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Beneficial estrogen-like effects of ginsenoside Rb1, an active component of *Panax ginseng*, on neural 5-HT disposition and behavioral tasks in ovariectomized mice

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ABSTRACT

Decreased 5-hydroxytryptamine (5-HT) concentration in the brain has been linked to central nervous system dysfunctions, especially in menopausal women. Ginsenoside Rb1, a potential phytoestrogen, has been shown to improve central nervous system dysfunctions, comparable to the estrogen treatment. To investigate the estrogen-like effects of ginsenoside Rb1 on neural 5-HT disposition and behavioral tasks, we quantified the concentrations of 5-HT and other related endogenous substances in the frontal cortex and striatum of ovariectomized mice. The activities of tryptophan hydroxylase (TPH), aromatic amino acid decarboxylase (AAAD) and monoamine oxidase (MAO) were also measured to evaluate the synthesis and metabolism of neural 5-HT. Our work shows that both ginsenoside Rb1 and estradiol increased the neural 5-HT concentration. Ginsenoside Rb1 and estradiol administration resulted in elevated TPH and depressed MAO activities, indicating that modulating the synthesis and metabolism of neural 5-HT successfully elevated 5-HT concentration. Ginsenoside Rb1 and estradiol also improved object recognition and decreased immobility time in the forced swimming test. However, a pretreatment with clomiphene (an estrogen receptor antagonist) blocked the beneficial effects of ginsenoside Rb1 and estradiol, suggesting that the estrogen-like effects of ginsenoside Rb1 were estrogen receptor-dependent.

1. Introduction

Decreased 5-hydroxytryptamine (5-HT) concentration in the brain is an induced factor for many psychiatric disorders (Graeff et al., 1996; Rueter et al., 1997). Some drugs elicit beneficial effects on central nervous system dysfunctions by modulating 5-HT synthesis, metabolism, reuptake and receptor binding in the brain (Hamon et al., 1981; MacQueen et al., 2001; Riederer et al., 2004).

Ginsenosides are the major active components in *Panax ginseng*. Accumulating evidences suggest that ginsenosides exert their pharmacological activities on the central nervous system by modulating neurotransmitters (Attele et al., 1999; Kimura et al., 1994; Tsang et al., 1985; Yuan et al., 1998; Zhang et al., 1990). Ginsenosides reversed the decrease of monoamine neurotransmitters in the hippocampus caused by chronic and mild stress (Dang et al., 2009). Both ginsenoside Rb1 and Rg1 were capable of partially reversing scopolamine-induced amnesia by improving cholinergic activity (Radad et al., 2004). Additionally, ginsenoside Rg1 increased the dopamine level in the striatum of 1-methy-4-phenyl-1,2,3,6-tetrahydropyridine-treated C57BL6 mice and attenuated the decrease of the dopamine level induced by 6-hydroxydopamine in rats (Wang et al., 2009; Xu et al., 2009). Ginsenoside Rb1 and Rg1 have also been shown to facilitate glutamate release from PC12 cells and primary hippocampal neurons (Liu et al., 2010; Xue et al., 2006). Ginsenoside Rb1 is one of the primary ginsenosides exhibiting several pharmacological actions in central nervous system. However, the potential effects of ginsenoside Rb1 on neural 5-HT remain elusive. To comprehensively evaluate the effects of ginsenoside Rb1 on neural 5-HT disposition, we measured the concentrations of 5-HT, 5-hydroxyindoleacetic acid (5-HIAA), tryptophan and kynurenine in the brain of ovariectomized mice treated with ginsenoside Rb1 either acutely or chronically. And we also measured the activities of synthesis and metabolism enzymes of neural 5-HT, including tryptophan hydroxylase (TPH), aromatic amino acid decarboxylase (AAAD) and monoamine oxidase (MAO).

Central nervous system dysfunctions were ameliorated by supplementary exogenous estrogen in menopausal women, which might be due to its interaction with the 5-HT neurotransmission (Imwalle et al., 2005; Pandaranandaka et al., 2009). Furthermore, estrogen induced changes in 5-HT receptor binding and metabolism in the brain, which contributed to the regulations of affection and cognition (Amin et al., 2005; Centeno et al., 2007). However, due to side effects of hormone therapy, many women turn to phytoestrogens as an alternative to

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hormone replacement therapy. Ginsenosides, especially their aglycone part, are similar to several steroids in structure, including female hormones, so they have been shown to possess estrogen-like activities (Chan et al., 2002; Cho et al., 2004; Lau et al., 2008; Lee et al., 2003; Lu et al., 2008). However, unlike estradiol and other phytoestrogens (Chen and Wong, 2004), the binding affinity of ginsenoside Rb1 to the estrogen receptor is unspecific. Its estrogenic activities in the absence of a direct interaction with estrogen receptor suggest that ginsenoside Rb1 may activate estrogen receptor *via* a ligand-independent pathway (Cho et al., 2004). In the present study, we investigated the estrogen-like effects of ginsenoside Rb1 on neural 5-HT disposition by measuring neurotransmitters, enzyme activities and behavioral tasks in ovariectomized mice when treated with estradiol and clomiphene (an estrogen receptor antagonist).

2. Materials and methods

2.1. Animals

All animal experiments were performed under a license granted by the Jiangsu Science and Technology Office (China) with approval from the Animal Ethics Committee of China Pharmaceutical University. Every effort was made to minimize the stress on the mice. Female Swiss-Hauschka mice weighing 18 to 22 g were used throughout these studies (China Pharmaceutical University experiment animal center, Nanjing, China).

They were housed at 22 ± 3 °C on a 12 h dark/light cycle. All mice were provided with water and standard chow *ad libitum* for one week prior to ovariectomy. Female mice were ovariectomized and handled gently two weeks to remove the influence of endogenous estrogen before experiments began.

2.2. Chemicals

Ginsenoside Rb1 (purity 99%) was purchased from QingZe Co. Ltd (Nanjing, China), estradiol, *m*-hydroxybenzylhydrazine (NSD-1015), clomiphene, pargyline, selegiline and clorgyline were all obtained from Sigma-Aldrich Co. Ltd (Shanghai, China). Amino acids, 5-HT, 5-HIAA, 5-hydroxytryptophan (5-HTP), kynurenine, dopamine and norepinephrine were all purchased from WanQing Co. Ltd (Nanjing, China). Other chemicals and solvents were of analytical grade and purchased from Nanjing Chemical Reagent Co. Ltd (Nanjing, China). All chemicals were dissolved in saline, except for ginsenoside Rb1 and estradiol which were dissolved in saline containing 20% sesame oil (v/v).

2.3. Tryptophan depletion

Decreased 5-HT concentration in the brain was achieved by tryptophan depletion. Acute tryptophan depletion was accomplished using a tryptophan-free amino acid mixture administration (Ardis et al., 2009; Fadda et al., 2000). The tryptophan-free amino acid mixture was comprised of 15 amino acids: L-alanine (5.1 g), L-arginine (4.9 g), L-cystine (2.7 g), glycine (3.2 g), L-histidine (3.2 g), L-isoleucine (8.0 g), L-leucine (13.1 g), L-lysine (11.0 g), L-methionine (3.0 g), L-phenylalanine (5.7 g), L-proline (12.2 g), L-serine (6.4 g), L-tyrosine (6.9 g), L-valine (8.5 g) and L-threonine (6.1 g). The tryptophan-containing amino acid mixture consisted of the same amino acids mentioned above plus tryptophan (2.3 g). The tryptophan-containing and tryptophan-free amino acid mixtures were suspended in water (5 g per 9 ml) immediately before intragastric administration. Ovariectomized mice were fasted for 14 h followed by intragastric administration (20 ml/kg) with either tryptophan-containing or tryptophan-free amino acid mixture. Chronic tryptophan depletion was achieved using a modified tryptophan-free amino acid diet (Fadda, 2000; Uchida et al., 2007). The tryptophan-free amino acid diet had the following compositions (per 100 g of food): zein (2%), ammonium citrate (2.3%), gelatin (2.85%), tryptophan-free amino acid mixture (10%; also used in acute tryptophan depletion), Hegsted salt (4%), choline chloride (0.2%), maize oil (10%), sucrose (68.4%) and complete vitamin integration (0.25%; thiamine HCl, riboflavin, pyridoxine HCl, nicotinic acid, calcium pantothenate, folic acid, biotin and vitamins B12, A, D3, E and K). The tryptophan-containing amino acid diet had the same composition as the tryptophan-free amino acid diet except 0.7% tryptophan in place of an equivalent amount of sucrose. The control mice were fed with the tryptophan-containing amino acid diet. In the chronic tryptophan depletion mice, water and the tryptophan-free amino acid diet were freely available for 7 days.

2.4. HPLC analysis

At the indicated time points, mice were decapitated, and then the brain was removed after a rapid dissection of the cranium. Brain samples were kept at −80 °C until the analysis. The frontal cortex and striatum were weighed and homogenized in saline. An aliquot of the homogenates were used to determine the concentrations of 5-HT, 5-HIAA, 5-HTP, tryptophan, kynurenine, dopamine and norepinephrine. The rest homogenates were used in assays for AAAD and MAO activities. An aliquot of homogenates were deproteinized on ice in 10% perchloric acid containing ascorbic acid (50 μM) and ethylenediamine tetraacetic acid (EDTA, 200 mM). After vortexing, the homogenates were centrifuged at 15,000 rpm for 10 min at 4 °C. The supernatants (30 μl) were used for high pressure liquid chromatography-fluorescence (HPLC-FLU) detection to determine the concentrations of 5-HT, 5-HIAA, 5-HTP, tryptophan, dopamine and norepinephrine in the brain (Lu et al., 2003; Wei et al., 2009). A reverse-phase C_{18} column was used. The mobile phase was methanol–water (10: 90), including 0.1 M sodium acetate, 0.1 M EDTA and 0.144% acetic acid (v/v). The flow rate was 1 ml/min. The λex was 290 nm and the λem was 330 nm.

The kynurenine concentration was quantified by HPLC-UV as described previously (Heyes and Quearry, 1988; Holmes, 1988). Briefly, the same supernatants were analyzed using a reverse-phase C_{18} column and a mobile phase containing 0.1 mM ammonium acetate, 0.1 M acetic acid and 2% acetonitrile. The absorbance was measured at 365 nm using a UV detector.

2.5. Experiment design for measuring neurotransmitters

2.5.1. Acute treatment

Ovariectomized mice were assigned to one of seven groups (n = 8 per group) as described in Table 1: (i) control group: mice were subjected to intragastric administration with tryptophan-containing amino acid mixture; (ii) model group: mice were subjected to intragastric administration with the tryptophan-free amino acid mixture; (iii) ginsenoside Rb1 group: mice were subjected to intragastric administration with the tryptophan-free amino acid mixture, followed by an intravenous injection with ginsenoside Rb1; (iv) estradiol group: mice were subjected to intragastric administration with the tryptophan-free amino acid mixture, followed by an intravenous injection with estradiol; (v) clomiphene + ginsenoside Rb1 group: similar treatment as in "(iii) ginsenoside Rb1 group" except that clomiphene was injected intraperitoneally 20 min before the intravenous injection with ginsenoside Rb1; (vi) clomiphene + estradiol group: similar treatment as in "(iv) estradiol group" except that clomiphene was injected intraperitoneally 20 min before the intravenous injection with estradiol; (vii) clomiphene group: similar treatment as in "(ii) model group" except that clomiphene was injected intraperitoneally 20 min before the intravenous injection with vehicle. In acute studies, the drug doses were as follows: ginsenoside Rb1 (20 mg/kg), estradiol (0.2 mg/kg) and clomiphene (8 mg/kg). The brain samples were collected 2 h after injection with ginsenoside Rb1 and estradiol.

Table 1
Experiment group overview of the acute treatment in experiment design for measuring neurotransmitters.

Group	$t_{-20\ min}$	$t_{-2\ min}$	$t_{0\ min}$	$t_{120\ min}$
(i)	Vehicle	Tryptophan-containing amino acid mixture	Vehicle	Decapitation
(ii)	Vehicle	Tryptophan-free amino acid mixture	Vehicle	Decapitation
(iii)	Vehicle	Tryptophan-free amino acid mixture	Ginsenoside Rb1	Decapitation
(iv)	Vehicle	Tryptophan-free amino acid mixture	Estradiol	Decapitation
(v)	Clomiphene	Tryptophan-free amino acid mixture	Ginsenoside Rb1	Decapitation
(vi)	Clomiphene	Tryptophan-free amino acid mixture	Estradiol	Decapitation
(vii)	Clomiphene	Tryptophan-free amino acid mixture	Vehicle	Decapitation

$t_{-20\ min}$: an intraperitoneal injection with clomiphene (8 mg/kg) and vehicle 20 min before the drug administration; $t_{-2\ min}$: an intragastric administration with amino acid mixtures (0.2 ml/10 g) 2 min before the drug administration; $t_{0\ min}$: an intravenous injection with ginsenoside Rb1 (20 mg/kg) and estradiol (0.2 mg/kg); $t_{120\ min}$: sacrificed at 120 min after the drug administration, and the brain was collected to analysis.

2.5.2. Chronic treatment

Ovariectomized mice were assigned to one of seven groups (n = 8 per group) as shown in Table 2: (i) control group: mice were fed with the tryptophan-containing amino acid diet for 7 days; (ii) model group: mice were fed with the tryptophan-free amino acid diet for 7 days; (iii) ginsenoside Rb1 group: mice were fed with the tryptophan-free amino acid diet for 7 days, combined with intraperitoneal injections with ginsenoside Rb1 for 7 days (twice a day); (iv) estradiol group: mice were fed with the tryptophan-free amino acid diet for 7 days, combined with intraperitoneal injections with estradiol for 7 days (twice a day); (v) clomiphene + ginsenoside Rb1 group: similar treatment as in "(iii) ginsenoside Rb1 group" except that clomiphene was injected intraperitoneally 20 min before intraperitoneal injections with ginsenoside Rb1 for 7 days (twice a day); (vi) clomiphene + estradiol group: similar treatment as in "(iv) estradiol group" except that clomiphene was injected intraperitoneally 20 min before intraperitoneal injections with estradiol for 7 days (twice a day); (vii) clomiphene group: similar treatment as in "(ii) model group" except that clomiphene was injected intraperitoneally 20 min before intraperitoneal injections with vehicle for 7 days (twice a day). In chronic studies, the drug doses were as follows: ginsenoside Rb1 (10 mg/kg/day), estradiol (0.1 mg/kg/day) and clomiphene (4 mg/kg/day). Brain samples were collected 2 h after the first injection with ginsenoside Rb1 and estradiol on the 7th day.

2.5.3. Preventive treatment

Ovariectomized mice were assigned to one of seven groups (n = 8 per group) as described in Table 3: (i) control group: mice were subjected to intraperitoneal injections with vehicle for 14 days (twice a day), after a three-day washout, the tryptophan-containing amino acid mixture was intragastricly administered; (ii) model group: mice were subjected to intraperitoneal injections with vehicle for 14 days (twice a day); (iii) ginsenoside Rb1 group: mice were subjected to intraperitoneal injections with ginsenoside Rb1 for 14 days (twice a day); (iv) estradiol group: mice were subjected to intraperitoneal injections with estradiol for 14 days (twice a day); (v) clomiphene + ginsenoside Rb1 group: similar treatment as in "(iii) ginsenoside Rb1 group" except that clomiphene was injected intraperitoneally 20 min before intraperitoneal injections with ginsenoside Rb1 for 14 days (twice a day). (vi) clomiphene + estradiol group: similar treatment as in "(iv) estradiol group" except that clomiphene was injected intraperitoneally 20 min before intraperitoneal injections with estradiol for 14 days (twice a day). (vii) clomiphene group: similar treatment as in "(ii) Model group" except that clomiphene was injected intraperitoneally 20 min before intraperitoneal injections with vehicle for 14 days (twice a day). After a three-day washout, the tryptophan-free amino acid mixture was intragastricly administered in Groups (ii)–(vii). In preventive studies, the drug doses were as follows: ginsenoside Rb1 (10 mg/kg/day), estradiol (0.1 mg/kg/day) and clomiphene (4 mg/kg/day). Brain samples were collected at 0 h and 2 h after intragastric administration with the tryptophan-containing and tryptophan-free amino acid mixtures.

2.6. TPH activity assay

2.6.1. Acute treatment

Mice were assigned to the similar treatment as in Section 2.5.1 except that NSD-1015 (AAAD inhibitor, 100 mg/kg) was injected intraperitoneally 30 min before the injection with ginsenoside Rb1 and estradiol. Brain samples were collected 2 h after the injection with ginsenoside Rb1 and estradiol, and the TPH activity was determined by the accumulation of 5-HTP in the brain (pmol/mg tissue).

2.6.2. Chronic treatment

Mice were assigned to the similar treatment as in Section 2.5.2 except that NSD-1015 (AAAD inhibitor, 100 mg/kg) was injected intraperitoneally 30 min before the first injection with ginsenoside Rb1 and estradiol on the 7th day. Brain samples were collected 2 h after the first injection with ginsenoside Rb1 and estradiol on the 7th day, and the TPH activity was determined by the accumulation of 5-HTP in the brain (pmol/mg tissue).

2.6.3. Preventive treatment

Mice were assigned to the similar treatment as in Section 2.5.3 except that NSD-1015 (AAAD inhibitor, 100 mg/kg) was injected intraperitoneally 30 min before the intragastric administration with the amino acid mixtures. Brain samples were collected 2 h after the

Table 2
Experiment group overview of the chronic treatment in experiment design for measuring neurotransmitters.

	$t_{7\ days}$	$t_{-20\ min}$	$t_{0\ min}$	$t_{120\ min}$
(i)	Tryptophan-containing amino acid diet	Vehicle	Vehicle	Decapitation
(ii)	Tryptophan-free amino acid diet	Vehicle	Vehicle	Decapitation
(iii)	Tryptophan-free amino acid diet	Vehicle	Ginsenoside Rb1	Decapitation
(iv)	Tryptophan-free amino acid diet	Vehicle	Estradiol	Decapitation
(v)	Tryptophan-free amino acid diet	Clomiphene	Ginsenoside Rb1	Decapitation
(vi)	Tryptophan-free amino acid diet	Clomiphene	Estradiol	Decapitation
(vii)	Tryptophan-free amino acid diet	Clomiphene	Vehicle	Decapitation

$t_{7\ days}$: tryptophan-containing and tryptophan-free amino acid diets for 7 days; $t_{-20\ min}$: intraperitoneal injections with clomiphene (4 mg/kg/day) and vehicle 20 min before the drug administration for 7 days (twice a day); $t_{0\ min}$: intraperitoneal injections with ginsenoside Rb1 (10 mg/kg/day) and estradiol (0.1 mg/kg/day) for 7 days (twice a day); $t_{120\ min}$: sacrificed at 120 min after the first drug administration on the 7th day, and the brain was collected to analysis.

Table 3
Experiment group overview of the preventative treatment in experiment design for measuring neurotransmitters.

	$t_{1\_-20\ min}$	$t_{1\_0\ min}$	$t_{3\ days}$	$t_{2\_0\ min}$	$t_{2\_120\ min}$
(i)	Vehicle	Vehicle	Washout	Tryptophan-containing amino acid mixture	Decapitation
(ii)	Vehicle	Vehicle		Tryptophan-free amino acid mixture	Decapitation
(iii)	Vehicle	Ginsenoside Rb1		Tryptophan-free amino acid mixture	Decapitation
(iv)	Vehicle	Estradiol		Tryptophan-free amino acid mixture	Decapitation
(v)	Clomiphene	Ginsenoside Rb1		Tryptophan-free amino acid mixture	Decapitation
(vi)	Clomiphene	Estradiol		Tryptophan-free amino acid mixture	Decapitation
(vii)	Clomiphene	Vehicle		Tryptophan-free amino acid mixture	Decapitation

$t_{1\_-20\ min}$: intraperitoneal injections with clomiphene (4 mg/kg/day) and vehicle 20 min before the drug administration for 14 days (twice a day); $t_{1\_0\ min}$: intraperitoneal injections with ginsenoside Rb1 (10 mg/kg/day) and estradiol (0.1 mg/kg/day) for 14 days (twice a day); $t_{3\ days}$: the washout time; $t_{2\_0\ min}$: an intragastric administration with amino acid mixtures (0.2 ml/10 g) on the experiment day; $t_{2\_120\ min}$: sacrificed at 120 min after an intragastric administration with amino acid mixtures on the experiment day, and the brain was collected to analysis.

intragastric administration with the amino acid mixtures, and the TPH activity was determined by the accumulation of 5-HTP in the brain (pmol/mg tissue).

2.7. AAAD activity assay

AAAD activity was measured as described previously (Gilbert et al., 1995). Briefly, brain samples were homogenized with ice-cold saline. The reaction was initiated by incubating an aliquot of the homogenates with a buffer containing 50 mM sodium phosphate buffer (pH 7.2), 0.1 mM EDTA, 0.17 mM ascorbic acid, 1 mM β-mercaptoethanol, 0.1 mM pargyline, 10 μM pyridoxal-5′-phosphate and 500 μM 5-HTP for 20 min at 37 °C. The reaction was stopped by adding ice-cold perchloric acid (10%), and 5-HT concentration was determined using HPLC-FLU. The protein concentration was determined according to the Lowry method (Lowry et al., 1951). The collection of brain samples and the generation of homogenates are outlined in Section 2.5. The AAAD activity is represented by the concentration of 5-HT in the homogenate (nmol/mg protein/20 min).

2.8. MAO activity assay

The MAO enzyme activity was performed as described previously (Chakrabarti et al., 1998). On the day of analysis, brain samples were homogenized with ice-cold saline. Sample aliquots were pre-incubated at 37 °C for 15 min with selegiline (to assay MAO-A activity) or clorgyline (to assay MAO-B activity) dissolved in phosphate buffer solution to a final concentration of 1 μM. The assay was initiated by the addition of kynuramine (dissolved in phosphate buffer solution to a final concentration of 22 μM) and stopped 20 min later by the addition of trichloroacetic acid (10%). Reaction mixture was centrifuged at 15,000 *g* for 15 min, and the supernatant were mixed with an equal volume of 1 M sodium hydroxide. The concentration of 4-hydroxyquinoline was measured on the FLU-spectrometer at 315 nm excitation and 380 nm emission. The protein concentration was determined according to the Lowry method (Lowry et al., 1951). The collection of brain samples and the generation of homogenates are outlined in Section 2.5. The MAO activity is represented by the concentration of 4-hydroxyquinoline in the homogenate (nmol/mg protein/20 min).

2.9. Behavioral testing

2.9.1. Object recognition memory

The novel object recognition task was conducted as described previously (Rosa et al., 2003; Schroder et al., 2003). Briefly, the task was carried out in a 40 cm × 50 cm open field surrounded by 50 cm high walls made of white plywood with a frontal glass wall. At the beginning of the experiment, all mice were given the opportunity to freely explore the open field for 5 min. During this habituation session, the apparatus was empty, and the light intensity was even throughout the apparatus (about 30 lx). Twenty-four hours after habituation, mice underwent training during which they were placed in the center of the area for 5 min, and two identical objects (object A; Duplo Lego Toys) were positioned in two adjacent corners 10 cm from the walls. One hour after training, mice underwent testing during which they were allowed to explore the open field for 5 min in the presence of one familiar (A) and one novel (B) object. All objects had similar textures, colors and sizes, but distinctive shapes. Between each trial, the open field box and the objects were washed with a 10% ethanol solution. The discrimination index was measured by the difference in the amount of

Table 4
The neurotransmitter concentration in the brain of ovariectomized mice after the acute treatment.

	Control	Model	Ginsenoside Rb1	Estradiol	Clomiphene + ginsenoside Rb1	Clomiphene + estradiol	Clomiphene
Frontal cortex							
Tryptophan	66.3 ± 5.8	34.2 ± 5.9[a]	33.9 ± 5.1[a]	32.3 ± 5.3[a]	27.9 ± 4.1[a]	32.3 ± 4.1[a]	28.3 ± 4.3[a]
Kynurenine	0.68 ± 0.19	0.54 ± 0.12	0.65 ± 0.16	0.66 ± 0.13	0.68 ± 0.10	0.58 ± 0.13	0.54 ± 0.09
5-HT	5.23 ± 0.21	4.20 ± 0.27[a]	4.81 ± 0.31[b]	4.88 ± 0.23[b]	4.36 ± 0.24[ac]	4.32 ± 0.25[ac]	4.21 ± 0.24[a]
5-HIAA	14.5 ± 1.6	11.1 ± 1.0[a]	7.8 ± 1.3[ab]	7.1 ± 0.9[ab]	10.6 ± 1.2[ac]	10.8 ± 1.4[ac]	10.2 ± 1.9[a]
5-HIAA/5-HT	2.76 ± 0.31	2.64 ± 0.42	1.62 ± 0.28[ab]	1.45 ± 0.32[ab]	2.43 ± 0.31[c]	2.50 ± 0.34[c]	2.42 ± 0.36
Dopamine	4.29 ± 0.37	4.08 ± 0.46	4.11 ± 0.29	4.42 ± 0.42	4.03 ± 0.28	4.41 ± 0.32	4.21 ± 0.47
Norepinephrine	5.52 ± 0.53	5.31 ± 0.24	5.42 ± 0.35	5.19 ± 0.36	5.42 ± 0.29	5.25 ± 0.37	5.21 ± 0.39
Striatum							
Tryptophan	45.1 ± 5.2	28.2 ± 4.3[a]	27.9 ± 4.1[a]	26.6 ± 5.1[a]	22.1 ± 3.4[a]	25.2 ± 3.8[a]	23.8 ± 4.2[a]
Kynurenine	0.45 ± 0.13	0.51 ± 0.09	0.49 ± 0.08	0.43 ± 0.11	0.51 ± 0.13	0.54 ± 0.14	0.53 ± 0.12
5-HT	5.11 ± 0.34	4.56 ± 0.32[a]	4.95 ± 0.29[b]	4.91 ± 0.23[b]	4.57 ± 0.26[ac]	4.51 ± 0.28[ac]	4.67 ± 0.33[a]
5-HIAA	8.4 ± 1.2	7.9 ± 1.1	6.0 ± 1.1[ab]	5.8 ± 0.8[ab]	8.2 ± 0.6[c]	7.9 ± 0.9[c]	8.2 ± 1.1
5-HIAA/5-HT	1.84 ± 0.21	1.77 ± 0.27	1.21 ± 0.20[ab]	1.18 ± 0.24[ab]	1.79 ± 0.28[c]	1.75 ± 0.18[c]	1.76 ± 0.26
Dopamine	7.24 ± 0.56	7.01 ± 0.48	7.32 ± 0.51	7.19 ± 0.45	7.21 ± 0.39	7.48 ± 0.48	7.35 ± 0.33
Norepinephrine	3.01 ± 0.26	3.43 ± 0.31	3.21 ± 0.43	3.15 ± 0.32	2.89 ± 0.37	3.08 ± 0.28	3.11 ± 0.41

All results are expressed as mean ± S.E.M. (n = 8). [a]$P<0.05$ vs Control group, [b]$P<0.05$ vs Model group, [c]$P<0.05$ vs similar treatment without clomiphene. The unit of concentrations of endogenous neurotransmitters was pmol/mg tissue.

Table 5
The neurotransmitter concentration in the brain of ovariectomized mice after the chronic treatment.

	Control	Model	Ginsenoside Rb1	Estradiol	Clomiphene + ginsenoside Rb1	Clomiphene + estradiol	Clomiphene
Frontal cortex							
Tryptophan	72.3 ± 6.3	20.3 ± 4.3[a]	18.6 ± 2.7[a]	23.5 ± 3.2[a]	23.4 ± 3.9[a]	18.4 ± 2.4[a]	19.5 ± 3.2[a]
Kynurenine	0.67 ± 0.13	0.69 ± 0.21	0.72 ± 0.18	0.59 ± 0.23	0.65 ± 0.16	0.68 ± 0.21	0.59 ± 0.19
5-HT	5.37 ± 0.32	4.17 ± 0.26[a]	4.69 ± 0.38[ab]	4.78 ± 0.28[ab]	4.09 ± 0.35[ac]	3.97 ± 0.40[ac]	4.17 ± 0.34[a]
5-HIAA	15.7 ± 2.6	11.5 ± 1.2[a]	9.1 ± 1.1[ab]	9.4 ± 0.9[ab]	12.8 ± 1.7[ac]	11.9 ± 1.2[ac]	12.7 ± 0.9[a]
5-HIAA/5-HT	2.77 ± 0.34	2.76 ± 0.41	1.94 ± 0.38[ab]	1.97 ± 0.45[ab]	3.13 ± 0.34[c]	3.00 ± 0.37[c]	3.05 ± 0.34
Dopamine	4.81 ± 0.36	4.67 ± 0.41	4.45 ± 0.51	4.73 ± 0.33	4.61 ± 0.24	4.68 ± 0.35	4.61 ± 0.56
Norepinephrine	6.21 ± 0.53	6.02 ± 0.46	6.43 ± 0.46	6.39 ± 0.32	6.10 ± 0.25	6.36 ± 0.36	6.03 ± 0.53
Striatum							
Tryptophan	54.3 ± 4.6	21.4 ± 3.2[a]	24.7 ± 3.5[a]	19.9 ± 4.2[a]	21.7 ± 3.9[a]	23.6 ± 4.7[a]	25.1 ± 3.2[a]
Kynurenine	0.45 ± 0.09	0.56 ± 0.11	0.52 ± 0.14	0.46 ± 0.09	0.49 ± 0.08	0.56 ± 0.12	0.46 ± 0.08
5-HT	5.13 ± 0.38	4.43 ± 0.21[a]	4.96 ± 0.28[b]	4.88 ± 0.34[b]	4.48 ± 0.33[ac]	4.51 ± 0.21[ac]	4.52 ± 0.31[a]
5-HIAA	9.2 ± 1.5	8.8 ± 1.2	5.9 ± 1.3[ab]	6.1 ± 1.7[ab]	8.4 ± 1.8[c]	8.8 ± 2.1[c]	7.9 ± 1.6
5-HIAA/5-HT	1.79 ± 0.32	1.98 ± 0.23	1.19 ± 0.33[ab]	1.25 ± 0.38[ab]	1.88 ± 0.28[c]	1.95 ± 0.31[c]	1.75 ± 0.35
Dopamine	7.14 ± 0.56	7.23 ± 0.39	6.98 ± 0.48	7.08 ± 0.51	7.31 ± 0.58	6.89 ± 0.71	7.21 ± 0.65
Norepinephrine	4.13 ± 0.43	4.01 ± 0.55	3.89 ± 0.36	4.23 ± 0.45	4.12 ± 0.35	4.15 ± 0.41	4.03 ± 0.34

All results are expressed as mean ± S.E.M. (n = 8). [a]$P<0.05$ vs Control group, [b]$P<0.05$ vs Model group, [c]$P<0.05$ vs similar treatment without clomiphene. The unit of concentrations of endogenous neurotransmitters was pmol/mg tissue.

time spent exploring the old and novel objects using the following equation: discrimination index = $(T_B - T_A)/(T_B + T_A)$. In this equation, T_A was the time spent exploring the familiar object A, and T_B was time spent exploring the novel objects B. Exploration was defined as sniffing or touching the object with the nose and/or forepaws. Mice were assigned to the same treatments as outlined in Section 2.5. In acute, chronic and preventive studies, the trainings were conducted 1 h after the completion of ginsenoside Rb1 and estradiol administration.

2.9.2. Forced swimming test

The forced swimming test was performed as described previously (Porsolt et al., 1978). Briefly, mice underwent a swimming-stress session for 15 min (pre-test). After 24 h, the mice were individually placed into glass cylinders (height: 25 cm, diameter: 10 cm, 10 cm of water) at 24 ± 1 °C for 6 min (test). A mouse was defined as immobile when it ceased to struggle and remained floating motionless in the water, making only small movement necessary to keep its head above water. The duration of the immobility was recorded during the last 4 min of the 6 min testing period. Mice were assigned to the same treatments as outlined in Section 2.5. In acute, chronic and preventive studies, the tests were conducted 1 h after the completion of ginsenoside Rb1 and estradiol administration.

2.9.3. Locomotor activity for mice

The assessment of locomotor activity was carried out on mice. Briefly, the locomotor activity of the mice was measured by an ambulometer with five activity chambers (ZL-2, Institute of Materia Medica, Chinese Academy of Medical Sciences, China). Mice were placed in the chambers and their paws contacted or disconnected the active bars producing random configurations those were converted into pulses. The pulses, which were proportional to the locomotor activity of the mice, were automatically recorded as the cumulative total counts of motor activity. Mice were placed in test chambers, 15 min prior to the evaluation for acclimatization and then locomotion counts were recorded for a period of 5 min. Mice were assigned to the same treatments as outlined in Section 2.5. In acute, chronic and preventive studies, the tests were conducted 1 h after the completion of ginsenoside Rb1 and estradiol administration.

Table 6
The neurotransmitter concentration in the brain of ovariectomized mice after the preventive treatment.

	Control	Model	Ginsenoside Rb1	Estradiol	Clomiphene + ginsenoside Rb1	Clomiphene + estradiol	Clomiphene
Frontal cortex							
Tryptophan	67.3 ± 6.8	27.9 ± 4.3[a]	21.5 ± 3.3[a]	24.3 ± 3.5[a]	20.8 ± 3.8[a]	25.3 ± 2.9[a]	29.1 ± 3.9[a]
Kynurenine	0.68 ± 0.14	0.71 ± 0.21	0.59 ± 0.09	0.63 ± 0.10	0.60 ± 0.12	0.66 ± 0.13	0.62 ± 0.09
5-HT	6.17 ± 0.38	5.18 ± 0.25[a]	5.79 ± 0.29[b]	5.85 ± 0.32[b]	5.23 ± 0.27[ac]	5.32 ± 0.31[ac]	5.35 ± 0.33[a]
5-HIAA	13.2 ± 2.1	8.9 ± 1.9[a]	6.5 ± 1.5[ab]	6.9 ± 1.2[ab]	9.2 ± 1.0[ac]	9.6 ± 1.3[ac]	9.3 ± 1.2[a]
5-HIAA/5-HT	2.14 ± 0.23	1.72 ± 0.25[a]	1.12 ± 0.34[ab]	1.18 ± 0.37[ab]	1.76 ± 0.41[c]	1.82 ± 0.35[c]	1.74 ± 0.45
Dopamine	4.19 ± 0.35	4.24 ± 0.41	4.54 ± 0.31	4.12 ± 0.29	4.02 ± 0.45	4.32 ± 0.35	4.21 ± 0.32
Norepinephrine	5.44 ± 0.41	5.21 ± 0.34	5.03 ± 0.45	5.14 ± 0.36	5.24 ± 0.36	5.54 ± 0.36	5.35 ± 0.42
Striatum							
Tryptophan	59.4 ± 5.8	23.4 ± 4.9[a]	21.5 ± 3.8[a]	26.4 ± 3.1[a]	20.4 ± 2.6[a]	23.5 ± 3.8[a]	22.8 ± 3.1[a]
Kynurenine	0.51 ± 0.09	0.46 ± 0.08	0.53 ± 0.10	0.58 ± 0.11	0.48 ± 0.08	0.51 ± 0.09	0.45 ± 0.07
5-HT	5.15 ± 0.34	4.48 ± 0.29[a]	4.89 ± 0.31[b]	4.96 ± 0.29[b]	4.57 ± 0.30[ac]	4.62 ± 0.24[ac]	4.52 ± 0.31[a]
5-HIAA	9.0 ± 1.1	8.6 ± 1.3	6.2 ± 0.9[ab]	6.5 ± 0.8[ab]	9.3 ± 1.5[c]	9.1 ± 1.7[c]	8.8 ± 1.4
5-HIAA/5-HT	1.74 ± 0.21	1.91 ± 0.32	1.27 ± 0.36[ab]	1.31 ± 0.29[ab]	2.04 ± 0.28[c]	1.97 ± 0.35[c]	1.95 ± 0.28
Dopamine	6.87 ± 0.44	6.75 ± 0.67	6.43 ± 0.53	6.35 ± 0.38	6.35 ± 0.36	6.78 ± 0.45	6.98 ± 0.47
Norepinephrine	3.89 ± 0.31	3.46 ± 0.26	3.56 ± 0.45	3.71 ± 0.46	3.78 ± 0.51	3.51 ± 0.41	3.34 ± 0.46

All results are expressed as mean ± S.E.M. (n = 8). [a]$P<0.05$ vs Control group, [b]$P<0.05$ vs Model group, [c]$P<0.05$ vs similar treatment without clomiphene. The unit of concentrations of endogenous neurotransmitters was pmol/mg tissue.

2.10. Data analysis

Data are expressed as mean ± standard error of mean (S.E.M). Differences between multiple groups were evaluated with one-way ANOVA and Post Hoc analysis using the SPSS software.

3. Results

3.1. Experiment design for measuring neurotransmitters

3.1.1. Acute treatment

As shown in Table 4, compared with the control group, the concentrations of tryptophan in the frontal cortex and striatum in all other groups were significantly lower. The uptake of tryptophan in the brain did not change after the administration with ginsenoside Rb1, estradiol and clomiphene. Therefore, decreased tryptophan concentration only correlated with the composition of the amino acid mixture. There was no significant difference in the kynurenine concentrations among the seven groups. Compared to the control group, 5-HT concentrations in the frontal cortex and striatum decreased in the model group, and both ginsenoside Rb1 and estradiol prevented the decrease of 5-HT concentrations. However, the beneficial effects of ginsenoside Rb1 and estradiol were abolished by a pretreatment with clomiphene. In the clomiphene group, 5-HT concentration did not change in the brain. As shown in Table 4, 5-HIAA concentration in the frontal cortex of the model group was lower than the control group, while no change was observed in the striatum. Compared with the model group, 5-HIAA concentrations were reduced in the ginsenoside Rb1- and estradiol-treated mice. Similarly, the 5-HIAA/5-HT ratios (an indirect index of MAO activity) were significantly decreased in the ginsenoside Rb1- and estradiol-treated mice. These significant changes in 5-HIAA concentrations and MAO activities induced by ginsenoside Rb1 and estradiol were reversed by a pretreatment with clomiphene. The concentrations of dopamine and norepinephrine in the frontal cortex and striatum were unchanged in all groups.

3.1.2. Chronic treatment

As indicated in Table 5, tryptophan concentrations in the frontal cortex and striatum were significantly reduced in all other groups compared to the control group. The kynurenine concentrations were similar in all groups, which might be due to the low kynurenine concentration in the brain, resulting in a minimal impact by tryptophan concentration. Compared with the control group, 5-HT concentration was significantly reduced in the model group. Ginsenoside Rb1 and estradiol resulted in increased 5-HT concentrations in the frontal cortex and striatum of ovariectomized mice with chronic tryptophan depletion, which were consistent with the results in the acute study. Increased 5-HT concentration was attenuated by the addition of clomiphene. As shown in Table 5, 5-HIAA concentrations and the 5-HIAA/5-HT ratios were markedly decreased in ginsenoside Rb1- and estradiol-treated mice compared with the model group. These decreased concentrations were prevented by a pretreatment with clomiphene. Dopamine and norepinephrine concentrations in the frontal cortex and striatum were not affected by administration with ginsenoside Rb1, estradiol and amino acid mixtures.

3.1.3. Preventive treatment

We also tested the effects of a single administration with tryptophan-free amino acid mixture in ovariectomized mice that were chronically administered with ginsenoside Rb1 and estradiol. 5-HT, 5-HIAA, tryptophan, kynurenine, dopamine and norepinephrine concentrations remained normal in every group before intragastric administration with the amino acid mixtures (data not shown), indicating that long-term injections with ginsenoside Rb1 and estradiol did not alter the concentrations of endogenous substances in the frontal cortex and striatum. As shown in Table 6, the tryptophan concentrations in the brain were depressed in groups treated with the tryptophan-free amino acid mixture. After intragastric administration with the tryptophan-containing and tryptophan-free amino acid mixtures, the kynurenine concentrations remained unchanged in all groups. The amino acid mixtures were given 3 days after the injections with ginsenoside Rb1 and estradiol, at which time, any circulating ginsenoside Rb1 and estradiol should be negligible. Two hours after intragastric administration with the tryptophan-free amino acid mixture, 5-HT concentration was reduced in the model group, while the 5-HT concentrations were not reduced in the ginsenoside Rb1- and estradiol-treated groups. These preventive effects were attenuated by pretreatments with clomiphene daily.

3.2. TPH activity assay

Ginsenoside Rb1 and estradiol enhanced 5-HTP accumulations in the frontal cortex and striatum in the acute, chronic and preventive protocols (Fig. 1A, B and C). The TPH activity *in vivo* was expressed by 5-HTP accumulation following a pretreatment with NSD-1015.

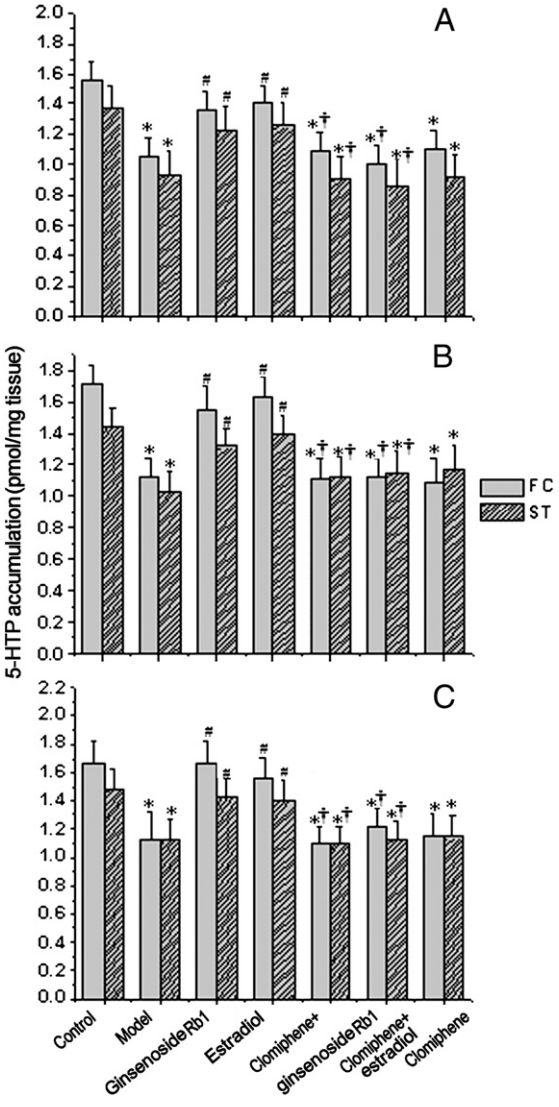

Fig. 1. The activities of TPH in the frontal cortex and striatum of ovariectomized mice injected with ginsenoside Rb1 and estradiol in different treatments. (A), a single intravenous injection with ginsenoside Rb1 and estradiol in the acute treatment; (B), multiple intraperitoneal injections with ginsenoside Rb1 and estradiol in the chronic treatment; (C), multiple intraperitoneal injections with ginsenoside Rb1 and estradiol in the preventive treatment. FC, frontal cortex; ST, striatum. $^{*}P<0.05$ vs control group, $^{\#}P<0.05$ vs model group, $^{\dagger}P<0.05$ vs similar treatment without clomiphene.

Compared to the model group, there were significant increases in the accumulation of 5-HTP in the brain of ginsenoside Rb1- and estradiol-treated mice. However, no change in the accumulation of 5-HTP was observed when animals were pretreated with clomiphene.

3.3. AAAD activity assay

In the enzyme kinetic assay, ginsenoside Rb1 and estradiol did not alter 5-HT concentrations in the frontal cortex and striatum in any group. In addition, the (5-HT + 5-HIAA)/5-HTP ratios *in vivo* were not significantly different across all groups.

3.4. MAO activity

In our studies, MAO-A and MAO-B activities between the model and the control group were comparable. However, ginsenoside Rb1 significantly reduced the activities of MAO-A and MAO-B in the frontal cortex and striatum of ovariectomized mice in various protocols, which were similar to estradiol treatment. These inhibitory effects were abolished by a co-treatment with clomiphene. Detailed observations are shown in Figs. 2 and 3.

3.5. Behaviors test

3.5.1. Object recognition memory

The effects of tryptophan depletion on object recognition are shown in Fig. 4, Compared to the control group, object recognition was impaired in the model group before the training in acute, chronic and preventive treatments. However, discrimination performance was significantly increased after administration with ginsenoside Rb1 and estradiol. These effects were abolished when ovariectomized mice were pretreated with clomiphene.

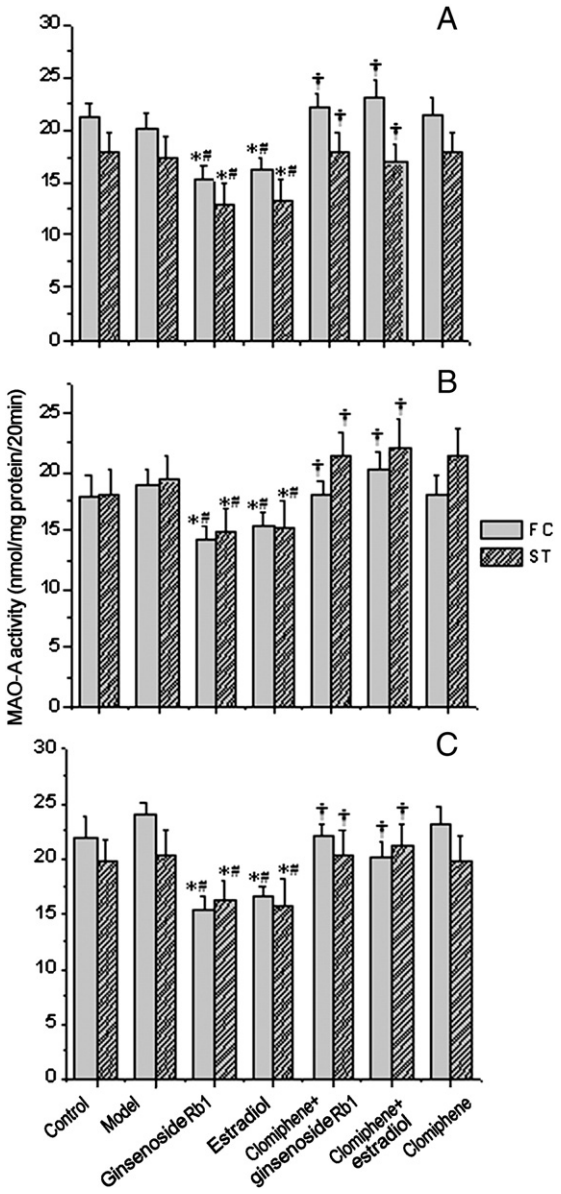

Fig. 2. The activities of MAO-A in the frontal cortex and striatum of ovariectomized mice injected with ginsenoside Rb1 and estradiol in different treatments. (A), a single intravenous injection with ginsenoside Rb1 and estradiol in the acute treatment; (B), multiple intraperitoneal injections with ginsenoside Rb1 and estradiol in the chronic treatment; (C), multiple intraperitoneal injections with ginsenoside Rb1 and estradiol in the preventive treatment. FC, frontal cortex; ST, striatum. $^{*}P<0.05$ vs control group, $^{\#}P<0.05$ vs model group, $^{\dagger}P<0.05$ vs similar treatment without clomiphene.

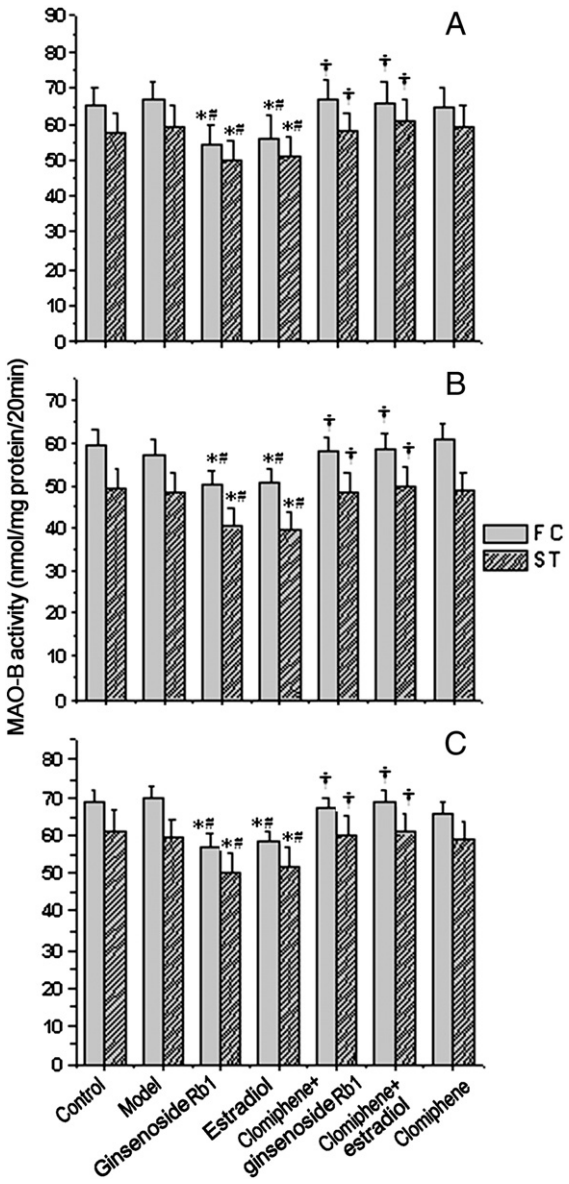

Fig. 3. The activities of MAO-B in the frontal cortex and striatum of ovariectomized mice injected with ginsenoside Rb1 and estradiol in different treatments. (A), a single intravenous injection with ginsenoside Rb1 and estradiol with in acute treatment; (B), multiple intraperitoneal injections with ginsenoside Rb1 and estradiol in the chronic treatment; (C), multiple intraperitoneal injections with ginsenoside Rb1 and estradiol in the preventive treatment. FC, frontal cortex; ST, striatum. $^{*}P<0.05$ vs control group, $^{\#}P<0.05$ vs model group, $^{\dagger}P<0.05$ vs similar treatment without clomiphene.

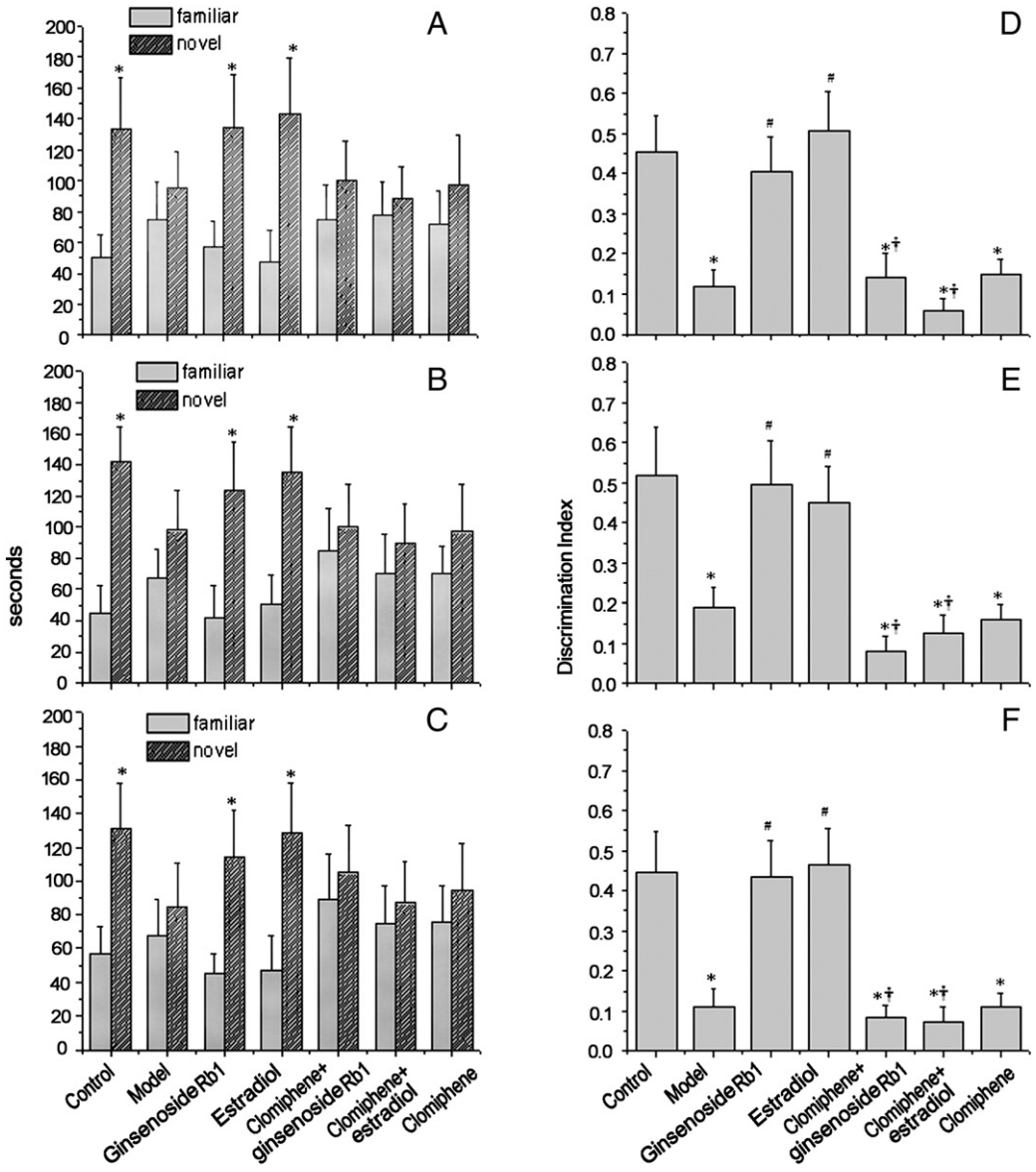

Fig. 4. The effects of ginsenoside Rb1 and estradiol on tryptophan depletion-induced deficit in the object recognition task. (A and D), a single intravenous injection with ginsenoside Rb1 and estradiol in the acute treatment; (B and E), multiple intraperitoneal injections with ginsenoside Rb1 and estradiol in the chronic treatment; (C and F), multiple intraperitoneal injections with ginsenoside Rb1 and estradiol in the preventive treatment. (A, B, and C), exploration time of a familiar object and a novel object in the 3-min test. (D, E, and F), discrimination index of a familiar object and a novel object in the 3-min test. $^{*}P<0.05$ vs control group, $^{\#}P<0.05$ vs model group, $^{\dagger}P<0.05$ vs similar treatment without clomiphene.

3.5.2. Forced swimming test

The anti-depressive effects of ginsenoside Rb1 and estradiol were evaluated in the forced swimming test for ovariectomized mice (Fig. 5). Compared to the control group, the mice in the model group had no obvious change in immobility time. In other groups, ginsenoside Rb1 and estradiol reduced the duration of immobility in the forced swimming test, and these effects were reversed by a pretreatment with clomiphene.

3.5.3. Locomotor activity

Locomotor activity was assessed at the various regimes. There was no significant difference in locomotion counts in different groups.

4. Discussion

In recent years, ginsenoside Rb1 has been shown to improve behavioral dysfunctions (Dang et al., 2009; Itoh et al., 1989; Zhang et al., 1990), and we speculated that these effects could be partially mediated by the 5-HT pathway in the central nervous system. We investigated the effects of ginsenoside Rb1 on the 5-HT concentration in the brain for two reasons: i) ginsenoside Rb1 is a primary monomer of ginsenosides, and it has beneficial pharmacological actions in the central nervous system; and ii) ginsenoside Rb1 is transported into the brain easily, as shown in tissue distribution studies (data not shown). Furthermore, ginsenoside Rb1 metabolism is hindered when it is administered via an intravenous or intraperitoneal injection, but it can be extensively metabolized by intestinal tract flora (Han et al., 2006). Thus, we administered ginsenoside Rb1 via an intravenous or intraperitoneal injection to investigate the effects of ginsenoside Rb1 on neurotransmitters.

Compared with other pharmacological models of 5-HT depletion, tryptophan depletion was chosen because it is specific and non-destructive. Our studies show that ginsenoside Rb1 enhances the 5-HT pathway in the brain of ovariectomized mice after 5-HT depletion. Ginsenoside Rb1 elevated 5-HT concentrations in a range of experimental conditions: after the acute tryptophan depletion, after the chronic tryptophan depletion and as a pretreatment during the acute

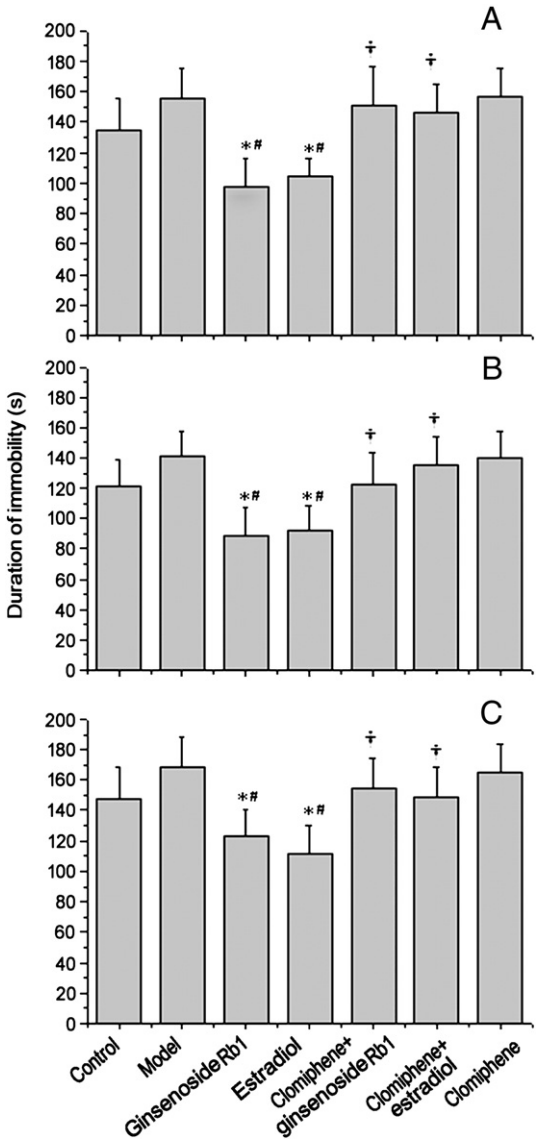

Fig. 5. The effects of ginsenoside Rb1 and estradiol on the duration of immobility in the forced swimming test. (A), a single intravenous injection with ginsenoside Rb1 and estradiol in the acute treatment; (B), multiple intraperitoneal injections with ginsenoside Rb1 and estradiol in the chronic treatment; (C), multiple intraperitoneal injections with ginsenoside Rb1 and estradiol in the preventive treatment. $^{*}P<0.05$ vs control group, $^{\#}P<0.05$ vs model group, $^{\dagger}P<0.05$ vs similar treatment without clomiphene.

tryptophan depletion. Ginsenoside Rb1 and estradiol did not significantly alter kynurenine pathway, which is another pathway involved in tryptophan metabolism. However, the 5-HT pathway is the major route for tryptophan metabolism in the brain. The kynurenine pathway plays a minor role unless it is activated by proinflammatory cytokines, such as interferon-g and tumor-necrosis factor-α, and this is generally associated with several pathologic states (atherosclerosis, congestive heart failure and rheumatoid arthritis) that have a common chronic inflammatory component (Popov et al., 2006; Takikawa et al., 1999). These could explain why kynurenine concentrations were unchanged in our studies.

Ginsenoside Rb1 and estradiol led to elevated 5-HT concentration, indicating that 5-HT synthesis and/or metabolism may have been affected. Thus, we evaluated the activities of TPH, AAAD and MAO in the frontal cortex and striatum. TPH is the rate-limiting enzyme for 5-HT biosynthesis, and it is a possible target for the modulation of neural 5-HT level. We found that ginsenoside Rb1 could enhance TPH activity, resulting in increased 5-HTP accumulation. Accumulating evidences suggest that TPH activity is regulated by enzyme activation and enzyme induction. In another report, *P. ginseng* inhibited the exercise-induced increase of TPH activity in the dorsal raphe of rats (Min et al., 2003), thereby suggesting that TPH is regulated bidirectionally.

AAAD is also involved in 5-HT biosynthesis. Because AAAD exhibits a smaller K_m and a larger V_{max} of AAAD, it may play a minor role in the modulation of 5-HT concentration under normal conditions. In these experiments, the activity of AAAD was unchanged. In addition, based on the observed (5-HT + 5-HIAA)/5-HTP ratios in our studies, we assumed that the activity of AAAD was relatively constant *in vivo*, although detailed evaluations have not been performed.

In addition to TPH and AAAD, the effects of ginsenoside Rb1 on MAO were also studied. MAO has two isoforms (A and B) that differ in their substrate specificity and sensitivity to specific inhibitors. MAO-A is inhibited by clorgyline and preferentially oxidizes noradrenaline and 5-HT, whereas MAO-B is inactivated by selegiline and preferentially targets phenylethylamine (Youdim and Bakhle, 2006). Kynuramine is an unspecific substrate that is metabolized by MAO-A and MAO-B. In these studies, ginsenoside Rb1 inhibited MAO-A and MAO-B in different experiments, thereby suggesting that ginsenoside Rb1 may modulate MAO-A and MAO-B activities through a common mechanism. It remains unclear whether these effects are based on a temporary enzyme inhibition or a permanent enzyme modification through a direct interaction or an indirect feedback mechanism. As indicated in Table 4–6, the metabolism of 5-HT (measured by 5-HIAA/5-HT) was depressed in ginsenoside Rb1-treated mice. These findings indicate that ginsenoside Rb1 not only increased TPH activity but also decreased the MAO activity, which synergistically contributed to increased 5-HT concentration.

Estradiol and an estrogen receptor antagonist were used to better understand the interaction between ginsenoside Rb1 and the 5-HT pathway. Estradiol gave similar results as ginsenoside Rb1. The beneficial effects of ginsenoside Rb1 and estradiol were both abolished by the pretreatment with clomiphene, suggesting that the estrogen receptor may mediate the observed effects of ginsenoside Rb1. Recent studies have indicated that ginsenoside Rb1 can activate estrogen receptor in a ligand-independent manner by a variety of stimuli, including the insulin-like growth factor I (Lee et al., 1997), epidermal growth factor (Kato et al., 1995) and serum (Karas et al., 1998).

The object recognition test was chosen to assess the effects of ginsenoside Rb1 and estradiol on memory performance after tryptophan depletion, because tryptophan depletion has been shown to affect memory in learning tasks (Riedel et al., 1999). The effects of tryptophan depletion on memory function have also been demonstrated in other studies (Rubinsztein et al., 2001; Schmitt et al., 2000). In the present study, when the mice were challenged by tryptophan depletion before the test, object recognition was impaired. These indicate that the concentrations of tryptophan and 5-HT in the brain were related to object recognition performance in ovariectomized mice. These results demonstrate that tryptophan depletion can be used as a 5-HT depletion model in the object recognition task. Estrogen has been shown to improve cognitive effects in several behavioral models (Aenlle et al., 2009; Iivonen et al., 2006) and through multiple possible mechanisms (Fernandez et al., 2008; Inagaki et al., 2010). In our studies, estradiol improved object recognition by increasing 5-HT concentration. Previous research has suggested that ginsenoside Rb1 has beneficial effects on behavior disorders (Kim et al., 1999). Similar to estradiol, ginsenoside Rb1 could also improve the impaired object recognition induced by tryptophan depletion, which was abolished by the pretreatment with clomiphene. These results indicate that ginsenoside Rb1 has an estrogen-like effect in the object recognition task. Ginsenoside Rb1 has also been shown to exhibit neuroprotective effects by reversing the cholinergic deficit caused by scopolamine (Radad et al., 2004), thereby increasing brain-derived neurotrophic factor (Gao et al., 2010) and attenuating beta-amyloid protein-induced

neurotoxicity (Qian et al., 2009). In our studies, ginsenoside Rb1 improved object recognition, which was likely due to enhanced 5-HT turnover. The elevation of neurotransmitter concentrations (5-HT, noradrenaline, and histamine) resulted in elevated cyclic Adenosine monophosphate (cAMP) concentration, which can reverse cAMP-mediated deficit in behaviors that result from a shortage in 5-HT (Rutten et al., 2007). Our results demonstrated that the systemic administration of ginsenoside Rb1 and estradiol before the training improved novel recognition tested 1 h after the training (short term memory) in mice, while no effects were observed for other behavioral parameters such as the training performance and the total amount of time spent exploring both objects.

The forced swimming test was selected to investigate the antidepressant effects of ginsenoside Rb1 and estradiol in ovariectomized mice with tryptophan depletion. In our studies, substantial depletions of tryptophan and 5-HT in the brain had no significant effect on immobility time. These results demonstrated that 5-HT concentration in the brain did not correlate with the immobility time in behavior experiments. Although 5-HT has been linked to the onset and development of depression, there are evidences that some 5-HT depletion models (para-chlorophenylalanine) do not show consistent depressive-like symptoms in the forced swimming test or in learned helplessness (Anisman et al., 1979; Cervo and Samanin, 1991; Martin et al., 1990). This disparity may result from the complexity of depression, which involves abnormalities of the sympathetic nervous system as well as in the endocrine and immune systems (Szelenyi and Selmeczy, 2002). Another consideration for this disparity is that the decreased 5-HT concentration in the brain could not reach a sufficient concentration to induce depression. In previous studies, estrogen had important neuroprotective effects against depression induced by several stimulations (Nakagawasai et al., 2009; Zhu et al., 2009). Similar to estradiol, ginsenoside Rb1 significantly reduced the duration of immobility, which was independent of tryptophan depletion in our studies.

In summary, we provided evidences that ginsenoside Rb1 enhances neural 5-HT concentration in ovariectomized mice by modulating the activities of TPH and MAO. The ginsenoside Rb1-induced elevation in 5-HT concentration and its neuroprotective effects were shown to be estrogen receptor-dependent.

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