1. Introduction

Major depressive disorder is a serious mental disorder characterized by a pervasive and persistent low mood that is accompanied by low self-esteem and a loss of interest or pleasure in normally enjoyable activities. Until recently, the pathophysiology of depression has not been well characterized. Monoamine deficiency, hypothalamic–pituitary–adrenal axis hyperactivity and neurotrophin reduction are involved in the pathophysiology of depression. The neurotrophin hypothesis of depression, which was proposed by Duman et al. (1997), has attracted increased attention due to its anti-inflammatory properties (Li et al., 2015a,b). However, it is unclear whether the neurotrophic system is involved in the antidepressant-like effects of apigenin.

Flavonoid intake has alleviated depression-like symptoms in both clinical trials and experimental models (Volz, 1997; Ekeanyanwu and Njoku, 2015). Apigenin (4′,5,7-trihydroxyflavone), a flavonoid widely presents in many plants, has various pharmacological effects, including antioxidant, antitumor, anti-inflammatory and nephroprotective activities. Previous studies found that apigenin exerted antidepressant-like effects in rodents (Nakazawa et al., 2003; Yi et al., 2008). Recently, studies demonstrated that apigenin produced antidepressant-like effects due to its anti-inflammatory properties (Li et al., 2015a,b). However, it is unclear whether the neurotrophic system is involved in the antidepressant-like effects of apigenin. Therefore, our present study was aimed to investigate whether the antidepressant-like effect of apigenin was mediated by BDNF expression.
and performed in accordance with the published guidelines of the China Council on Animal Care.

2.2. Chemicals and reagents

Corticosterone was purchased from TCI Development Co., Ltd. (Tokyo, Japan). Apigenin (purity > 98% by HPLC) was purchased from Shandong Huike Botanical Development Co., Ltd. (Xi’an, China). Fluoxetine hydrochloride was purchased from Changzhou Siyao Pharmaceuticals Co., Ltd. (Changzhou, China).

2.3. Drugs treatment

Mice were randomly divided into five groups (n=8): control-vehicle group, corticosterone-vehicle group, fluoxetine group (20 mg/kg) and apigenin groups (20 and 40 mg/kg). All groups except the control-vehicle group received a corticosterone injection for 21 days. The corticosterone was dissolved in physiological saline containing 0.1% dimethyl sulfoxide (DMSO) and 0.1% Tween-80, and injected subcutaneously (s.c) once daily at a dose of 40 mg/kg (Ali et al., 2015). The control-vehicle group was injected with the same volume of physiological saline containing 0.1% DMSO and 0.1% Tween-80. Apigenin and fluoxetine were suspended in physiological saline and administrated by oral gavage 30 min prior to the corticosterone injection for the following 21 days.

2.4. Sucrose preference test

The sucrose preference test was carried out at the end of corticosterone treatment according to the previous report (Mao et al., 2014). Briefly, before the test, the mice were trained to adapt to sucrose solution (1%, w/v): two bottles of sucrose solution were placed in each cage for 24 h, and then one bottle of sucrose solution was replaced with water for 24 h. After the adaptation, the mice were deprived of water and food for 24 h. The test was conducted at 9:30 a.m. in which the mice were housed in individual cages and had free access to two bottles containing sucrose solution and water, respectively. After 24 h, the weights of the consumed sucrose solution and water were recorded.

2.5. Forced swimming test

The forced swimming test was performed after 24 h of sucrose preference as described in detail elsewhere (Porsolt et al., 1977), with some modification. Briefly, mice were individually placed in a glass cylinder (20 cm in height, 14 cm in diameter) filled with 10-cm high water (25 ± 2 °C). All animals were forced to swim for 6 min, and the duration of immobility was recorded during the final 4 min interval of the test. The immobility period was regarded as the time spent by the mouse floating in the water without struggling and making only those movements necessary to keep its head above the water. The test sessions were recorded by a video camera and scored by an observer blind to treatment.

2.6. Blood and tissue sampling

Animals were killed by decapitation one day after the forced swimming test. To avoid fluctuations on hormone levels due to circadian rhythm, animals were bled at 10:00 a.m. to 12:00 p.m. on the day of sacrifice. The brain region of hippocampus was isolated immediately, and then stored at −80 °C for later analysis of BDNF measurement.

2.7. Serum corticosterone assay

Blood was immediately collected on ice and separated in a refrigerated centrifuge at 4 °C. Serum was stored at −20 °C until assays were performed. Serum corticosterone levels were measured using an enzyme immunoassay kit (Enzo Life Sciences, Plymouth Meeting, USA).

2.8. RT-PCR

Total RNA was isolated from hippocampus using Trizol reagent following the manufacturer’s instructions. Reverse transcription was performed using moloney murine leukemia virus reverse transcriptase for complementary DNA synthesis. Real-time PCR reactions were performed using a SYBR Premix Ex Taq Kit in ABI-7500 system. The BDNF (forward 5′-TTATTTCATACCTCGGTTGC-3′; reverse 5′-TGTCAGCCAGTGATGTCG-3′) and the internal control GAPDH (forward 5′-TGAGCCGGTATGACGTATG-3′; reverse 5′-CAGTCTTTGCTGGCAGTAT-3′) primers were used. The fluorescence signal was detected at the end of each cycle. Melting curve analysis was used to confirm the specificity of the products. The results were analyzed by the 2−ΔΔCt method.

2.9. Hippocampal BDNF protein levels

Each hippocampus tissue was weighed and homogenized in 20 μl/mg tissue of lysis buffer containing 137 mM NaCl, 20 mM Tris–HCl (pH 8.0), 1% NP40, 10% glycerol, 1 mM PMSF, 10 μg/ml aprotinin, 1 μg/ml leupeptin, 0.5 mM sodium vanadate. The homogenate was centrifugated at 16,000g for 30 min at 4 °C, and the supernatant was collected and stored at −80 °C until assay. Protein levels of samples were measured using the Lowry Method. BDNF protein was measured using BDNF ELISA kit (Boster, China) according to the protocol of the manufacturer.

2.10. Statistical analysis

Data are presented as mean ± S.E.M. and analyzed by one-way ANOVA followed by post-hoc Dunnett’s test. A value of P < 0.05 was considered statistically significant.

3. Results

3.1. Effects of apigenin on sucrose preference induced by corticosterone

Chronic exposure to corticosterone induced a significant decrease [F(1,14)=8.66, P < 0.05] on the sucrose preference (Fig. 1). Treatment with apigenin (40 mg/kg) and fluoxetine (20 mg/kg) significantly increased the sucrose preference in mice exposed to corticosterone treatment (P < 0.05, P < 0.05, respectively).

![Fig. 1. Effects of apigenin and fluoxetine on sucrose preference in mice exposed to repeated corticosterone injection. **P < 0.01 vs control-vehicle group. *P < 0.05 and **P < 0.01 vs corticosterone-vehicle group.](image-url)
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