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Genistein alleviates anxiety-like behaviors in post-traumatic stress disorder model through enhancing serotonergic transmission in the amygdala

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ABSTRACT

Post-traumatic stress disorder (PTSD) is a chronic psychiatric disorder, characterized by intense fear, and increased arousal and avoidance of traumatic events. The current available treatments for PTSD have limited therapeutic value. Genistein, a natural isoflavone, modulates a variety of cell functions. In this study, we tested anti-anxiety activity and underlying mechanisms of genistein in a PTSD rat model. The rats were trained to associate a tone with foot shock delivery on day 0, then fear conditioning was performed on day 7, 14 and 21. Genistein (2–8 mg/kg) was injected intraperitoneally daily for 7 days. The anti-anxiety effects of genistein were measured by contextual freezing behavior and elevated plus maze. By the end of the experiments, the amygdala was extracted and subject to neurochemistry analysis. Genistein alleviated contextual freezing behavior and improved performance in elevated plus maze dose-dependently in PTSD rats. Furthermore, in these rats, genistein enhanced serotonergic transmission in the amygdala, including upregulation of tryptophan hydroxylase, serotonin, and phosphorylated (p)-CaMKII and p-CREB, as well. Genistein exerts anti-anxiety effects on a PTSD model probably through enhancing serotonergic system and CaMKII/CREB signaling pathway in the amygdala.

1. Introduction

Post-traumatic stress disorder (PTSD), a chronic psychiatric disorder, develops immediately or long time after exposure to traumatic events. PTSD is characterized by intense fear, increased arousal, avoidance and re-experience of traumatic events (Jak et al., 2016). Several neurochemical systems are implicated in pathophysiology of PTSD, including serotonin (5-TH), noradrenaline, glutamate, gamma-aminobutyric acid (GABA), cannabinoids and others (Kelmendi et al., 2016). Among them, altered serotonin is a core neurochemical feature of PTSD (Jak et al., 2016; Kelmendi et al., 2016), and contributes to multiple PTSD-related behaviors, including hypervigilance, impulsivity, aggression, hostility, depression (Sherin and Nemeroff, 2011).

The key fear circuitry is composed of the amygdala, the hippocampus and the ventromedial prefrontal cortex, and serotonergic transmission in this circuitry attracts most attention in PTSD research (Jak et al., 2016; Kelmendi et al., 2016). A direct evidence for a cardinal role of serotonin in PTSD is that sertraline and paroxetine, two selective serotonin reuptake inhibitors (SSRIs), are the most commonly prescribed drug for PTSD treatment (Berger et al., 2009). SSRIs relieve PTSD symptoms, such as depression and insomnia or nightmares, through strengthening serotonergic system. However, their therapeutic effects are only observed in around 60% of PTSD patients and are often accompanied by undesired side-effects (Berger et al., 2009). Although serotonin plays a pivotal role in PTSD, the current serotonin mimetic treatments are not optimal. Therefore, it is prudent to seek a better alternative pharmacological treatment for PTSD.

Genistein, a natural isoflavone derived from soybean, has a wide range of bioactivities. It is an estrogenic agonist, and has beneficial effect on low estrogen-related depression (Dixon and Ferreira, 2002; Polkowski and Mazurek, 2000). It has antioxidant, immuno- and anti-inflammatory activities (Polkowski and Mazurek, 2000). Moreover, it also inhibits tyrosine kinase, and regulates diverse intracellular signal transductions (Polkowski and Mazurek, 2000). Several studies demonstrated that genistein had anti-depression effect in experimental animals probably via activating serotonergic pathway (Estrada-Camarena et al., 2003; Kageyama et al., 2010; Sapronov and Kasakova, 2008). Taken together, we hypothesize that genistein could benefit PTSD treatment because it effectively relieves depression, a symptom commonly seen in PTSD patients (Kessler et al., 1995).

In the present study, we preformed fear-conditioning to establish a PTSD model, and tested anti-anxiety like behaviors of genistein on this

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model. We found that genistein attenuated anxiety behaviors in PTSD rat model. Further neurochemistry and western blot assay showed that genistein enhanced serotoninergic transmission in the amygdala: up-regulating TPH, elevating serotonin levels, and promoting serotonin receptor-related CaMKII/CREB signaling pathway. These results provide experimental basis for the notion that genistein could have therapeutic potential for PTSD treatment.

2. Methods

2.1. Animals and housing

Adult Sprague-Dawley male rats (250–350 g) were purchased from SLAC (Shanghai, China) and housed in a room at 22 ± 1 °C under a 12 h dark-light cycle. To eliminate the interference of gender on the observed results, only male rats were employed in this study. Rats were accessed to food and water ad libitum. All experimental procedures were in accordance with guidelines of Medical College of Taizhou University for the care and use of laboratory animals.

2.2. PTSD rat model

A PTSD model was established based on a previous study with some modifications (Ji et al., 2014). The experimental device used for PTSD rat model consists of a plexiglass transparent chamber (length × width × height: 30 × 26 × 22 cm) with stainless steel grid floor. The grid floor was connected with foot shock delivery system. During pre-test sessions, rats were placed in the chamber for 30 min daily for 7 days. In main test sessions, on day 0, after 5 min acclimation in the chamber, rats in PTSD group were given a tone (30 s, 5 kHz, 75 dB) followed by five electrical foot shocks (2 mA, 2 s) over a 15 min period with variable inter-shock intervals. Control rats only received the tone (30 s, 5 kHz, 75 dB) without foot shock. On day 7, 14 and 21, all animals were placed back to the same chambers for 5 min and presented the same tone without foot shock. In this session, the rats were monitored with a video camera, and the time spent on active movement and freezing was calculated off-line.

2.3. Elevated plus maze

Elevated plus maze constitutes two open arms and two closed arms. The stand for the elevated plus maze was approximately 90 cm above the floor, and each arm is 11 cm in width and 65 cm in length. The elevated plus maze was performed according to previous study (Walf and Frye, 2007). Briefly, rats were placed in the center of elevated plus maze with head facing to one of the open arms, and were allowed to roam freely in four arms of the apparatus for 5 min. The time spent in closed arm vs. open arm, and total open arm entries were recorded by a video camera during 5 min test session.

2.4. Western blot analysis

Proteins from the amygdala were extracted and analyzed with western blot assay. Primary antibodies included anti- tryptophan hydroxylase (TPH) (Sigma, St. Louis, MO, USA), anti-CaMKII (Abcam, Cambridge, MA, USA), anti-phosphorylated CaMKII (Abcam, USA), anti-CREB (Sigma, USA), anti-phosphorylated CREB (Abcam, USA) and GAPDH (Sigma, USA). The antibody–protein complexes were visualized by chemiluminescent reagents. The band densities were quantified by imaging quantification. Ratios of the band density for the protein of interest to that for GAPDH were calculated. All biochemical examinations were conducted on day 7.

2.5. High-Performance Liquid Chromatography (HPLC)

The amygdala was dissected and placed in cold phosphate buffered saline (PBS), added perchloric acid to a final concentration of 0.1 M, and homogenized. Tissue homogenates were sonicated followed by centrifuge. The supernatants were used for HPLC. Injected samples should be completely clear, filter if needed. Concentration of serotonin (5-HT) was detected using Eicom HTEC 500 high performance liquid chromatography system according to previous report (Ebenezer et al., 2016).

2.6. Drugs

Genistein (Sigma, St. Louis, MO, USA) was first solubilized in dimethyl sulfoxide (DMSO) with a concentration of 0.1 w/v, and then diluted with normal saline and administered by intraperitoneal injection (2–8 mg/kg). After foot shock, rats received genistein or vehicle (normal saline) injection once per day for 7 days starting from day 0 immediately after 5 min test session.

2.7. Statistical analysis

Data were presented as mean ± SD. One-way ANOVA or two-way ANOVA were performed as indicated. p value less than 0.05 was considered significant. These analysis was carried out using Prism (GraphPad Software, Inc; San Diego, CA; version 5.0).

3. Results

3.1. Genistein alleviated contextual freezing behavior in PTSD rats

Rat pre-exposed with 5 foot shocks as stressors displayed significant increase in contextual freezing time at day 7, 14 and 21 (Fig. 1), indicating pre-exposure of foot shocks induced anxiety-like behavior in the stressed rats. Genistein (2, 4 and 8 mg/kg, as suggested in previous studies (Castro et al., 2012; Kim et al., 2011)) was injected intraperitoneally once per day for 7 days starting from day 0 when rats were given 5 foot shocks during 15 min session. Repeated treatment with low-dose of genistein (2 mg/kg) did not alter freezing time in stressed rats at day 7, 14 and 21, respectively (Fig. 1). In contrast, the freezing time was significantly reduced in stressed rats treated with higher doses of genistein (4 mg/kg and 8 mg/kg) for 7 days (Fig. 1). Moreover, genistein at a dose of 8 mg/kg reversed freezing time back to control levels in stressed rats at day 21 (Fig. 1). These data indicate that chronic treatment of genistein improves anxiety-like behavior in the PTSD rats.

3.2. Genistein attenuated natural anxiety in elevated plus maze test

The anti- anxiety effect of genistein was further evaluated in elevated plus maze tests. Rats pre-exposed to 5 foot shocks spent much less

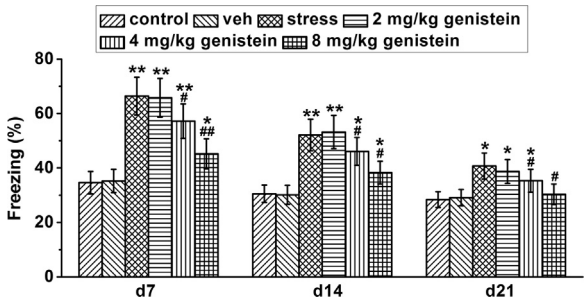

Fig. 1. Genistein relieved contextual anxiety-like behavior in PTSD rat model. Effects of genistein (2–8 mg/kg) on the contextual freezing behavior were examined in the PTSD rats. The percentages of freezing time in these groups (8 rats in each group) were measured on day 7, day 14, and day 21, respectively. Data were presented as mean ± SD. Two-way ANOVA analysis was used following a Tukey's post hoc test. $^{*}p < 0.05$ and $^{**}p < 0.01$ versus Control group, $\#p < 0.05$ and $\#\#p < 0.01$ versus stress group.