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Rosmarinic acid ameliorates PTSD-like symptoms in a rat model and promotes cell proliferation in the hippocampus

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ABSTRACT

Rosmarinic acid (RA) is an important component of Chinese herbal medicine treatments and has been demonstrated to exert therapeutic effects in mood disorders. The present study was designed to assess the effects of RA on post-traumatic stress disorder (PTSD)-like symptoms, hippocampal cell proliferation and phosphorylation extracellular regulated protein kinases (pERK1/2) expression. We found that administration of RA (10 mg/kg) alleviated PTSD-like symptoms in rats exposed to an enhanced single prolonged stress (ESPS) paradigm and restored hippocampal proliferation and pERK1/2 expression. Interestingly, the effects of RA were inhibited by the blockage of the ERK signaling. These data support the use of RA for treating PTSD and indicate that the ERK1/2 signaling cascade may play a critical role in the therapeutic efficacy of RA in treating such conditions.

1. Introduction

Post-traumatic stress disorder (PTSD) is a biopsychosocial dysfunction that severely impacts quality of life (Berger et al., 2007; Clapp et al., 2008; Warshaw et al., 1993). With the increasing incidence of natural and humanitarian disasters, PTSD has become a major mental health issue (Ma et al.; Maes et al., 2001; Mak et al.; Trickey et al.). Currently, the first treatment choice for PTSD is antidepressants, especially selective serotonin reuptake inhibitors (SSRIs), which have demonstrated efficacy in symptom alleviation and relapse prevention in PTSD patients (Berger et al., 2009; Corchs et al., 2009; Schneier et al.). However, 'even when treated with this class of drugs, response rates rarely exceed 60% and less than 20–30% of the patients achieve full remission' (Berger et al., 2009; Stein et al., 2002; Zohar et al., 2002). Hence, efforts have been made to identify novel treatment approaches for PTSD.

The molecular and cellular mechanisms underlying PTSD are not fully understood. Recent structural neuroimaging studies have shown that hippocampal volumes are relatively low in PTSD patients as compared to the healthy or trauma-exposed controls (Bremner et al., 2008; Kitayama et al., 2005; Lindauer et al., 2006). In animal models of PTSD, hippocampal cell proliferation is suppressed (Hendriksen et al.; Kikuchi et al., 2008), and neuronal apoptosis is increased (Kaster et al. 2012; Li et al.). Furthermore, physical or psychosocial stress, the original cause of PTSD, could induce morphological changes in the hippocampus, including atrophy, loss of pyramidal neurons, and reduced neurogenesis in the dentate gyrus (Kim et al., 2007; Rosenbrock et al., 2005; Yang et al., 2007). Meanwhile, inhibited adult neurogenesis is implicated in impaired pattern separation underlies the overgeneralization often seen in PTSD (Clelland et al., 2009; Guo et al., 2011; Nakashiba et al., 2012). Taken together, inhibited hippocampal neurogenesis is not only a pathological change, but also the cause for some behavioral dysfunction in patients suffering PTSD. Then what are the modulators of neurogenesis during the pathological changes of PTSD patients? In a social isolation mouse model, which simulates aggression, fear and anxiety symptoms of the PTSD patients, Pibiri et al. demonstrated that decreased allopregnanolone (Allo) biosynthesis in corticolimbic circuit is associated with such behavioral dysfunctions (Pibiri et al., 2008). There is also a clinical report discovering that a down-regulation of Allo level in the cerebral fluid of the PTSD patients was correlated with increased re-experiencing and comorbid depressive symptoms (Rasmusson et al., 2006). Decreased Allo level is accompanied with

Abbreviations: PTSD, post-traumatic stress disorder; ESPS, enhanced single prolonged stress; RA, rosmarinic acid; p-ERK1/2, phosphorylation extracellular regulated protein kinases; BrdU, bromodeoxyuridine; Allo, allopregnanolone.

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suppressed hippocampal neurogenesis (Dranovsky et al., 2011) and behavioral dysfunctions (Pinna et al., 2003). Suggesting modulators of hippocampal neurogenesis like Allo might be pharmacological targets for treating PTSD patients. Therefore, the development of new pro-neurogenic drugs may have therapeutic potential for such patients (Pinna, 2013).

Rosmarinic acid (α-*O*-caffeoyl-3, 4-dihydroxyphenyl-lactic acid; RA) is one of the major polyphenolic ingredients of Perillae Herba (a component herb of such Kampo medicines as Xiang-Su-San in China) and has multiple biological activities, including anti-allergic and anti-inflammatory effects (Osakabe et al., 2004; Sanbongi et al., 2004). Moreover, there is evidence that RA produces an antidepressant-like effect in mice by promoting cell proliferation in the hippocampus (Ito et al., 2008). Given the association between inhibited hippocampal neurogenesis and PTSD, we hypothesized that RA may also be effective in reducing anxiety symptoms in a PTSD model in which rats are exposed to an enhanced single prolonged stress (ESPS) paradigm. The present study sought to determine whether early RA intervention could ameliorate stress-associated behaviors in ESPS-exposed rats and assessed the effects of RA treatment on the hippocampal neurogenesis.

2. Materials and methods

2.1. Animals

The experimental protocol used in this study was approved by the Ethics Committee for Animal Experimentation of the Fourth Military Medical University. All experiments were performed in accordance with the Guidelines for the Care and Use of Mammals in Neuroscience and Behavioral Research. Male Sprague Dawley (SD) rats (nearly 8 weeks old) were housed four per cage in an air-conditioned room with a 12:12-h light/dark cycle and had free access to food and water. Animals were allowed to acclimate for at least 10 days before the experiments.

2.2. Experimental designs

2.2.1. Experiment I

RA (536954, Sigma-Aldrich, St. Louis, MO, USA) was dissolved in saline. To investigate the therapeutic effects of early RA intervention in a PTSD rat model, rats were randomly assigned to six groups ($n = 8$ per group): sham + vehicle, sham + RA (L), sham + RA (H), ESPS + vehicle, ESPS + RA (L), and ESPS + RA (H). The rats in the ESPS + vehicle group were subjected to ESPS and then received saline for 14 days (1 ml/kg daily). The ESPS + RA (L) or ESPS + RA (H) group also experienced ESPS, but they received RA (5 or 10 mg/kg daily [L and H, respectively]) for 14 days. The sham + vehicle group received only saline, and the sham + RA (L) or sham + RA (H) group received only RA (5 or 10 mg/kg daily). After RA or vehicle treatment, open field and elevated plus maze tests were conducted. The animals were then sacrificed, and hippocampal phosphorylation extracellular regulated protein kinases (p-ERK1/2) expression was determined by Western blot analysis.

2.2.2. Experiment II

To assess the effect of U0126 (an ERK1/2 phosphorylation inhibitor) on anxiety-like behavior improvement and neurogenesis associated with RA, rats were randomly assigned to five groups: sham + vehicle, sham + RA, ESPS + vehicle, ESPS + RA, and ESPS + RA + U0126 ($n = 13$ per group).

The U0126 (#9903, Cell Signaling, Danvers, MA, USA) was dissolved in dimethyl sulfoxide (DMSO) and then diluted with saline (DMSO: saline = 1:4) before use. The animals in the sham + vehicle, sham + RA, ESPS + vehicle, and ESPS + RA groups were intraperitoneally injected with 0.6 ml/kg vehicle (DMSO:saline = 1:4), and then received saline or RA (10 mg/kg daily) 30 min later once a day for 14 days. The rats in the ESPS + RA(H) + U0126 group were intraperitoneally injected with 0.5 mg/kg U0126 once a day for 14 days 30 min prior to RA administration. The dose of U0126 in this experiment was selected based on previous studies (Du et al., 2010; Sironi et al., 2006). Ten days later, some of the animals ($n = 5$ per group) were intraperitoneally injected with 75 mg/kg bromodeoxyuridine (BrdU) once a day for 3 days, and then sacrificed and subjected to BrdU immunohistochemistry. The remaining animals ($n = 8$ per group) were subjected to behavioral tests and Western blot analysis 14 days later, as in Experiment I.

2.2.3. Experiment III

To investigate whether RA directly enhances hippocampal neural stem cell proliferation, we cultured hippocampal-derived neural stem cells from fetal Sprague Dawley rats (see Section 2.6). After stem cells were treated with different concentrations of RA, we measured the sphere diameters and cell viability after 5 days. U0126 was added into the cultured medium 30 min before RA treatment to determine whether ERK signaling was involved in RA-mediated effects on proliferation.

2.3. Behavioral paradigms

2.3.1. ESPS

The details of the ESPS procedure have been described in our previous study (Wang et al., 2009). Briefly, rats were restrained for 2 h, and this was immediately followed by forced swimming for 20 min in 24 °C water in a clear acrylic cylinder (24 cm in diameter and 50 cm in height). After 15 min of recuperation, the animals were exposed to diethyl ether until they lost consciousness and were then moved into a shock chamber. When they recovered (after about 30 min), a single electric foot shock was delivered (1 mA for 4 s) via metal grids installed in the bottom of the chamber.

2.3.2. Activity box

The apparatus consisted of a black acrylic plastic box placed in a soundproof box. The acrylic box formed a square area (47 × 47 cm), with walls 47 cm in height. The animals' performance was recorded from the soundproof box, which was illuminated by a red fluorescent light (30 W). During testing, each rat was initially placed in the center zone, and the total distance traveled was recorded for 15 min using an automatic analyzing system (TopScan, Clever Sys Inc., Reston, VA, USA).

2.3.3. Elevated plus maze test

This paradigm has been well validated for detecting responses to external stressful stimuli. The Plexiglas apparatus consisted of a plus-shaped platform elevated 50 cm above the floor. Two of the opposing arms (50 cm × 10 cm) were enclosed by side and end walls (40 cm high, closed arms). The other two arms did not have walls (open arms). At the beginning, animals were placed in the central area of the maze (10 × 10 cm) facing an enclosed arm. The initial 5 min of the rats' exposure to this environment was recorded with a video camera. The time spent and the numbers of entries into the open arms were used as indices of anxiety. These events were recorded by an investigator who was blinded to the treatment conditions. In addition, the percentage values of both parameters were also calculated in relation to the total time spent in all arms, as well as the total number of entries into all arms.

2.3.4. Freezing condition

Experiments were performed in two contexts: (i) the shock chamber and (ii) the neutral test context. For shock application, rats were placed into the shock chamber, and after 196 s, a single 4 s scrambled electric shock of 1 mA current, was applied to the feet via the metal grid and induced signs of pain (jumping or vocalizing). Stressed rats remained in the shock chamber for another 60 s before being returned to the home cages. To study conditioned fear, rats were re-exposed to the shock chamber (CS+) for 3 min without tone presentation or a