and water, except during observation in individual cages (25 × 18 × 13 cm), immediately after intraperitoneal (i.p.) injection of either *I*-5-HTP or geissoschizine methyl ether or intracerebroventricular (i.c.v.) injection of 8-OH-DPAT. The technique employed here for i.c.v. administration was the same as that originally described by Brittain and Handley (1967). The temperature of the room in which the animals were housed, treated and observed for the head twitch response or 5-HT syndrome was maintained at 23 ± 1 °C with constant humidity (55 ± 5%). The room was illuminated from 09:00 to 21:00. All behavioral studies were performed between 10:00 and 17:00. There were 10 mice per group in every experiment. All experiments were performed according to the Guide for Care and Use of Laboratory Animals at Tohoku Pharmaceutical University.

2.2. Measurement of [^{3}H]radioligand binding to mouse brain membranes

The binding affinities for various receptors were determined by means of ligand displacement assays using the conditions summarized in Table 1. Adult male ddY mice (22–25 g) were killed by decapitation and their brains were immediately removed on ice. The frontal cortex or cerebral cortex were dissected out and homogenized in 10 volumes of ice-cold buffer (Tris–HCl, 50 mM; EDTA, 5 mM; aprotinin, 0.5 mg/ml; pH 7.5). The homogenate was centrifuged for 15 min at 21,000 rpm (50,000 × *g*) at 4 °C and the supernatant was discarded. The pellet was washed by resuspension in seven volumes of the same buffer and recentrifugation. The final pellet was then resuspended in five volumes of 50 mM Tris–HCl buffer (containing 0.3 M sucrose); 1 ml aliquots were distributed to plastic vials (NUnc) and frozen at −80 °C (at final concentration of 10 mg/ml) until analysis. Protein content was measured according to the method of Bradford (1976) using bovine serum albumin as the standard.

Table 1
Radioligand receptor binding methods

Receptor	5-HT_{2A}[a]	5-HT_{2C}[b]	5-HT_{1A}[b]	α_1-Adrenoceptor[c]	D_2-Dopamine[d]
Ligand	[^{3}H]-ketanserin	[^{3}H]mesulergine	[^{3}H]8-OH-DPAT	[^{3}H]prazosin	[^{3}H]-spiperone
(Concentration, nM)	(0.75)	(1.0)	(1.0)	(0.4)	(0.4)
NSB[e]	ketanserin	mianserin	8-OH-DPAT	phentolamine	butaclamol
(Concentration, μM)	(10)	(1)	(1)	(10)	(1)
Inc.[f] (min)/°C	20/37	20/37	30/37	45/25	30/37
Buffer[g]	50 mM Tris	50 mM Tris	50 mM Tris + 4 mM $CaCl_2$	50 mM Tris	50 mM Tris
Tissue	frontal cortex	frontal cortex	cerebral cortex	cerebral cortex	cerebral cortex

[a] Leysen et al. (1981).
[b] Assie et al. (1993).
[c] Greenglass and Bremner (1979).
[d] Creese et al. (1977).
[e] NSB: compound for determination of nonspecific binding.
[f] Inc.: incubation time and temperature.
[g] Buffer: 50 mM Tris: 50 mM Tris HCl, 120 mM NaCl, 5 mM KCl, 2 mM $CaCl_2$, 1 mM $MgCl_2$, 0.1% ascorbic acid and 10 μM pargyline. All buffers were adjusted to pH 7.4 at 26 °C.

Aliquots (1 ml) of the membranes at a final protein concentration of 0.5 mg/ml in 50 mM Tris–HCl buffer were incubated with the compound of interest or with the nonspecific binding ligand in the presence of [^{3}H]radioligand. The binding interaction was terminated by filtration under vacuum through a Whatman GF/C glass fibre filter pretreated with 0.3% (w/v) polyethyleneimine, followed by five washes with 3 ml of 50 mM Tris–HCl buffer. The radioactivity retained on the filters was determined by liquid scintillometry in 5 ml of tritosol scintillation cocktail for 3 min at efficiency of 50%. The specific binding was defined as that displaceable by the nonspecific binding ligand. The percentage of the specific binding obtained with each concentration of the test compound was calculated and the IC_{50} (concentration that inhibits 50% of the specific binding) was determined by nonlinear regression analysis, using an interactive curve-fitting procedure to a simple Langmuir isotherm performed on an IBM-PC computer (McPherson, 1985). The affinity of competing compounds (K_i) was calculated from the IC_{50} values using the Cheng–Prusoff equation (Cheng and Prusoff, 1973). The data are expressed as mean ± standard error mean (S.E.M.).

2.3. Measurements of head twitch response and head weaving induced by I-5-HTP plus clorgyline

The technique used here for a single administration of geissoschizine methyl ether or vehicle was the same as that originally described by Tadano et al. (1989). Pretreatment with the monoamine oxidase inhibitor clorgyline (1 mg/kg i.p.) was performed 1 h before or with the i.p. injection of test compound or vehicle 30 min before the *I*-5-HTP treatment. The number of head twitches (characteristic rapid movements of the head with little or no involvement of the trunk) and head weaving was counted for 2 min at 10 min intervals from 10 to 90 min after the administration of *I*-5-HTP (75 mg/kg i.p.). The total number of head twitches and head weaving was counted for 90 min after the administration of *I*-5-HTP.

2.4. Measurement of head weaving induced by 8-OH-DPAT

Each mouse was allowed to adapt for 15 min in an observation cage before the i.p. injection of the test compounds or vehicle; 30 min after the i.p. administration of drugs, 8-OH-DPAT (in Ringer solution) was injected (5 μg/mouse, i.c.v.). The total number of individual head-weaves was counted for 5 min, immediately after the injection of 8-OH-DPAT.

2.5. Measurement of hypothermia

Mice were placed in clear plastic cages (25 × 18 × 13 cm) with bedding, and rectal temperature was determined using a thermometer (type PT class 1.0; Shibaura Electronics, Tokyo, Japan) with a rounded 2.5-mm diameter probe inserted 2 cm into the rectum. Sesame oil was used for lubrication during probe insertion. Mice were i.p. injected with geissoschizine methyl ether (20, 30 mg/kg), 8-OH-DPAT (1 mg/kg) or vehicle. The rectal temperature was measured every 10 min for 60 min.

2.6. Measurement of head twitch response induced by I-5-HTP alone (I-5-HTP potentiation)

Mice were injected by i.p. administration, 60 min for saline and 30 min for drugs or control or co-administration of geissoschizine methyl ether (20 mg/kg, i.p.) and fluoxetine (20 mg/kg, i.p.), before the administration of *I*-5-HTP (75 mg/kg, i.p.). The number of head twitches was counted for 2 min at 10 min intervals from 10 to 90 min after the administration of *I*-5-HTP. The total number of head twitches was counted for 90 min after the administration of *I*-5-HTP.

2.7. Measurement of locomotor activity

To measure the locomotor activity, each mouse was placed in an activity cage, which was connected to a CompACT.AMS DI-064 (Muromachi Kikai, Tokyo, Japan) activity meter. The mice were allowed to adapt to the cage 15 min prior to the injection of test compound. The locomotor activity was measured immediately after i.p. injection of geissoschizine methyl ether. The number of activity counts was recorded every 15 min in a 90-min period.

2.8. Drugs and chemicals

Geissoschizine methyl ether (Fig. 1) was isolated and purified from *U. sinensis* (Oliv.) Havil. at Tokyo University of Pharmacy and Life Sciences as previously described (Aimi et al., 1977; Mimaki et al., 1997). *I*-5-HTP (5-hydroxy-L-tryptophan), 8-OH-DPAT (8-hydroxy-2-(di-*n*-propyl-amino)tetralin hydrobromide), 5-HT (5-hydroxytryptamine creatine sulfate), prazosin hydrochloride, butaclamol hydrochloride and phentolamine hydrochloride were purchased from Sigma (St. Louis, MO, USA); ketanserin tartrate and clorgyline HCl were from Research Biochemical International (RBI, Natick, MA, USA); and fluoxetine HCl was from Eli Lilly (Indianapolis, IN, USA). [^{3}H]8-OH-DPAT (234 Ci/mmol), [^{3}H]ketanserin ([ethylene-^{3}H]ketanserin HCl) (63.3 Ci/mmol), and [^{3}H]mesulergine (86 Ci/mmol) were purchased from Amersham Pharmacia Biotech (UK). [^{3}H]prazosin (83.8 Ci/mmol) and [^{3}H]spiperone (66.7 Ci/mmol) were obtained from DuPont/NEN Research Products (Wilmington, MA, USA). All other chemicals were of analytical grade. Geissoschizine methyl ether, *I*-5-HTP, ketanserin tartrate and fluoxetine were suspended in 0.5% polyoxyethylene sorbitan monooleate (Tween 80), 8-OH-DPAT was dissolved in Ringer solution (NaCl 147 mM, KCl 4.4 mM, $CaCl_2$ 2.7 mM) and clorgyline was dissolved in saline solution.

2.9. Data analysis

Results of experiments are expressed as mean ± S.E.M. [confidence interval 95%] where N expresses the number of assays or the number of animals used in the experiments. The statistical significance of difference between two means ($P < 0.05$) was estimated by Student's two-tailed t test for paired data. Comparisons were made by one-way analysis of variance (ANOVA) combined with Scheffe's test. P values < 0.05 were regarded as significant.

3. Results

3.1. Effects of geissoschizine methyl ether on the binding of various [^{3}H]radioligands to mouse brain membranes

The inhibition of the binding of specific [^{3}H]radioligands to mouse brain membranes induced by geissoschizine methyl ether is shown in Fig. 2. This compound (0.03–100 μM) more potently inhibited the 5-HT receptors (5-HT_{1A} and 5-$HT_{2A/2C}$) than dopamine D_2 or α_1-adrenoceptor. The inhibition of specific [^{3}H]8-OH-DPAT, [^{3}H]mesulergine and [^{3}H]ketanserin binding to mouse brain membranes due to geissoschizine methyl ether is shown in Table 2.

3.2. Effects of geissoschizine methyl ether on I-5-HTP plus clorgyline-induced head twitch response and head weaving

In previous findings (Tadano et al., 1989), *I*-5-HTP pretreated with clorgyline (a monoamine oxidase A-selective inhibitor) was found to significantly increase the frequency and total number of head-twitches in mice. Here, mice were i.p. administered with vehicle (Tween 80) or geissoschizine methyl ether (5, 10, 20 and 30 mg/kg) and the head twitch response was determined as detailed in Section 2. Fig. 3 demonstrates that after pretreatment with

Table 2
The binding affinity of geissoschizine methyl ether to various receptors of mouse brain membranes

Receptor	[^{3}H]Ligand	K_i (μM)
5-HT_{2A}	ketanserin	1.4
5-HT_{2C}	mesulergine	0.9
5-HT_{1A}	8-OH-DPAT	0.8
D_2	spiperone	> 15
α_1	prazosin	> 10

Results are expressed as apparent inhibition constants K_i (μM) corrected for ligand occupancy using the Cheng–Prusoff equation.

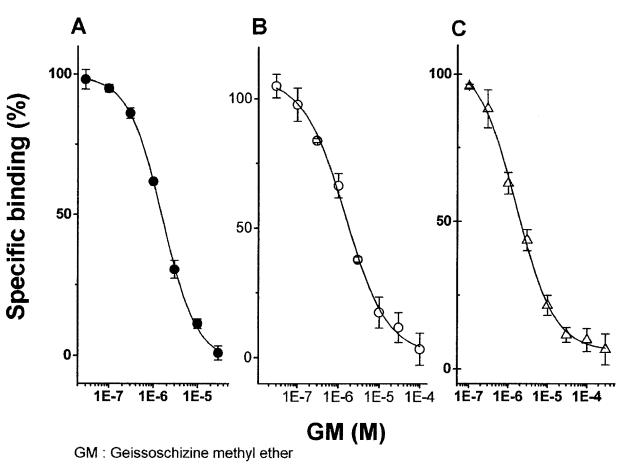

Fig. 2. Concentration–inhibition curve for geissoschizine methyl ether in (A) [^{3}H]8-OH-DPAT, (B) [^{3}H]mesulergine and (C) [^{3}H]ketanserin binding to mouse brain membrane preparation. Mouse brain membrane preparation was incubated with [^{3}H]ligand and the indicated concentration of geissoschizine methyl ether. Each point is the mean of at least three experiments and the vertical lines show S.E.M.

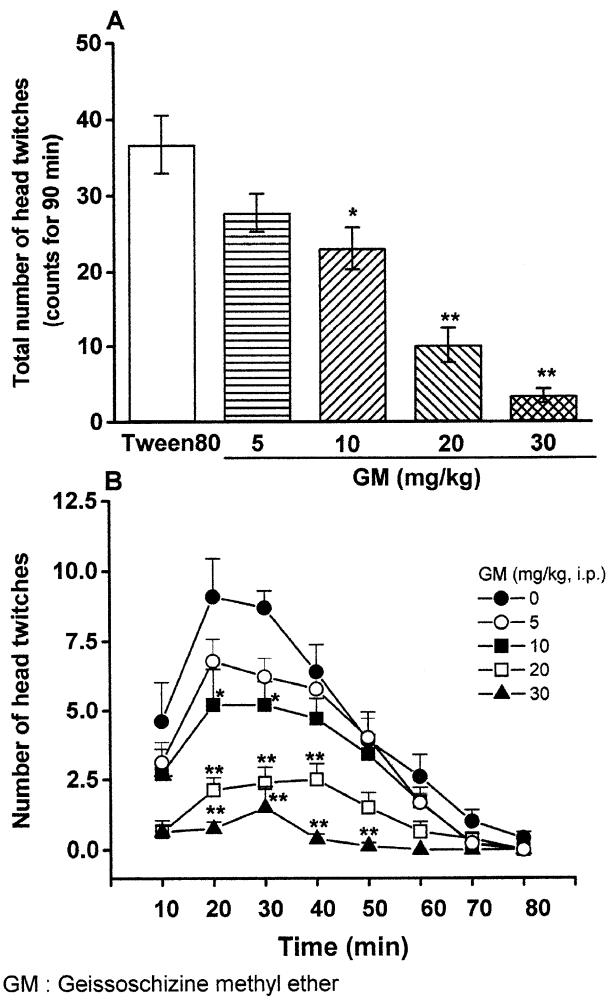

Fig. 3. Effect of geissoschizine methyl ether in various concentrations (5, 10, 20 and 30 mg/kg i.p.) on *I*-5-HTP plus clorgyline-induced head twitch response in mice. (a) Each point represents the mean number of head twitches for 2 min. (b) Each column shows the total number of head twitches counted for 90 min. Vertical lines show the S.E.M. ($n = 10$). $^*P < 0.05$ or $^{**}P < 0.01$ indicates a significant difference from vehicle control.

geissoschizine methyl ether [$F(4,40) = 22.61$, $P < 0.01$] (5 mg/kg, $P = 0.27$; 10 mg/kg, $P = 0.02$; 20 mg/kg, $P < 0.01$; and 30 mg/kg, $P < 0.01$, i.p.), the total number of head-twitches induced by *I*-5-HTP plus clorgyline was reduced by geissoschizine methyl ether in a dose-dependent manner. In contrast, *I*-5-HTP (75 mg/kg i.p.) plus clorgyline (1 mg/kg i.p.)-induced head weaving did not significantly change by geissoschizine methyl ether with doses up to 20 mg/kg, i.p. (data not shown).

3.3. Effects of geissoschizine methyl ether on 8-OH-DPAT-induced head weaving

The head weaving induced by 8-OH-DPAT (5 μg/mouse, i.c.v.) for 5 min after i.p. administration of geissoschizine methyl ether ($t = -0.013$, $P = 0.99$) did not significantly change the total number of head weaving events compared to controls (Fig. 4).

3.4. Effects of geissoschizine methyl ether on mouse rectal temperature

Geissoschizine methyl ether (20, 30 mg/kg, i.p.) and 8-OH-DPAT (1 mg/kg, i.p.) consistently induced a hypothermic response with a typical duration of 60 min, which was maximal at 20 min after treatment. The rectal temperature at 20 min after geissoschizine methyl ether [$F(2,27) = 27.88$, $P < 0.01$] (20 mg/kg, $P < 0.01$ and 30 mg/kg, $P < 0.01$) or 8-OH-DPAT ($t = 5.34$, $P < 0.01$) was significantly decreased compared to the Tween 80-treated controls (Fig. 5).

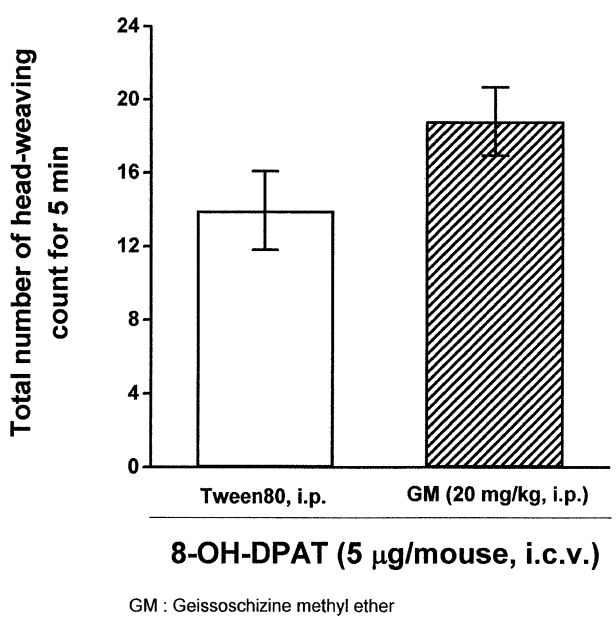

Fig. 4. Effect of geissoschizine methyl ether on 8-OHDPAT-induced head weaving in mice. Results are expressed as mean ± S.E.M. ($n = 10$).

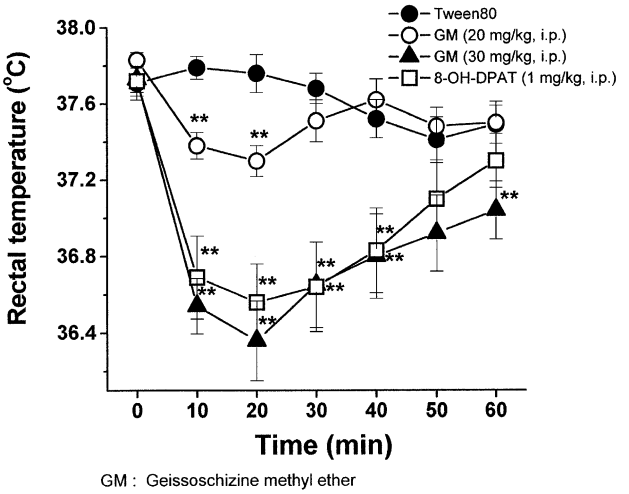

Fig. 5. Time course of the hypothermic effect of 8-OH-DPAT (1 mg/kg i.p.; △); geissoschizine methyl ether (20 mg/kg i.p., ○; 30 mg/kg i.p., ▲) in mice compared with vehicle control (●). Results are expressed as mean ± S.E.M. ($n = 10$). $^{*}P < 0.05$ or $^{**}P < 0.01$ indicates a significant difference from vehicle control.

3.5. Effects of geissoschizine methyl ether on potentiated I-5-HTP-induced HTR

Fig. 6 shows that *I*-5-HTP at a dose of 75 mg/kg, i.p. by itself causes no clear behavioral change, but *I*-5-HTP plus fluoxetine (20 mg/kg, i.p.) markedly increased the head twitch response ($t = -5.60$, $P < 0.01$). However, geissoschizine methyl ether (10 mg/kg, i.p.) did not sig-

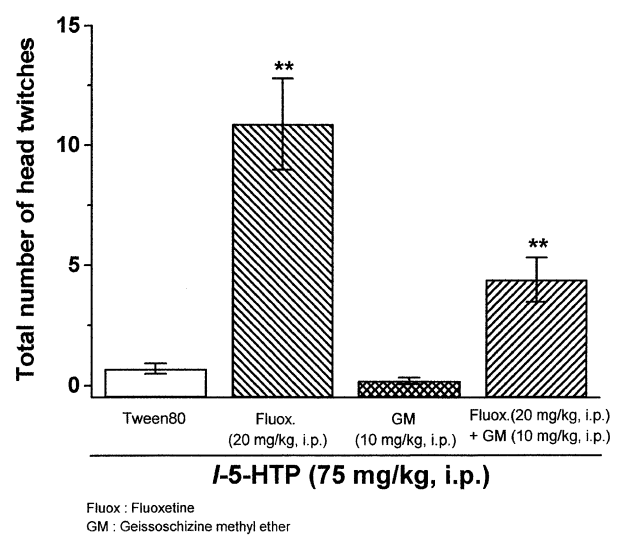

Fig. 6. Effect of fluoxetine or geissoschizine methyl ether alone and the co-administration geissoschizine methyl ether with fluoxetine on single administration of *I*-5-HTP-induced head twitch response. Each column shows the total number of head-twitches counted for 90 min, pretreated with fluoxetine (20 mg/kg i.p.), geissoschizine methyl ether (10 mg/kg i.p.), and co-administration of geissoschizine methyl ether (10 mg/kg i.p.) with fluoxetine (20 mg/kg i.p.). Vertical lines show the S.E.M. ($n = 10$). $^{**}P < 0.01$ indicates a significant difference from vehicle control.

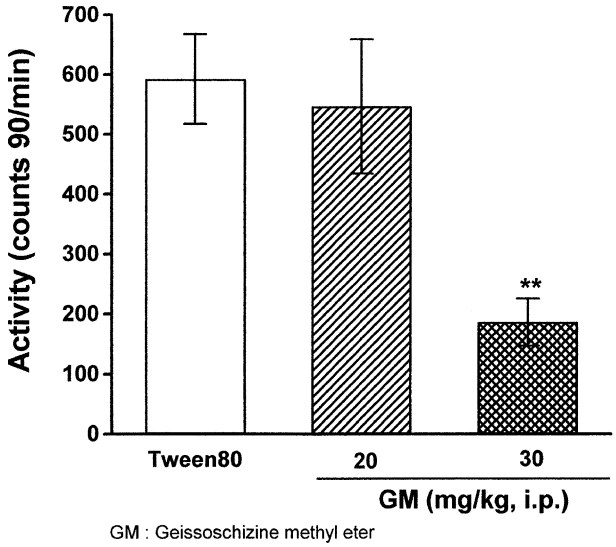

Fig. 7. Effect of geissoschizine methyl ether on mouse locomotor activity. Each column shows the total locomotor activity (count/90 min) after i.p. injection of geissoschizine methyl ether (20 and 30 mg/kg i.p.). Vertical lines show S.E.M. ($n = 10$). $^{**}P < 0.01$ vs. vehicle control.

nificantly change the head twitch response induced by *I*-5-HTP ($t = 1.99$, $P = 0.06$). On the other hand, geissoschizine methyl ether (10 mg/kg, i.p.) ($t = 3.16$, $P < 0.01$) suppressed the head twitch response induced by the combined administration of fluoxetine (20 mg/kg i.p.) and *I*-5-HTP (75 mg/kg i.p.).

3.6. Effects of geissoschizine methyl ether on locomotor activity

The effects of geissoschizine methyl ether on locomotor activity were examined and the data obtained were analyzed by one-way ANOVA test [$F(2.26) = 10.19$, $P < 0.01$]. The i.p. administration of geissoschizine methyl ether at 20 mg/kg did not significantly affect the locomotor activity ($P = 0.92$), but at a dose of 30 mg/kg it decreased the locomotor activity ($P < 0.01$) (Fig. 7).

4. Discussion

It has been reported that i.p. administration of *I*-5-HTP induces head twitch response and 5-HT syndromes such as head weaving (Glennon et al., 1991; Murphy et al., 1991; Lucki, 1992); interestingly, the number of head twitches is enhanced by the combination of *I*-5-HTP and clorgyline, a monoamine oxidase type A-selective inhibitor (Tadano et al., 1989). In the present study, we demonstrated that geissoschizine methyl ether, an alkaloid isolated from the water extracts of the hooks of *U. sinensis* (Oliv.) Havil, reduced the *I*-5-HTP plus clorgyline-induced head twitch response in a dose-dependent manner. The magnitude of head twitch response in the presence of geissoschizine methyl ether was significantly less than the response in the vehicle-treated control.

The in vitro binding studies indicated that geissoschizine methyl ether has affinity for [^{3}H]ketanserin binding to the 5-HT$_{2A}$ receptor with the K_i value of 1.4 μM. Furthermore, this compound also has affinity for [^{3}H]mesulergine binding to the 5-HT$_{2C}$ receptor with the K_i value of 0.9 μM. Since Hibert et al. (1991) suggested, based on a study using a molecular model, that 5-HT binds to the 5-HT$_{2A}$ and 5-HT$_{2C}$ receptors in similar agonist binding pockets, it is difficult to distinguish the role of 5-HT$_{2A}$, 5-HT$_{2B}$ and 5-HT$_{2C}$ receptors. However, several recent studies have indicated that the *I*-5-HTP-induced head twitch response is mediated by the 5-HT$_{2A}$ receptors, but not the 5-HT$_{2C}$ receptors (Schreiber et al., 1995). Additionally, the putative 5-HT$_{2C/2B}$ receptor selective antagonist SB 200646A (*N*-(1-methyl-5-indolyl)-*N*′-(3-pyridyl) urea hydrochloride) does not block DOI-induced head twitches (Kennett et al., 1994). Although there is sufficient evidence that the 5-HT$_{2A}$ receptors play a role in the positive effect of geissoschizine methyl ether on *I*-5-HTP-induced head twitches, the involvement of 5-HT$_{2C}$ receptors cannot be excluded.

We also studied the effect of geissoschizine methyl ether on the 5-HT$_1$ receptors, and found that it has affinity for [^{3}H]8-OH-DPAT binding to mouse cerebral cortex membranes (K_i value of 0.8 μM). However, both *I*-5-HTP plus clorgyline-induced and 8-OH-DPAT-induced head weaving were not affected by this compound. These results may indicate that this compound does not possess 5-HT$_1$ antagonist activities. Moreover, it has been reported that geissoschizine methyl ether has partial agonistic activity on the 5-HT receptor of guinea pig ileum, which is correlated to the 5-HT$_1$ receptor subtype (Kanatani et al., 1985). Therefore, in order to prove the 5-HT$_1$ receptors property of geissoschizine methyl ether, we examined the effect of geissoschizine methyl ether on the hypothermic response. Goodwin and Green (1985) reported that s.c. administration of 8-OH-DPAT (in doses up to 10 mg/kg) elicits hypothermia in mice but shows no apparent behavioral syndrome. This hypothermic response is due mainly to the activation of 5-HT$_{1A}$ receptors (Bill et al., 1991; Martin et al., 1992). In the present study, i.p. administration of geissoschizine methyl ether and 8-OH-DPAT (as a positive control) elicited a hypothermic response. Taken together, it is suggested that geissoschizine methyl ether possess the 5-HT$_{1A}$ receptor agonist but not antagonist activities.

A single treatment of 5-HT re-uptake blocker, such as citalopram or fluoxetine, significantly increases extracellular 5-HT levels in the rat frontal cortex (Hatanaka et al., 2000). In addition, the increase in synaptic concentrations of 5-HT via the inhibition of synaptic 5-HT re-uptake enhances the frequency of the *I*-5-HTP-induced head twitch response (Darmani and Reeves, 1996). The present find-

ings were similar to those reported by Takeuchi et al (1997), in which, the frequency of *I*-5-HTP-induced head twitch response was enhanced by fluoxetine, following *I*-5-HTP administration, compared with *I*-5-HTP alone. On the other hand, geissoschizine methyl ether did not enhance the head twitches induced by *I*-5-HTP. However, the combined effect of *I*-5-HTP and fluoxetine on head twitch response was decreased by i.p. administration of geissoschizine methyl ether. These results indicate that the effect of geissoschizine methyl ether on *I*-5-HTP-induced head twitch response may not involve uptake and accumulation in serotonergic neurons by the selective 5-HT uptake system.

Geissoschizine methyl ether has been reported to decrease locomotion elicited by methamphetamine or apomorphine when given orally at a dose of 100 mg/kg but has no significant effect on locomotor activity by itself (Sakakibara et al., 1999). This depression of locomotor activity may be due to mediation of the central dopamine system. In the present study, geissoschizine methyl ether did not affect the locomotor activity at doses up to 20 mg/kg i.p., while at a higher dose (30 mg/kg i.p.), it decreased the locomotor activity. These in vivo results were reflected in the in vitro receptor binding studies. Geissoschizine methyl ether has less affinity toward α_1-adrenoceptor or dopamine D_2 receptors than 5-HT_{1A}, 5-HT_{2C} and 5-HT_{2A} receptors in the mouse brain. Therefore, the present findings indicated that the effect of geissoschizine methyl ether at i.p. doses up to 20 mg/kg is not related to a direct effect on both α_1-adrenoceptor and dopamine D_2 receptors.

The structure of geissoschizine methyl ether contains a tetrahydro-β-carboline (THBC). The presence of the D-ring and the substituents of THBC on geissoschizine methyl ether might actually increase the affinity for 5-HT_{2A}, 5-HT_{2C} and 5-HT_{1A} receptors. Furthermore, the structure of geissoschizine methyl ether is also similar to rauwolscine, which has been reported to show selectivity for 5-HT_{2B} receptors (Wainscott et al., 1998). However, it has not yet been proven that the native 5-HT_{2B} receptor in the brain couples to phospatidylinositol hydrolysis (Barnes and Sharp, 1999).

In conclusion, these results suggest that geissoschizine methyl ether may possess a putative mixture of 5-HT_{1A} receptor agonist and 5-$HT_{2A/2C}$ receptor antagonist activities. Geissoschizine methyl ether inhibits the head twitch response by blocking the 5-HT_{2A} receptors and this may be, at least in part, by stimulation of the 5-HT_{1A} receptors in the central nervous system.

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