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ORIGINAL PAPER

Antidepressant Effects of the Ginsenoside Metabolite Compound K, Assessed by Behavioral Despair Test and Chronic Unpredictable Mild Stress Model

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Abstract
Depression is a major social and health problem worldwide. Compound K (CK), an intestinal metabolite of panaxadiol ginsenosides, has been demonstrated to possess significant pharmacological effects on the central nervous system (CNS). Here, we set up this study to investigate the antidepressant effect of CK, and to explore the potential mechanisms underlying this activity. The behavioral despair model and chronic unpredictable mild stress (CUMS) model were established in mice or rats, respectively. Forced swimming test (FST), tail suspension test (TST) and locomotor activity were performed in mice, while the open-field test, food consumption and sucrose preference were assessed in rats. To investigate the underlying mechanism, the levels of endogenous noradrenaline, dopamine (DA), 5-hydroxytryptamine (5-HT) and their metabolites in the prefrontal cortex (PFC) and hippocampus were detected by HPLC coupled with electron detector. The dopamine degradation enzyme (COMT and MAO) expression was measured by western blot. The BDNF and NGF expression were investigated by immunohistochemical staining analysis. The results showed CK (10, 30 mg/kg) intragastric administration for 14 days significantly shorten the immobility time in FST and TST, which could be partially reversed by a D1 receptor antagonist Sch23390. For CUMS rats, CK alleviated the depressant-like behaviors, including decreased food consumption, spontaneous locomotor activity and lower sucrose preference, while WAY-100635, a 5-HT_{1A} receptor antagonist, could attenuate this effect. In addition, CK increased the levels of 5-HT, DA and their metabolites in the PFC and hippocampus of CUMS rats, and could reverse overexpression of MAO_B in PFC and hippocampus. CK also increased the GSH and GPx activity in the hippocampus and PFC. The IHC results revealed the BDNF and NGF expression were increased in CK-treated rats. The obtained results indicate that CK exhibits antidepressant effects in rodents, which may be due to the regulation of monoamine neurotransmitter concentration, enhancement of antioxidant capacity, as well as increase of neurotrophin expression in the CNS.

Keywords Compound K · Depression · Monoamine neurotransmitter · BDNF · NGF

Introduction

Depression, one of the most common mental disorders, imposes a substantial health burden on society. With the rapid development of modern society, increasing fierce competition and the fast pace of life increase the risk of major depression [1, 2]. However, the mechanism of depression is not fully understood. Traditionally, it is well-documented that the alteration of monoaminergic neurotransmitters contributes to the neurochemical pathophysiology of depression [3–5]. Recently, a leading hypothesis of depression suggests that neurotrophic factors and neurogenesis play critical roles in mediating behavioral responses to antidepressants [6, 7]. A number of studies have demonstrated that stress reduces BDNF expression and its activity in the hippocampus, and this reduction can be reversed by treatment with antidepressant drugs [7].

Ginseng root (*Panax* ginseng C.A. Meyer, Araliaceae) is one of the most popular herbal medicines in China for thousands of years, which extracts shows an antidepressant-like effects clinically. Yamada et al. found that ginsenoside

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Rb1 and its metabolite, compound K, are antidepressant-like components of the ginseng root, and that 5-HT_{2A} receptors may play an important role in mediating the antidepressant-like effect of Rb1 [8]. Bo Jiang et al. reported that ginsenoside Rg1 has antidepressant activity via activation of the BDNF signaling pathway and up-regulation of hippocampal neurogenesis [9]. Several clinical studies indicate that ginseng extracts do improve the depressive state in postmenopausal women, compared with the placebo group [10, 11]. However, it is noteworthy that gensenosides are poorly absorbed from gut, as major ginsenosides are highly glycosylated, whereas ginsenoside metabolite compound K (CK) is easily absorbed. Given its remarkable pharmaceutical activities and readily intestinal absorption, CK has attracted more attention in its pharmacology and therapeutics.

Ginsenoside compound K [20-*O*-β-(glucopyranosyl)-20(S)-protopanaxadiol], the main intestinal bacterial metabolite of ginsenosides (chemical structure shown in Fig. 1) [12], has multiple pharmacological properties, such as anti-inflammatory [13], hepatoprotective [14, 15], antidiabetic [16], antitumor and potential health-promoting effects. Recently, there is growing interest in the neuronal functions of CK apart from its role mentioned above, such as cognition improvement, neuroprotection and neurotransmission modulation (review by [17]). Although CK processes potent neuro-protection effects, there is just only evidence suggests an antidepressant role in the menopausal mice [8]. They found CK dose-dependently prevented the prolongation of immobility time in the forced swimming test. However, it is well documented that the forced swim test measures coping strategies to acute inescapable stress, which is usually used for initial screen antidepressant drugs. Given the unique pharmacokinetics and pharmacology properties, we consequently undertake to further investigation on the antidepressant effect of CK and the underlying mechanism.

In the present study, we first assessed the antidepressant effects of CK, using various models of depression, including the forced swimming test (FST), tail suspension test (TST) and chronic mild stress. Further, to determine the mechanisms underlie the above behavioral effects, we measured the function of monoaminergic system, anti-oxidative status and neurotropic factors expression in the CNS.

Materials and Methods

Drugs and Reagents

Compound K (CAS:39262-14-1) was provided by Sichuan Wickqi biotechnology co., LTD., (HPLC ≥ 98%). 5-hydroxytryptamine (5-HT), 5-hydroxyindoleacetic acid (5-HIAA), dopamine (DA), homovanillic acid (HVA) and 3,4-dihydroxyphenylacetic acid (DOPAC) are HPLC-MS grade and purchased from Sigma–Aldrich (St. Louis, MO, USA). Sch23390 and WAY-100635 were obtained from Selleck Chemicals (Texas, USA). Glutathione peroxidase (GPx) assay kit, glutathione (GSH) assay kit and superoxide dismutase assay kit were from Nanjing Jiancheng Bioengineering Institute (Nanjing, China). Ammonium formate, sodium tetraborate, benzoyl chloride and formic acid were purchased

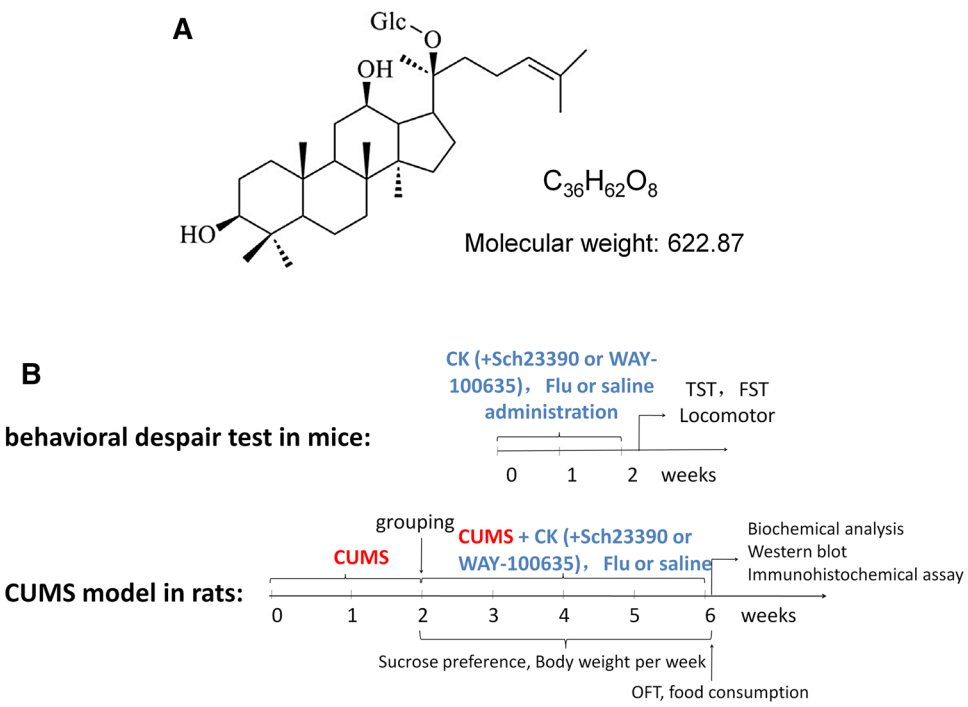

Fig. 1 Chemical structure of compound K (**a**) and Schematic representation of the experimental procedure (**b**). *CUMS* chronic unpredictable mild stress, *FST* forced swimming test, *TST* tail suspension test, *Flu* fluoxetine, *OFT* open-field test

from Sigma–Aldrich (St. Louis, MO, USA). LC/MS grade acetonitrile was purchased from Thermo Fisher.

Animals

Male KM mice, weight 18–22 g, provided by the Experimental Animal Center of the Academy of Military Medical Sciences, certificate number: SCXK (Army) 2016-0004; SD rats, male, weight 180–200 g, by Beijing Huafu Kang Biotechnology Co., Ltd., certificate number: SCXK (Beijing) 2016-004. Animals are kept in the environment of light on at 08:00, at room temperature of 22 ± 2 °C and free to drink water for 3 days.

Experimental Designs

Sixty mice were randomly divided into 5 groups (n = 12), including blank control group, positive drug fluoxetine group and CK (3, 10, 30 mg/kg) groups. Fluoxetine or CK was administered intragastrically once daily for consecutive 14 days. TST was performed on day 13, FST on day 14.

Eighty rats were randomly divided into two groups (12 for the control group and 68 for the CUMS group). After two weeks of CUMS, the 60 rats with less than 70% Sucrose consumption in CUMS group were picked out, and then were split into 5 groups, including the CUMS group, CK (3, 10 and 30 mg/kg) groups and fluoxetine (20 mg/kg) group. Fluoxetine or CK was then administered once daily for 4 weeks. After all the behavioral experiments, the rats were decapitated immediately. Six brain tissues homogenates from each group were used for western-blot assay and detection of monoamine neurotransmitters, while other six brain tissues were used for immunohistochemistry.

In order to verify whether the monoaminergic system is involved in the antidepressant effect of CK, the following experimental groups were studied: (1) control; (2) 30 mg/kg CK (i.g.); (3) 0.05 mg/kg Sch23390 (s.c.); (4) 0.2 mg/kg WAY-100635 (s.c.); (5) CK + Sch23390; (6) CK + WAY-100635. Control group was treated with physiological saline (10 mL/kg, i.g.). The two antagonists were applied 30 min before CK administration. All that 6 groups were subjected to both acute stress (mice, n = 12) and CUMS (rats, n = 12), of which drugs were administered for 2 weeks before the desperate test, and for 4 weeks for CUMS model.

Tail Suspension Test (TST)

The mice were fixed on a Tail Suspension Monitor at a distance of 2 cm from the tail tip, which was in a suspended state and the head was more than 10 cm from the ground. After 2 min adaption, the immobility time was recorded for 4 min. The criteria of immobility are that the animals stop struggling and the body was vertically suspended.

Forced Swim Test (FST)

The mice were placed in an organic glass drum (height: 25 cm, diameter: 12 cm) filled with water (depth of 10 cm, water temperature 23 ± 2 °C). All animals were allowed to swim freely for 6 min, and the duration of immobility in the last 4 min was recorded. Each mouse was judged to be immobile when it stopped struggling, remaining floating motionless in water, and making only those movements necessary to keep its head above water.

CUMS Procedure

The CUMS model was set up as described from previous research with a slight modification [18, 19]. Rats were exposed to different stressors for 6 weeks, including: overnight illumination (continuous illumination for 36 h), fasting for 24 h, water-depriving for 24 h, tilted cage with 45° for 24, behavior restrictions for 1 h, damp bedding, white noise for 24 h, cold water swimming for 5 min (15 °C), rotation on a shaker for 10-min and LED light strobe stimulation. Each stress stimulus is discontinuous and irregular. Sucrose preference test and weight were measured once a week. After 6 weeks, the open field test and food intake were measured.

Sucrose Preference Test

On the first day, animals are free to drink two bottles of 1% sucrose water. The next day, one of the sucrose water was replaced by ordinary distilled water. After 24 h of fasting, the animals were given one bottle of 1% sucrose water and the other bottle of normal tap water (100 mL). After 1 h drinking, the consumption was calculated by weighing the weight of the drinking bottle. The sucrose preference was calculated by the following formula:

$$\text{Sucrose preference} = \frac{\text{sucrose consumption}}{\text{water consumption+sucrose consumption}} \times 100\%$$

Open-Field Test (OFT)

Locomotor activities of mice were measured by OFT. The floor of the apparatus (100 × 100 × 50 cm) with a black line equally divided into 25 squares. The floor and walls of the apparatus are black. The locomotion activities were evaluated by recording the number of grid crossing (three claws of mice into the grid count once) by a camera. Each animal was measured for 10 min.

Measurement of NE, 5-HT and DA Levels in the Hippocampus and Prefrontal Cortex

After all the behavioral experiments, the brain was decapitated immediately, and the prefrontal cortex and the hippocampus were quickly stripped on the ice tray, and then placed in a refrigerator. The samples were prepared using perchloric acid solution (0.1 M $HClO_4$, 0.5 mM EDTA-Na_2, 0.1% L-Cysteine) as described previously. The homogenate was centrifuged at 12,000 rpm (4 °C) for 20 min. The chromatographic conditions were as follows: DiamonsilTMC18 200 × 4.6 mm, inner diameter 5 μm; mobile phase: 85 mmol/L citric acid, 100 mmol/L sodium acetate, 0.2 mmol/L EDTA-Na_2, 1.2 mmol/Sodium ethanesulfonate, methanol (85:15, V / V), pH 3.7. Electrochemical detector working electrode for the glass of carbon, the reference electrode for the solid Ag/AgCl; detection voltage of + 0.7 V; flow rate of 1.0 mL/min; column temperature of 37 °C. External standard curves were used to quantify the amounts of monoaminergic transmitters including NE, 5-HT, 5-HIAA, DA, DOPAC and HVA in each sample, calculated using the area under the curve.

Western-Blot Assay

Rats were sacrificed by cervical dislocation. The hippocampus and prefrontal cortex samples were dissected, weighed, and then homogenized on ice in homogenization buffer containing 50 mM Tris (pH 7.4), 150 mM NaCl, 1% Triton X-100, 0.5 mM EDTA, 0.1% SDS and protease inhibitor mixture. Total protein was extracted from brain tissue through grinder followed RIPI lysate (BI YUN TIAN Biotechnology Institute P0013B). Consequently, the protein concentration of the sample was determined by BCA (BCA Protein Assay Kit, BI YUN TIAN Biotechnology Institute P0012S) method. 10% SDS–PAGE electrophoresis was employed with a 40 μg sample volume and 70/120V constant voltage for 150 min, which followed by electrotransfering to a PVDF membrane (0.45 μm). The membrane was blocked by 5% skim milk under 37 °C for 1 h. After slightly washing, the membrane was incubated with murine internal reference protein β-actin antibody (1:500, Santa Cruz Biotechnology sc-47792), anti-MAO_A rabbit antibody (1:1000, Santa Cruz), anti-MAO_B rabbit polyclonal anti-body (1:500, Santa Cruz) and COMT polyclonal antibody (1:500, Bio-rad) in TBST plus 5% milk, followed by incubation with horseradish peroxidase-conjugated IgG as the secondary antibody. The membrane was then washed with TBST buffer (3 × 10 min) before ECL developing reagent added. Finally, the membrane was scanned by UVP gel image analysis system and the results were indicated by the ratio of the target protein bands' gray value and the internal reference protein β-actin bands' gray value.

Evaluation of BDNF and NGF Expressions in the Hippocampus and Prefrontal Cortex

The animals were intraperitoneally injected with 100 g/L chloral hydrate (40 mg/kg) for anesthesia, and perfused transcardially with saline (30 mL) followed by 4% paraformaldehyde for 30 min (phosphate saline 0.1 M, pH 7.4). The brain tissue was fixed in 40 g/L paraformaldehyde solution for 24 h and then immersed in 150 and 300 g/L sucrose phosphate buffer (4 °C) respectively. After cryopreservation, the brain tissues were cut into coronal sections at a thickness of 20 μm with frozen microtome according to the anatomical map of the brain.

After rehydration and blockage with goat serum for 1 h, the sections were incubated with rabbit polyclonal anti-BDNF antibody (1:00, Santa Cruz), rabbit polyclonal anti-NGF antibody (1:500, Santa Cruz) individually and subsequently reacted with the biotinylated secondary antibody at 37 °C for 1 h then cover slipped and mounted with anti-fade mounting medium. The hippocampal CA1 and CA3 regions and the prefrontal cortex were examined.

Statistical Analysis

Statistical analysis was carried out by SPSS 13.0 software (SPSS Inc., Chicago, IL, USA). All values were expressed as mean ± SEM. Student's *t* test was used to compare the difference between two experimental groups, while one-way ANOVA was used to compare the differences among three or more groups, with post-hoc Tukey's HSD test or Dunnett's *t* test for individual group comparisons. To evaluate the interaction between groups, these data were analyzed by two-way repeated-measures ANOVA followed by Fisher's LSD test. The level of significance was set at $p < 0.05$.

Results

CK Attenuates Depressive-Like Behavior of Mice

To preliminary evaluate antidepressant activity of CK, the desperate behavior test in mice was performed. One-way ANOVA of both TST and FST data revealed significant differences among the five groups (TST: $F_{(4, 55)} = 0.52$, $p < 0.05$; FST: $F_{(4, 55)} = 1.70$, $p < 0.05$). Further post-hoc analysis showed significant difference among CK-treated groups and control group in TST, and the immobility time was significantly decreased in CK groups (10 and 30 mg/kg, $p < 0.01$) in FST (Fig. 2a, b). Fluoxetine, as a positive control, could significantly shorten the immobility time,

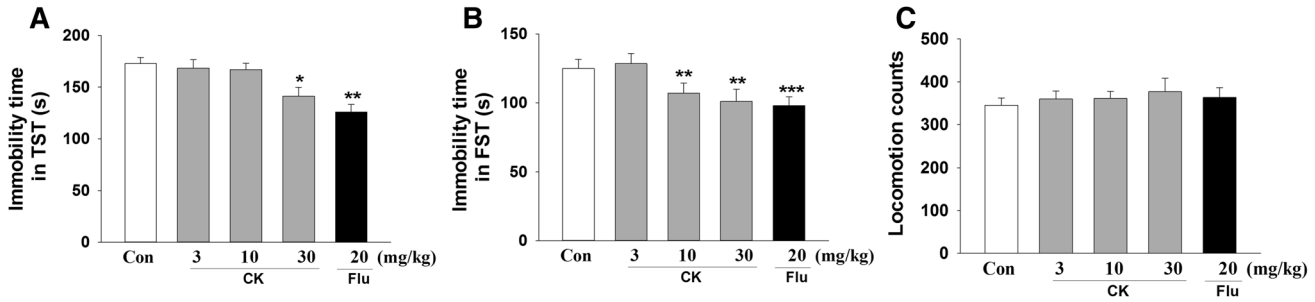

Fig. 2 The effects of CK or fluoxetine on tail suspension test (**a**), forced swimming test (**b**), locomotor activity (**c**) in mice. Fluoxetine or CK was administered intragastrically once daily for consecutive 14 days. *FST* chronic forced swimming test, *TST* tail suspension test. Data represent means ± SEM (n = 11–12/group). For statistical significance, $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ versus control group

compared with the control group in both TST and FST. In addition, after 14 days of administration, CK had no significant effect on the locomotion activity, which indicated that the effects of CK on FST and TST were not related to excitability (Fig. 2c).

CK Attenuates Sucrose Preference, OFT, Food Consumption of CUMS Rats

As shown in Fig. 3a, a independent-sample T test showed the sucrose preference was significantly reduced in the

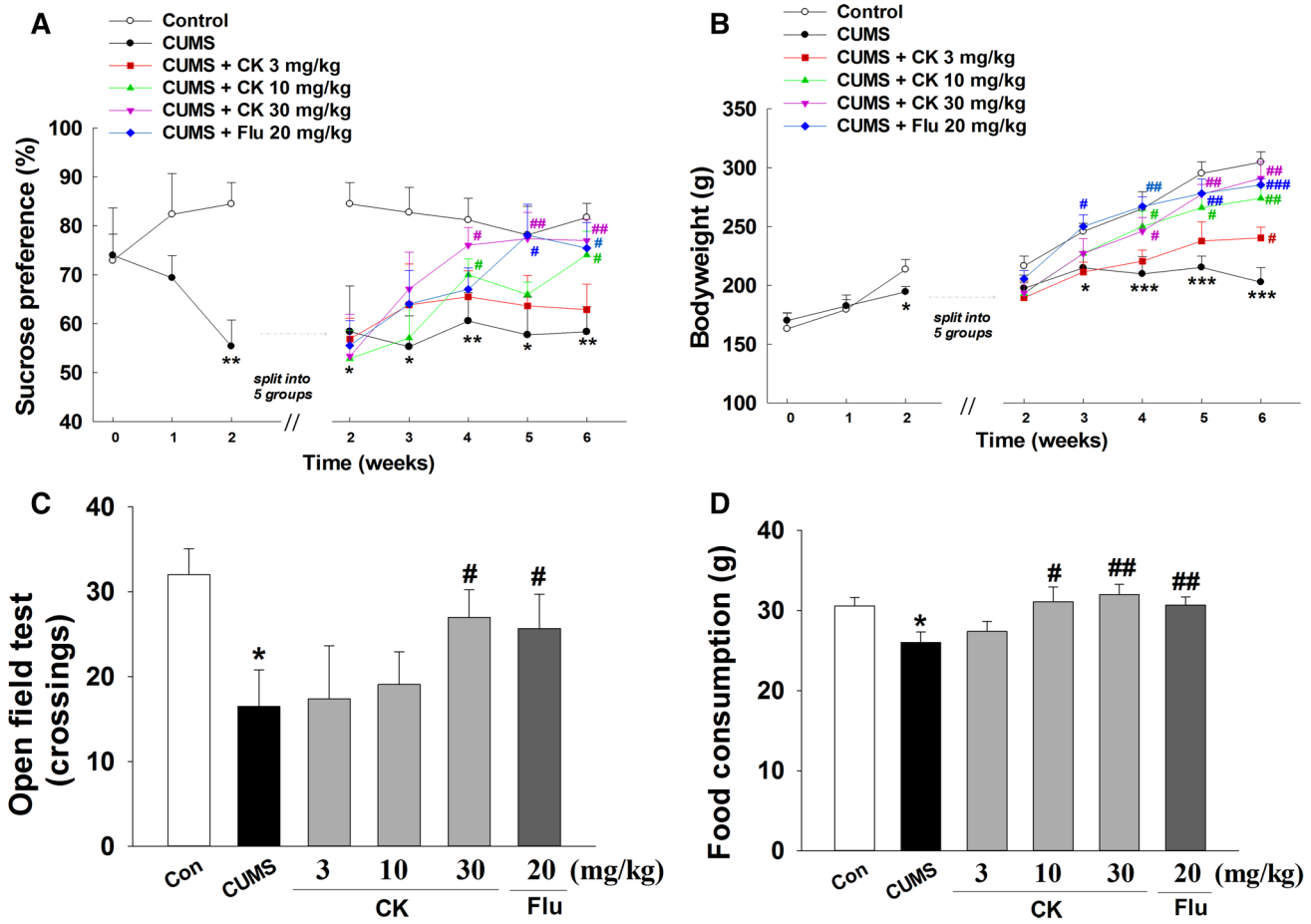

Fig. 3 The effects of CK or fluoxetine on sucrose preference test (**a**), body weight (**b**), open field test (**c**) and food consumption (**d**) in CUMS rats. *CUMS* chronic unpredictable mild stress, *Flu* fluoxetine. The values are expressed as mean ± SEM (n = 10–12/group). For statistical significance, $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ compared with the control group; $^{\#}p < 0.05$, $^{\#\#}p < 0.01$, $^{\#\#\#}p < 0.001$ compared with the CUMS group

CUMS group after 2-week CUMS ($p < 0.01$), indicating that the CUMS model successfully established. A one-way ANOVA showed significant increase of the sucrose preference at 2–4 week CK administration (4th week: $F_{(5,65)} = 15.52$, $p < 0.01$). After 4-week administration, CK significantly increased the sucrose preference to 74.07 ± 4.66% (10 mg/kg, $p < 0.05$) and 76.98 ± 3.39% (30 mg/kg, $p < 0.01$). As shown in Fig. 3b, it was observed that CK significantly increased bodyweight compared to CUMS group (6th week: 3 mg/kg, $p < 0.05$; 10 and 30 mg/kg, $p < 0.01$).

After 4 weeks treatment with CK or fluoxetine, the OFT and food consumption were investigated (Fig. 3c, d). The one-way ANOVA test revealed significant difference in OFT ($F_{(5,65)} = 9.39$, $p < 0.01$) and in food consumption ($F_{(5,65)} = 6.08$, $p < 0.05$). The frequency of crossings and food consumption of CUMS rats were significantly lower than those of the control rats ($p < 0.05$). Compound K at 30 mg/kg or fluoxetine could significantly increase that to control levels.

CK Modulates the Function of Monoaminergic System in the Hippocampus and PFC of CUMS Rats

The concentrations of NE, 5-HT, DA and their metabolites in the hippocampus were presented in Table 1. A one-way ANOVA showed significant difference of DA levels ($F_{(5,30)} = 9.07$, $p < 0.05$) and 5-HT levels ($F_{(5,30)} = 53.14$, $p < 0.001$). CUMS exposure resulted in the decrease of hippocampal DA and 5-HT levels ($p < 0.05$, $p < 0.001$, respectively). Compared to CUMS group, CK (3, 10, 30 mg/kg) increased DA levels ($p < 0.05$), while CK (3, 30 mg/kg) increased 5-HT levels in the hippocampus (each $p < 0.05$). Further, CK (10, 30 mg/kg) reversed the increased the ratio

Table 1 Effects of CK or fluoxetine treatment on monoamine neurotransmitter and their metabolites concentrations in hippocampus of CUMS rats

	Con	CUMS	CUMS + CK 3	CUMS + CK 10	CUMS + CK 30	CUMS + Flu 20
DA	46.85 ± 6.03	38.33 ± 6.78*	44.01 ± 7.65$^{\#}$	46.78 ± 11.45$^{\#}$	50.60 ± 8.03$^{\#\#}$	57.43 ± 8.47$^{\#\#}$
DOPAC	41.58 ± 4.88	39.55 ± 6.50	41.99 ± 6.74	38.70 ± 3.23	43.06 ± 6.96$^{\#}$	46.75 ± 5.23$^{\#}$
HVA	45.88 ± 5.07	42.39 ± 7.69	43.78 ± 1.61	42.22 ± 7.62	45.07 ± 8.30	47.00 ± 6.97
DOPAC/DA	0.89 ± 0.06	1.03 ± 0.12	0.95 ± 0.17	0.82 ± 0.15$^{\#}$	0.84 ± 0.09$^{\#}$	0.81 ± 0.07$^{\#}$
HVA/DA	0.97 ± 0.12	1.11 ± 0.23	0.98 ± 0.15	0.91 ± 0.13$^{\#}$	0.89 ± 0.07$^{\#}$	0.82 ± 0.18$^{\#\#}$
5-HT	177.17 ± 14.97	96.44 ± 12.18***	122.46 ± 9.25$^{\#}$	116.78 ± 15.91	152.55 ± 22.77$^{\#}$	168.84 ± 6.62$^{\#\#\#}$
5-HIAA	238.03 ± 24.82	193.4 6 ± 17.62*	191.18 ± 13.17	203.77 ± 21.53	211.13 ± 14.61$^{\#}$	258.91 ± 15.98$^{\#}$
5-HIAA/5-HT	1.34 ± 0.17	2.24 ± 0.28***	1.56 ± 0.14$^{\#\#\#}$	1.72 ± 0.10$^{\#}$	1.37 ± 0.15$^{\#\#\#}$	1.53 ± 0.26$^{\#\#}$
NE	73.13 ± 8.64	56.05 ± 9.65*	54.39 ± 11.86	60.98 ± 9.85	64.13 ± 10.90	69.75 ± 7.36$^{\#}$

Data are shown as mean ± SEM (n = 6 rats/group). Neurotransmitter concentrations were expressed as ng/g brain region wet weight

CUMS chronic unpredictable mild stress; *Flu* fluoxetine

*$p < 0.05$, ***$p < 0.001$ compared with control group; $^{\#}p < 0.05$, $^{\#\#}p < 0.01$, $^{\#\#\#}p < 0.001$ compared with CUMS group

Table 2 Effects of CK or fluoxetine treatment on monoamine neurotransmitter and their metabolites concentrations in prefrontal cortex of CUMS rats

	Con	CUMS	CUMS + CK 3	CUMS + CK 10	CUMS + CK 30	CUMS + Flu 20
DA	54.12 ± 6.04	49.92 ± 7.34	47.44 ± 7.56	51.98 ± 5.68	58.50 ± 8.64$^{\#}$	52.91 ± 5.36
DOPAC	62.95 ± 6.64	57.27 ± 5.15	59.29 ± 11.43	60.87 ± 6.80	63.61 ± 7.32$^{\#}$	58.30 ± 8.27
HVA	68.22 ± 7.72	69.34 ± 9.21	67.86 ± 4.46	72.10 ± 8.69	64.27 ± 6.07	66.80 ± 4.69
DOPAC/DA	1.16 ± 0.08	1.17 ± 0.10	1.26 ± 0.15	1.18 ± 0.07	1.10 ± 0.06	1.12 ± 0.09
HVA/DA	1.26 ± 0.11	1.40 ± 0.07	1.42 ± 0.12	1.41 ± 0.13	1.11 ± 0.14$^{\#}$	1.26 ± 0.17
5-HT	263.60 ± 21.45	121.75 ± 17.87***	143.24 ± 22.40	187.89 ± 14.96$^{\#}$	210.12 ± 20.79$^{\#\#\#}$	196.12 ± 16.52$^{\#}$
5-HIAA	256.37 ± 17.14	227.99 ± 22.71	214.94 ± 15.37	253.19 ± 21.56	287.16 ± 19.96$^{\#}$	281.80 ± 22.53$^{\#}$
5-HIAA/5-HT	0.97 ± 0.10	1.85 ± 0.26***	1.49 ± 0.17$^{\#}$	1.35 ± 0.13$^{\#}$	1.36 ± 0.21$^{\#\#}$	1.43 ± 0.18$^{\#}$
NE	102.57 ± 15.82	72.44 ± 8.80**	77.16 ± 10.01	75.33 ± 8.06	82.18 ± 9.90	95.51 ± 7.63$^{\#}$

Data are shown as mean ± SEM (n = 6 rats/group). Neurotransmitter concentrations were expressed as ng/g brain region wet weight

CUMS chronic unpredictable mild stress; *Flu* fluoxetine

*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ compared with control group; $^{\#}p < 0.05$, $^{\#\#}p < 0.01$, $^{\#\#\#}p < 0.001$ compared with CUMS group

of 5-HIAA/5-HT, DOPAC/DA and HVA/DA. In addition, the metabolites of DA and 5-HT were also increased in 30 mg/kg group. The concentrations of NE, 5-HT, DA and their metabolites in the PFC were presented in Table 2. One-way ANOVA showed significant difference in 5-HT levels ($F_{(5,30)} = 72.66$, $p < 0.001$), as well as DA levels ($F_{(5,30)} = 7.50$, $p < 0.05$). CK (3, 10, 30 mg/kg) increased the 5-HT levels and decreased the 5-HIAA/5-HT ration in PFC ($p < 0.05$). In addition, CUMS caused a reduction in NE levels in both hippocampus and PFC, which was not affected by CK administration.

We then examined the expression of monoamine neurotransmitter degradation enzymes (MAO_A, MAO_B and COMT) in the hippocampus and PFC (Fig. 4). The data showed that CUMS increased MAO_B expressions, while CK (30 mg/kg) could reverse this overexpression in the hippocampus and PFC. In addition, CK at 30 mg/kg could down-regulated the MAO_A expression in PFC. In contrast, One-way ANOVA showed no significant difference in COMT data.

In order to further investigate whether the monoaminergic system is involved in the antidepressant effect of CK, Sch23390, a D1 receptor antagonist and WAY-100635, a 5-HT_{1A} receptor antagonist were administrated before CK. In the desperate behavior test (Fig. 5a, b), the co-administration of Sch23390 and CK significantly increased the immobility time of TST and FST, compared to the CK group (one-way ANOVA, $p < 0.05$ for both), while Sch23390 itself did not show significant effect on the despair test. A two-way ANOVA showed a significant interaction between Sch23990 and CK (CK × Sch23390 interaction: $F_{(1, 44)} = 1.09$, $p < 0.05$), suggesting that D1 receptor may be involved in the role of CK in improving desperation behavior. WAY-100635 showed no significant interaction with CK in either TST or FST assay. As shown in Fig. 5c, d, for the CUMS model, WAY-100635 was able to attenuate the effect of CK on increasing glucose preference (CK × Sch23390 interaction: $F_{(1, 42)} = 2.17$, $p < 0.05$), whereas Sch23390 did not show this effect.

CK Reverses BDNF and NGF Decline in the Cortex and Hippocampus of CUMS Rats

As shown in Fig. 6, statistical analysis revealed that CUMS exposure resulted in the decline of BDNF and

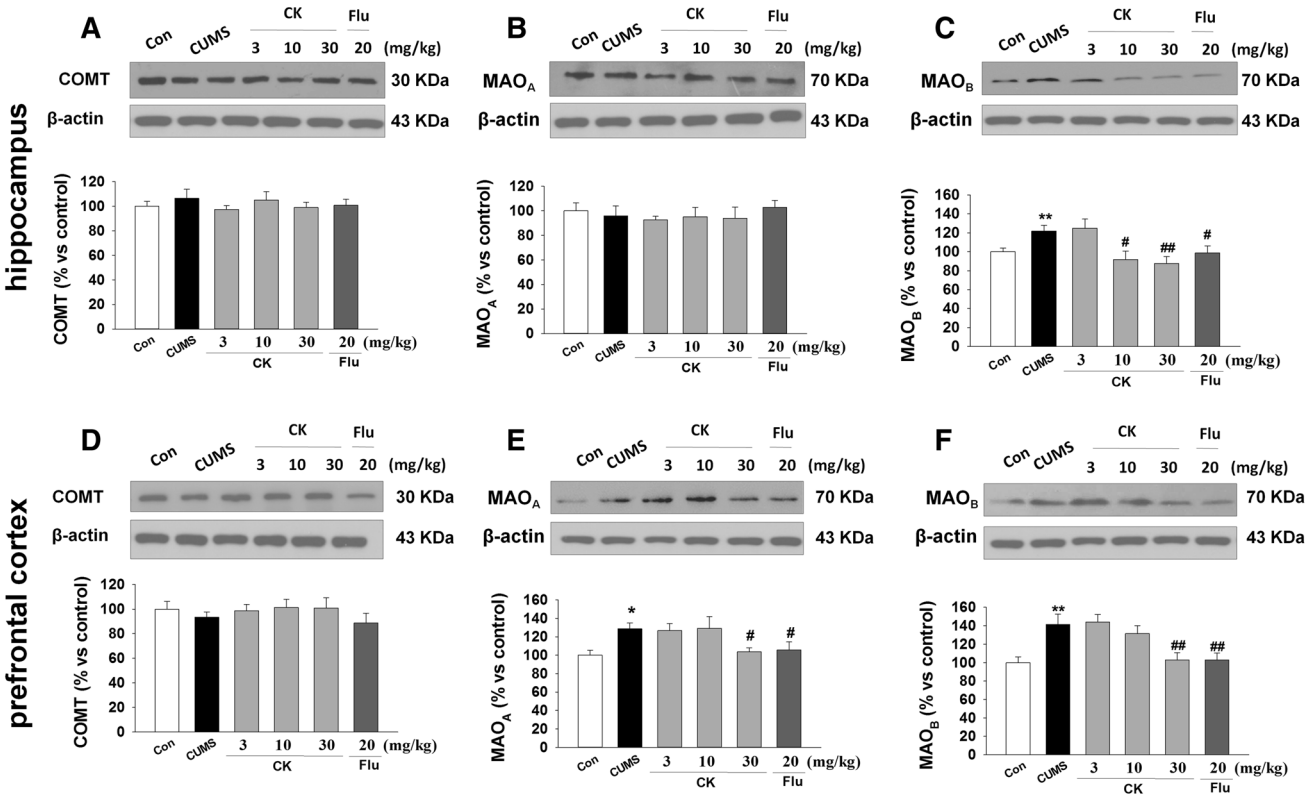

Fig. 4 The effects of CK on the degradation of monoamine neurotransmitters in hippocampus (**a, b** and **c**) and PFC (**d, e** and **f**), including COMT, MAO_A and MAO_B protein expression. *COMT* catechol-*O*-methyltransferase, *MAO* monoamineoxidase. The relative optical densities normalized to β-actin are shown below the bands. Data are represented as mean ± SEM (n = 4–6/group). $^{*}p < 0.05$, $^{**}p < 0.01$ versus control group; $^{\#}p < 0.05$, $^{\#\#}p < 0.01$ versus CUMS group

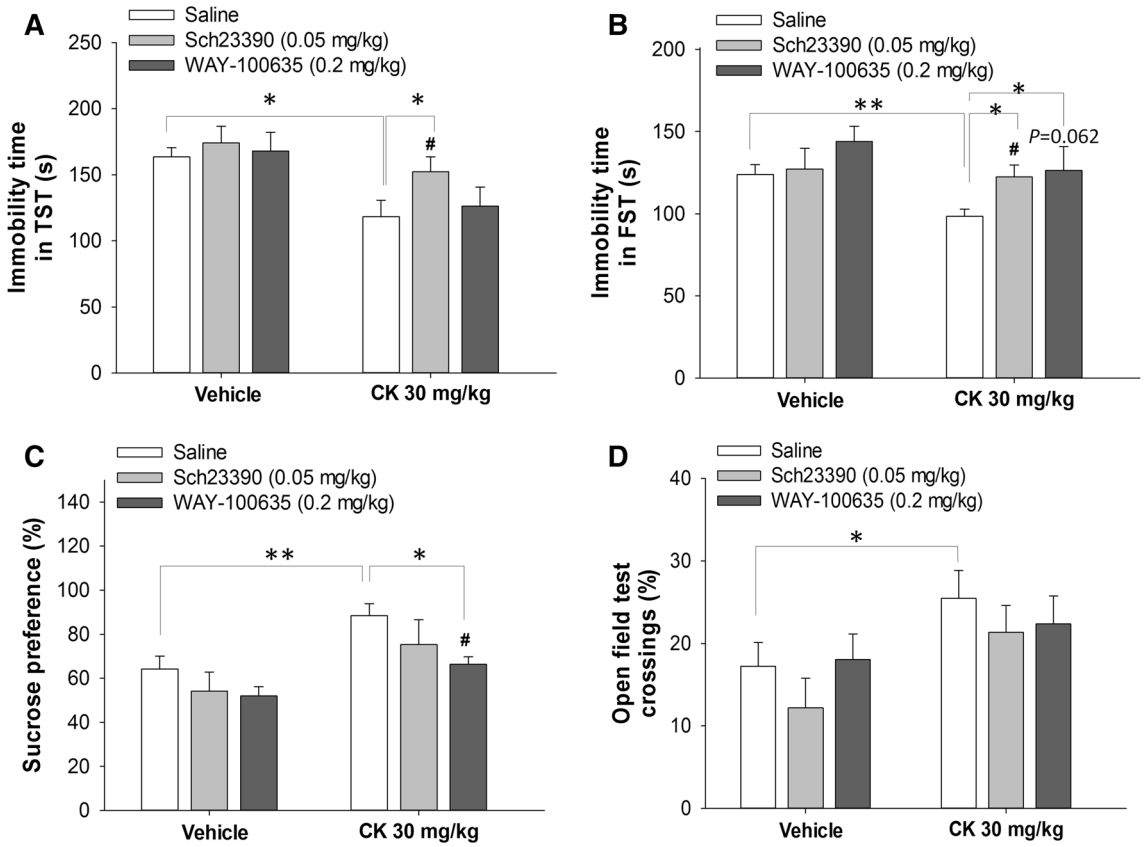

Fig. 5 The effects of D1 antagonist Sch23390 and 5-HT_{1A} antagonist WAY-100635 on CK's antidepressant behavioral effects. Sch23390 (0.05 mg/kg) or WAY-100635 (0.2 mg/kg) was administered (s.c.) 30 min before CK once daily for consecutive 14 days, and TST (**a**) was performed on day 13, FST (**b**) on day 14. For CUMS model study, after 2 weeks of CUMS, the antagonists were administrated respectively for 4 weeks, and then sucrose preference (**c**) and open field test (**d**) were performed. *FST* chronic forced swimming test, *TST* tail suspension test. Data represent means ± SEM (n = 12/group). $^{*}p < 0.05$, $^{**}p < 0.01$, one-way ANOVA with post-hoc Tukey's HSD test or Dunnett's *t* test; $^{\#}p < 0.05$, two-way repeated-measures ANOVA interaction

NGF protein levels in hippocampus (CA1 and CA3 region) and PFC (one-way ANOVA: $p < 0.05$ each) compared to control groups. Administration of CK produced an increment of BDNF and NGF levels. In contrast, more significant changes were found in the content of NGF, because even low doses of CK can also increase the level of NGF ($p < 0.05$).

CK Restores CUMS-Induced Depletion of GSH and GPx

To investigate whether CK can restore CUMS-induced depletion of endogenous antioxidants, we examined the activities of GSH, GPx and SOD in the hippocampus and PFC. As shown in Fig. 7, compared with control group, CUMS induced a significant reduction in GSH and GPx, but had no significant effect on SOD. Compared with CUMS group, CK significantly restored CUMS-induced depletion of GSH (30 mg/kg, in hippocampus and PFC, $p < 0.05$) and GPx (30 mg/kg, in hippocampus and PFC, $p < 0.05$; 3–30 mg/kg, in PFC, $p < 0.05$) levels.

Discussion

In the present study, the antidepressant effect of CK was evaluated in rodents. The data showed that administration of CK alleviated despair behaviors in both FST and TST. In addition, rats exposed to CUMS for 6 weeks showed decreased body weight, sucrose consumption, food intake and locomotor activity decline. Administration of CK significantly alleviated the CUMS-induced depression-like behaviors. Further, CK reversed CUMS-induced reduction of serotonin (5-HT) and dopamine (DA) levels in the rat PFC and hippocampus. Further, CUMS increased MAO expressions, while CK (10, 30 mg/kg) could reverse this overexpression. The BDNF and NGF expression in these brain regions were also increased by CK administration.

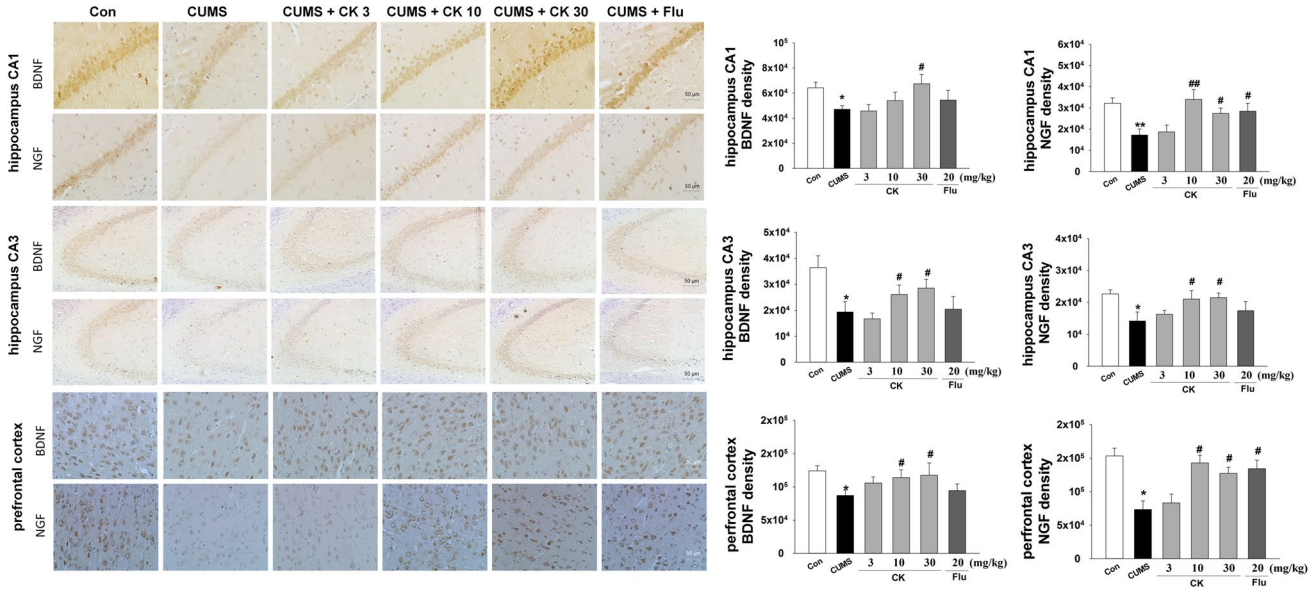

Fig. 6 The effect of CK on the BDNF and NGF expression in the hippocampus CA1, CA3 region and PFC of CUMS rats. Positive cells are represented as brown spots. Bar = 50 μm. *CUMS* chronic unpredictable mild stress, *BDNF* brain derived neurotrophic factor; *NGF* nerve growth factor. The level of staining density was quantified by Image-Pro Plus 6.0 and presented as mean ± SEM (n = 5–6/group). For statistical significance, $^{*}p < 0.05$, $^{**}p < 0.01$ compared with the control group; $^{\#}p < 0.05$, $^{\#\#}p < 0.01$, compared with the model group

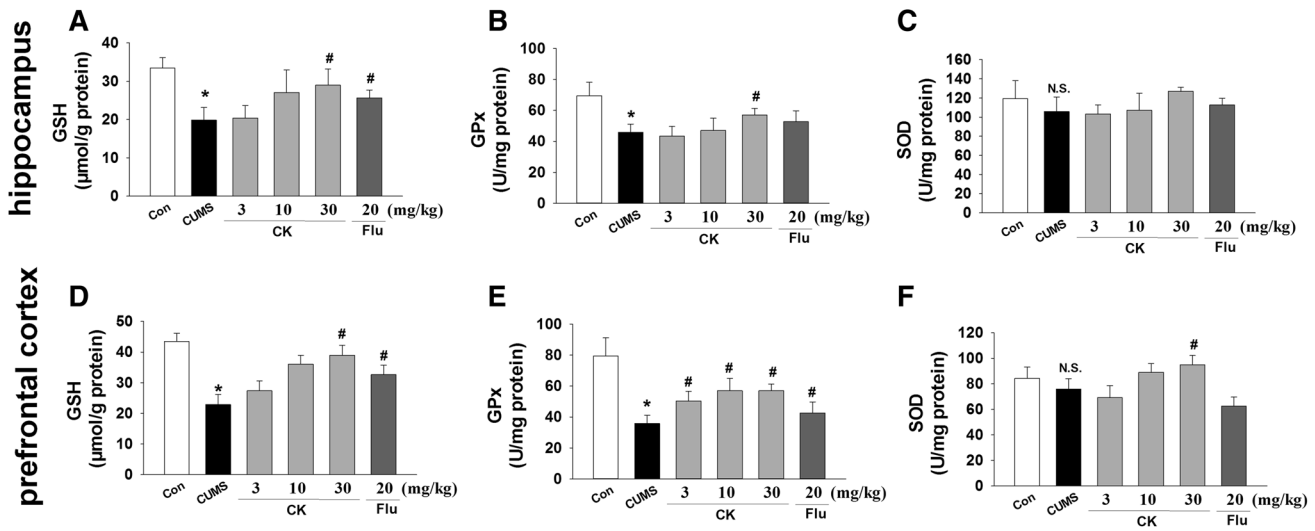

Fig. 7 The effect of CK on brain antioxidant status. **a**–**c** in hippocampus, **a** GSH; **b** GPx; **c** SOD; **d**–**f** in PFC, **d** GSH; **e** GPx; **f** SOD. *GSH* glutathione, *SOD* superoxide dismutase, *GPx* glutathione peroxidase. The values are expressed as mean ± SEM (n = 6/group). For statistical significance, $^{*}p < 0.05$ compared with the control group; $^{\#}p < 0.05$ compared with the CUMS group

The decrease of monoamine neurotransmitters, especially for 5-HT, is one of the most important causes of depression. We therefore examined the levels of monoamine neurotransmitters and their metabolites. The results showed that CK could increase the levels of DA and 5-HT, but no obvious selectivity was found. In addition, we found that CK had no significant effect on DA metabolites (DOPAC and HVA) or 5-HT metabolites (5-HIAA), suggesting that CK is more likely to enhance monoamine-neurotransmitters neuronal function. We also found that D1 receptor antagonist and 5-HT_{1A} receptor antagonist could attenuate the antidepressant effects of CK with different degrees. The effect of D1 receptor antagonists is more pronounced under acute stress, while the 5-HT_{1A} receptor in CUMS model. This complex result may be due to different depression models mimicking different

aspects of depression. However, these experimental results suggest that D1 and 5-HT_{1A} receptors may be involved in the antidepressant effects of CK. Interestingly, we also found that CK increased the expression of MAO_B in the hippocampus, but had little effect on COMT and MAO_A expression, indicating that regulation of monoamine neurotransmitters cannot be excluded and may be one of the mechanisms of antidepressant effect of CK.

Several studies found that the levels of BDNF in hippocampus and prefrontal cortex were significantly decreased in depressive patients [20]. Several antidepressants could promote the survival of neurons by increasing BDNF expression, synaptic plasticity and neurogenesis [21, 22]. Hiroyuki Koike's study showed that the BDNF-TrkB pathway is a potential target for the development of novel antidepressants [23]. Neuronal growth factor (NGF), as the first discovered neurotrophic factor, with neuroprotective activity, promotes neuronal differentiation and induces nerve fiber directional growth and regeneration. Ritabrata et al. [24] found that the expressions of NGF and TrkA in hippocampus of depressive model rats were significantly decreased, while ERK1/2 and AKT, the downstream of TrkB, were down-regulated. The improvements in BDNF and NGF levels provide new insights into the treatment of depression. This study found that CK can increase the levels of BDNF and NGF, which may be one of the possible mechanisms of CK antidepressant. Similar to CK, other ginseng active components also have the function of regulating BDNF and NGF. A series of studies have found that S111 increases the level of BDNF in the hippocampus and has a comparable antidepressant activity with fluoxetine [25]. Another study found that ginsenoside Rg1 upregulated CUMS rat hippocampus BDNF signaling pathway and increased dendritic spine density [26]. Therefore, increasing the neurotrophic factor may be a common mechanism of ginsenoside's antidepressant effects.

Oxidative stress in the brain is a result of accumulated free radical damage that produces signs of molecular, cellular, and clinical pathological phenotypes when the neural antioxidant defenses of the organism are inadequate to counter the ROS, which are constantly produced in the processes of respiration and energy production [27]. A series of evidence shows oxidative stress is closely related to depression [28, 29], that damages the mitochondria and causes neuronal apoptosis in the cortex and hippocampus. Antioxidants are considered to be potential therapeutics for neuropsychiatric disorders [30]. In the present study, we found CUMS induced a significant reduction in GSH and GPx in brain, which is consist with previous studies [31, 32]. Based on the potent antioxidant capacity of CK [33], we examined its effects on CUMS-induced brain antioxidant enzymes. We found that CK could increase the levels of GSH and GPx in the brain, but had little effect on SOD activity. Therefore, improving the antioxidant capacity of the central nervous system may also be involved in the antidepressant effects of CK.

A large number of studies have shown that ginseng or its extract exhibit antidepressant effects, but most studies focus on common ginsenosides. Wang et al. [34] have found that ginsenoside Rb1 has a potent antidepressant effect by regulating the noradrenergic, serotontergic and dopaminergic neurotransmitters in the central nervous system. Another study of Rb1 found that Rb1 exerted antidepressant effects by affecting 5-HT_{2A} receptors [8]. Further, a series of studies have shown that Ginseng total saponins (GTS) alleviated the depressant behaviors, which can not only improve the concentration of monoamine neurotransmitters and BDNF in the hippocampus [35], but also enhance the GSK-3β inhibitory phosphorylation [36]. In addition, the antioxidant effect of the acidic polysaccharide portion of ginseng was observed in depressant model rats, similar to many of the current antidepressant and mood stabilizing drugs [37]. 20(S)-protopanaxadiol (S111), other intestinal ginsenoside intestinal metabolites, exhibited antidepressant-like activity as potent as fluoxetine. The results of the present study show that ginseng antidepressant active substances may also include CK.

Here, as the first report of the antidepressant effect of CK in CUMS model, we found this effect may be due to increasing of monoamine neurotransmitters and upregulating the expression of BDNF and NGF. But other possible mechanisms cannot be rule out. Mounting evidence has confirmed the neuroprotective effect of CK. For example, CK exhibited strong anti-inflammatory activity in LPS or Aβ-induced microglia, by inhibiting the release of inflammatory factors [38]. Some studies have suggested that CK's anti-inflammatory effect is mediated by regulation of MAPKs, ROS and NF-κB pathways [39]. In addition, CK can also regulate the central neurotransmitters release, especially the GABAergic nervous system. Bae et al. found that CK (10 μM) increased the spontaneous release of GABA in the hippocampal CA1 region through synaptic membrane calcium release from Ca^{2+} stores [40]. In addition, CK also has the characteristics of GABA agonists and the characteristics of NMDA receptor antagonists [41]. It is well known that GABA enhances neuronal activity and increases the level of GABA neurotransmitter with antidepressant effects. Thus, anti-inflammatory in CNS and regulation of amino acid neurotransmitters may be the mechanism of CK's antidepressant activity as well.

In summary, the obtained results indicate that CK exhibits antidepressant effects, which may be due to the regulation of monoamine neurotransmitter concentration, enhancement of antioxidant capacity, as well as increase of neurotrophin expression in the CNS.

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