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Antidepressant-like behavioral, neurochemical and neuroendocrine effects of naringenin in the mouse repeated tail suspension test

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ABSTRACT

Our previous study demonstrated that the citrus bioflavonoid naringenin ameliorated behavioral alterations via the central serotonergic and noradrenergic systems in the tail suspension test (TST) induced mice. To better understand its pharmacological activity, mice were submitted to three 6 min-TSTs one week apart (Day 1: test, Day 7: retest 1, Day 14: retest 2) followed by hippocampal glucocorticoid receptor (GR), monoamine neurotransmitters and serum corticosterone measurement. The results suggested that repeated TST detected the gradual increase in the efficacy of naringenin over time, additionally 1-day (20 mg/kg), 7-day (10, 20 mg/kg) and 14-day (5, 10, 20 mg/kg) naringenin treatment markedly decreased the immobility time. Moreover, administration of naringenin for 14 days (20 mg/kg) increased hippocampal serotonin (5-HT), norepinephrine (NE) and GR levels, and reduced serum corticosterone levels in mice exposed to the repeated TST. Overall, the present study indicated that the re-exposure would facilitate the detection of the anti-immobility effects of antidepressant drugs in the mouse TST, and clearly demonstrated that the antidepressant-like effect of naringenin may be mediated by an interaction with neuroendocrine and neurochemical systems.

1. Introduction

According to the World Health Organization, depression is a serious emotional disorder causing a huge burden on health worldwide. A number of theories have been proposed to explain the pathogenesis of depression, including monoamine deficiency, hyperactivity of the hypothalamic-pituitary-adrenal (HPA) axis, neurodegeneration and others (Delgado, 2000; Maes et al., 2009; Mizoguchi et al., 2003).

As the most classic pathophysiological theory of depression, the monoamine hypothesis holds that depression results from low levels of serotonin (5-HT), norepinephrine (NE), dopamine (DA) or all of them in the central nervous system (Delgado, 2000). A vast amount of evidence obtained from clinical and preclinical studies indicated that levels of monoamine neurotransmitters in the brain increased compared with that of controls after antidepressant treatments (Elhwuegi, 2004).

Besides monoamine deficiency, hypersecretion of glucocorticoids and dysregulation of glucocorticoid receptor (GR) function are also involved in the pathogenesis of depression (Juruena et al., 2004). Glucocorticoids (principally cortisol in humans and corticosterone in rodents), a vital steroid hormone released in response to stress, is found to be in the regulation of the HPA axis (Zunszain et al., 2011). Its levels are markedly higher than those of controls in depressed patients (Manthey et al., 2011) and stress-induced depression-like rodents (Yi et al., 2008a,b);. The increased activity of the HPA axis in depression has been attributed to reduction of glucocorticoid receptors in the brain regions involved in the feedback inhibition mechanism, especially in the hippocampus (Pariante and Miller, 2001). Antidepressants reduce HPA axis activity by increasing the negative feedback on the HPA axis by up-regulating glucocorticoid receptor and suppressing glucocorticoids (Yau et al., 2007).

These observations above indicate that successful antidepressants treatment might normalize changes in neurochemical and neuroendocrine actions in depression. Therefore, glucocorticoid, GR as well as monoamine neurotransmitters play important roles in the new antidepressant agent development.

In our previous studies, 1-day naringenin treatment was found to decrease the immobility time only in the mouse tail suspension test (TST) but not in the forced swimming test (FST), without altering the

Abbreviations: BCA, bicinchoninic acid; BSA, bovine serum albumin; cDNA, complementary DNA; dNTP, deoxyribonucleoside triphosphate; DOPAC, 3,4-Dihydroxyphenylacetic acid; EDTA, ethylenediaminetetraacetic acid; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; HPLC-ECD, high-performance liquid chromatography with electrochemical detection; HRP, horseradish peroxidase; 5-HIAA, 5-Hydroxyindoleacetic acid; MHPG, 4-Hydroxy-3-methoxyphenylglycol; DA, dopamine; FST, forced swimming test; GR, glucocorticoid receptor; HPA, hypothalamic-pituitary-adrenal; M-MLV, moloney murine leukemia virus; NE, norepinephrine; OFT, open-field test; PVDF, polyvinylidene fluoride; RT-PCR, reverse transcription polymerase chain reaction; 5-HT, serotonin; SDS-PAGE, sodium dodecyl sulfate polyacrylamide gel electrophoresis; TST, tail suspension test; TBST, Tris-buffered saline with Tween; TPH, tryptophan hydroxylase; TH, tyrosine hydroxylase.

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locomotor activity in mice, exhibited antidepressant-like effects via monoaminergic systems (Yi et al., 2010). However, the antidepressant-like mechanism involved in the aspects of neurochemical and neuroendocrine interaction, as well as behavioral results after 7-day and 14-day treatment, remains unknown. In addition, Mezadri et al. (2011) have demonstrated that repeated FST reduced the number of animals used, and was suitable to detect short and long-term effects of antidepressant agents. For these reasons we reproduced a repeated TST model and used it for evaluation of the antidepressant-like effect and mechanism of naringenin after 14-day treatment.

Therefore, the aim of the present study was to further investigate possible mechanism of naringenin actions on hippocampal monoamine neurotransmitter and GR levels, and serum corticosterone levels in mice exposed to repeated TST.

2. Materials and methods

2.1. Animals

Male ICR mice (24 ± 2 g) were purchased from the Laboratory Animal Centre, Fujian Medical University, Fujian Province, PR China. Animals were housed 8 per cage (320 × 180 × 160 cm) under a normal 12-h/12-h light/dark schedule with the lights on at 07:00 a.m. and had free access to tap water and food pellets. Ambient temperature and relative humidity were maintained at 22 ± 2 °C and at 55 ± 5%, and given a standard chow and water *ad libitum* for the duration of the study. The animals were allowed 1 week to acclimatize themselves to the housing conditions before the beginning of the experiments. All procedures were performed in accordance with the published guidelines of the China Council on Animal Care (Regulations for the Administration of Affairs Concerning Experimental Animals, approved by the State Council on October 31, 1988 and promulgated by Decree No. 2 of the State Science and Technology Commission on November 14, 1988).

2.2. Chemicals

Naringenin (purity = 98.3% by HPLC) was obtained from Shanxi Huike Botanical Development Co., Ltd. (1 S)-(+)-10-camphorsulfonic acid, 5-HT, DA, 5-Hydroxyindoleacetic acid (5-HIAA), 4-Hydroxy-3-methoxyphenylglycol (MHPG), 3,4-Dihydroxyphenylacetic acid (DOPAC) standards were purchased from Sigma–Aldrich Co. NE standard was obtained from the National Institute for the Control of Pharmaceutical and Biological Products. Fluoxetine hydrochloride was purchased from Changzhou Siyao Pharmaceuticals Co., Ltd. The anti-GR antibody and the respective secondary antibody were purchased from Santa Cruz Biotechnology Inc.

2.3. Drug treatments

Animals were divided into control and five experimental groups (8 animals per group) as follows: one vehicle-control (0.9% physiological saline), one vehicle-TST (0.9% physiological saline), one fluoxetine (15 mg/kg) and three naringenin treatments (5, 10, 20 mg/kg). All treatments were administered by oral (p.o.) gavage in a volume of 10 ml/kg body weight. Drugs were administered by oral gavage for 14 consecutive days. The treatment protocol of dose, pre-exposure time and administration route used for naringenin and fluoxetine was adopted according to our previous study (Yi et al., 2009, 2010). Naringenin was suspended in saline with 10% (v/v) Tween-80 (polyoxyethylene sorbitan monooleate), and administered 60 min before being tested in the TST on day 1, day 7 and day 14. Fluoxetine was dissolved in 0.9% physiological saline and also administered 60 min with the same regimen as naringenin.

2.4. TST

The TST was conducted as previously described (Steru et al., 1985), with some modification. Briefly, mice were individually suspended by tail with a clamp (1 cm from the tip of the end) in a box (25 × 25 × 30 cm) with the head 5 cm from the bottom. Testing was carried out in a darkened room with minimal background noise. A mouse was suspended for a total of 6 min, and the duration of immobility was recorded during the final 4 min interval of the test. Mice were considered immobile only when they hung passively and completely motionless. The test sessions were recorded by a video camera and scored by an observer blind to treatment.

2.5. Open-field test (OFT)

To exclude the possibility of false positive results of naringenin in the TST, mice administered with the same regimen as in the TST was evaluated in the OFT (Mao et al., 2008). The apparatus consisted of a wooden box measuring 40 × 40 × 30 cm, with the floor divided into 25 equal squares (8 × 8 cm). The number of squares crossed with all paws (crossings) was counted in a 3-min session. The apparatus was cleaned with a detergent and dried after occupancy by each mouse. The test sessions were recorded by a video camera and scored by an observer blind to treatment.

2.6. Blood sampling and tissue extraction

Animals were killed by decapitation after the final TST. To avoid fluctuations on hormone levels due to circadian rhythm, animals were bled at 12:00 p.m.–13:00 p.m. on the day of sacrifice. As the hippocampus is rich in glucocorticoid receptor (McEwen, 1973) and is expected to be particularly associated with cognitive abnormalities (learning and memory impairments) that are seen in depressive patients (Berton and Nestler, 2006), the hippocampus was isolated immediately, and then stored at −80 °C for later analysis of GR and monoamine neurotransmitter levels.

2.7. Serum corticosterone assay

Blood was 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).

2.8. Reverse transcription polymerase chain reaction (RT-PCR)

Total RNA was isolated from hippocampus using Trizol reagent following the manufacturer's instructions. The concentration and purity of RNA were measured by the optical density at 260 and 280 nm using spectrophotometer. Reverse transcription was performed with 1 μg RNA using Moloney murine leukemia virus (M-MLV) reverse transcriptase for complementary DNA (cDNA) synthesis. Amplification of cDNA by PCR was performed in 25 μL reactions containing 8 μl cDNA, 1 μl forward and 1 μl reverse primers (10 μM, forward 5′-CAAAGCCG-TTTCACTGTCC-3′; reverse 5′-ACAATTTCACACTGCCACC-3′; 314 bp), 2.5 μl PCR × 10 buffer containing $MgCl_2$, 0.5 μl deoxyribonucleoside triphosphate (dNTP) mixture (10 mM), 0.5 μl Taq polymerase (2.5 U) and 11.5 μl sterile ddH_2O. In general, PCR was performed with a preheating cycle at 95 °C for 5 min, denaturation, annealing and elongation were carried out at 95 °C for 45 s, at 55 °C for 30 s, and at 72 °C for 1 min, respectively. The reactions were repeated for 31 cycles. The PCR products were resolved by a 1.5% agarose gel electrophoresis and quantified by the Bio-Rad ChemiDoc XRS Gel Documentation system and Bio-Rad Quantity One software. The results were normalized to the mRNA expression level of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) in each sample.