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Antidepressant-like effects of salidroside on olfactory bulbectomy-induced pro-inflammatory cytokine production and hyperactivity of HPA axis in rats

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ABSTRACT

Salidroside (SA) is the primary bioactive marker compound in the standardized extracts from *Rhodiola rosea*. Although it has potential antidepressant activity in a rat behavioral despair model, the mechanisms of antidepressant effect for SA remain unclear. The objective of this study was to evaluate the antidepressant effects of SA and to discuss the potential mechanisms in olfactory bulbectomized (OBX) rats. SA of 20, 40 mg/kg (p.o.) for 2 weeks notably alleviated OBX-induced hyperactivity in open field test, decreased immobility time in TST and FST. Chronic treatment with SA could remarkably reduce TNF-α and IL-1β levels in hippocampus. Western blot showed that SA could markedly increase glucocorticoid receptor (GR) and brain-derived neurotrophic factor (BDNF) expression in the hippocampus. Besides, SA could also attenuate corticotropin-releasing hormone (CRH) expression in hypothalamus, as well as reducing significantly the levels of serum corticosterone. In conclusion, this study demonstrated that OBX rats treated with SA could significantly improve the depressive-like behaviors. The antidepressant mechanisms of SA might be associated with its anti-inflammatory effects and the regulation of HPA axis activity. Reversal of abnormalities of GR may be partly responsible for those effects. These findings suggested that SA might become a beneficial agent to prevent and treat the depression.

1. Introduction

Depression is a severe affective disorder characterized by a prevalent and persistent low mood that is accompanied by inappropriate guilt, low self-esteem, hopelessness and thoughts of death or suicide, which brings a heavy burden to sufferers, their families and society (de Bodinat et al., 2010). It is the fourth major cause of morbidity worldwide at present and it will become the second by 2020 according to the World Health Organization (Kessler et al., 2003; Kessler et al., 2011). Although there are many clinical effective antidepressant medications, most of them often generate severe side effects (Berton and Nestler, 2006). Therefore, it is necessary to develop new antidepressant drugs with lower adverse effects and better efficacy.

Increasing evidence has indicated that the malfunction of glucocorticoid receptor (GR) might be involve in the hyperactivity of hypothalamus–pituitary–adrenal (HPA) axis, which is one of the main biochemical changes in major depression (Pariante and Miller, 2001; van Rossum and Lamberts, 2006). Under normal physiological conditions, the HPA axis activity can be normalized via the endogenous glucocorticoid-mediated negative feedback inhibition, which is largely dependent on the function of GR in several brain regions (Mizoguchi et al., 2003). However, previous researches suggest that depressed individuals exhibit the reduction of GR mRNA and protein expression in various brain regions and that then causes an increase in glucocorticoid levels (Webster et al., 2002; Cubala and Landowski, 2006). Moreover, GR transgenic mouse or mice with GR down-regulation also significantly induce depressive-like behavior that is accompanied by increased glucocorticoid levels and reduce the sensitivity of dexamethasone, possibly due to impaired GR-mediated feedback inhibition on the HPA axis (Barden, 1999; Kronenberg et al., 2009). Interestingly, many studies have demonstrated that the hyperactivity of HPA axis in both depressed patients and animals can be improved by antidepressant treatment (Nikisch et al., 2005; Pan et al., 2010). Therefore, abnormalities of GR may contribute to the development of depression and normalization of GR may improve the depressive symptoms.

Recently, mounting data show that activation of inflammatory responses and release of pro-inflammatory cytokines (PICs) may play a critical role in the pathogenesis of depression (Maes et al., 2012). Increased levels of PICs, such as tumor necrosis factor-alpha (TNF-α) and interleukin-1 beta (IL-1β), have been found in both the periphery

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and brain of depressed patients (Maes et al., 2009). These PICs could not only sharply stimulate the release of corticotropin-releasing hormone (CRH) and then activate the HPA axis (Maes et al., 1993; Miller et al., 2009; Song et al., 2009), but could also reduce GR expression (Pariante and Miller, 2001) and influence GR function through abated GR translocation, protein–protein interactions and binding to glucocorticoid response elements (GREs) (Pace et al., 2007). Thus, antidepressants possess the enhancement of GR expression and function, and anti-inflammatory activity may have better efficacy for treatment of depression.

The bilateral OBX in rats has been extensively accepted as an effective animal model of depression (Song and Leonard, 2005; Morales-Medina et al., 2013). OBX causes numerous behavioral changes such as increase locomotor activity after being exposed to a novel environment and increase immobile behavior in the behavioral despair tests (Song and Leonard, 2005; Bhatt et al., 2013). Interestingly, these alterations induced by OBX are not dependent of anosmia (van Riezen et al., 1977), but may rely on the disrupted connections in the olfactory-limbic circuitry, enlarged third ventricles, and decreased hippocampal volume (Jesberger and Richardson, 1988; Kelly et al., 1997; Song and Leonard, 2005), which might be responsible for OBX-induced behavioral abnormalities. Moreover, OBX can also cause the dysregulation of HPA axis (Cairncross et al., 1977), and increase inflammatory reactions and PICs, including IL-1β and TNF-α, in several brain regions (Myint et al., 2007; Rinwa and Kumar, 2013). These changes are not only similar to clinical symptoms of human depression, but also can be improved by repeated long-term antidepressant treatment, thus this model is usually used to study the pathophysiology of depression and to screen the antidepressants (Eisenstein et al., 2010; Oral et al., 2013). Salidroside (SA) (p-hydroxy-phenethyl-β-D-glucoside, $C_{14}H_{20}O_7$) (Fig.1), a major bioactive marker substance in the standardized extracts (SHR-5) from *R. rosea*, has many biological properties such as anti-fatigue (Huang et al., 2009), anti-oxidative (Ma et al., 2009) and anti-inflammatory (Li et al., 2013). Recent studies have suggested that SA exhibits anti-stress and antidepressant-like activity in immobilization stress models and behavioral despair models respectively (Panossian et al., 2007; Panossian et al., 2008). However, the mechanisms of antidepressant effect for SA are still unclear.

Based on the above evidences, we investigated whether SA treatment could improve the depressive-like behaviors of OBX rats and explored the possible antidepressant mechanisms. In this study, the effects of SA on TNF-α and IL-1β levels in hippocampus, serum corticosterone concentrations, mRNA expression of CRH in hypothalamus and hippocampal GR, and brain-derived neurotrophic factor (BDNF) protein expression in OBX rats were examined for elucidating its underlying mechanisms.

2. Materials and methods

2.1. Drugs and assay kits

Salidroside (Purity ≥ 98%) (Nanjing Zelang Medical Technology Co. Ltd., China), was diluted in normal saline, amitriptyline hydrochloride (Hunan DongtingPharm. Co. Ltd., China), ELISA kits (R&D, Minneapolis, MN, USA), RNA Isolater Total RNA Extraction Reagent (Vazyme Biotech Co., Ltd., Nanjing, China), EasyScript® First-Strand cDNA Synthesis SuperMix (TransGen Biotech Co., Ltd., Beijing, China), TransStart® Green qPCR SuperMix UDG (TransGen Biotech Co., Ltd., Beijing, China) and primers used in the present research were synthesized by GenScript (Nanjing) Co., Ltd. (Nanjing, China).

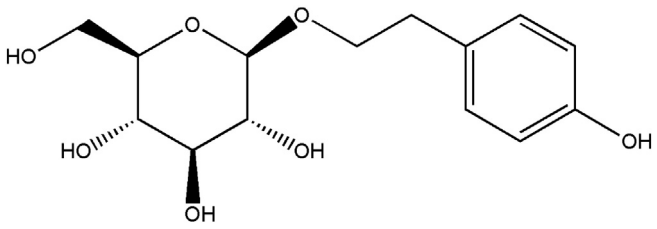

Fig. 1. Chemical structure of salidroside.

2.2. Animals

Adult male Sprague-Dawley rats, weighting 200–220 g, were from the Laboratory Animal Center of Zhejiang Province, China. Rats were randomly housed six per cage for at least 7 days to adapt to the animal room environment before the experiment started. All rats were kept in polypropylene cages remained at 22 ± 1 °C temperature and 12-h light/dark cycle with free access to water and food. All procedures, animal feed and care were performed strongly in agreement with the National Institutes of Health Guide for Care and Use of Laboratory Animals (Publication No. 85-23, revised 1985).

2.3. OBX surgery

After one week adaptation period, bilateral OBX surgery was carried out in rats (n = 50) narcotized with Nembutal (50 mg/kg, i.p.) as depicted previously (Xu et al., 2005). In brief, after being fixed on the brain with stereotaxic instrument (Stoelting Co., USA), the skull of rats were exposed and then (2 mm) burr holes were drilled through the skull at 7 mm anterior to the bregma and 2 mm to either side of the midline. Then olfactory bulbs were destroyed with a blunt hypodermic needle and removed by suction, then haemostatic sponge (Stypro, Absorbable hemostatic sponge, China) was inserted into the holes to prevent bleeding and the skin was sutured. Sham-operated rats received the same surgical process but destruction and removal of bilateral olfactory bulbs. To avoid infection, procaine penicillin powder was added to the wound before closed the scalp and each rat received penicillin (40,000-IU/kg, i.m.) once a day for 3 consecutive days after surgery. All rats were housed separately in cages for 2 weeks to recover from the surgery prior to drug treatments, and they were given extra care by the experimenter to reduce aggressive behavior in the recovery period. Moreover, a few rats died in the operation due to hyperesthesia, and several rats were excluded for the poor health condition after surgery.

2.4. Drug treatments and experimental design

Rats were divided into five groups with 8–9 rats in each: Sham control group (normal saline), OBX-vehicle control group (normal saline), OBX-amitriptyline treated group (10 mg/kg), OBX-SA treated groups (20 and 40 mg/kg, the effective doses for behavioral responses). All drugs were administered intragastrically once daily for 2 weeks. The present study was performed for 4 weeks, and the detailed experimental design was showed in Fig.2.

2.5. Behavioral detection

2.5.1. Open field test (OFT)

On day 29 after the OBX, the OFT was measured to estimate the effect of SA on hyperactivity in OBX rats. The open field apparatus (100 × 100 × 40 cm) consisted of a black metallic enclosure with a white open floor and was divided into 25 equal sectors by red lines. Starting with the center of the device, each rat was permitted to explore freely for 5 min (Borre et al., 2012). The test was performed in a quiet, darkened room and two 60-W light bulbs were suspended 90 cm above the center of the apparatus to provide bright illumination. The crossing times (entering into a new sector with four paws), the rearing times (erecting on its hind legs) and the grooming times (scratching its face with the forepaws) were recorded by a trained observer who was blind to the experimental group.