Neural Mechanisms Underlying Antidepressant-Like Effects of Glycyrrhizae Radix in Rats

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Glycyrrhizae radix (GR) is an herbal medicine commonly used in East Asia for treating a variety of diseases, including stomach disorders. In this study, the antidepressant-like activity of GR was investigated using the forced swimming test (FST) in rats. After the FST, the expression of c-Fos and corticotrophin releasing factor (CRF) was assessed by immunochemistry of brain samples from the paraventricular nucleus of the hypothalamus (PVN). The results of the FST showed that a high dose (400 mg/kg) of extract was very effective in reducing immobility($P<0.01$), and increased climbing. In addition, treatment with GR (400 mg/kg) significantly decreased the expression of c-Fos and CRF in the PVN, compared to controls. In conclusion, the findings of this study demonstrated that GR effectively reduced behavioral and physiological depression responses in an animal model of depression, suggesting that GR might be useful in the treatment of clinical depression.

Key words : Glycyrrhizae radix (GR) depression, forced swimming test (FST) corticotrophin releasing factor (CRF), c-Fos

Introduction

Depression is a common, debilitating, life-threatening illness with an increased morbidity and mortality. Furthermore, the World Health Organization has reported that depression is the fourth leading cause of disability worldwide, exceeded only by lower respiratory infections, perinatal conditions and HIV/AIDS[1]. Current antidepressant medications, including various monoamine reuptake inhibitors, monoamine oxidase inhibitors and serotonin reuptake inhibitor and tricyclic antidepressant, have proven to be effective and are available in the clinical setting. However, they have disadvantages such as a slow onset of action, relatively low response rates and side effects. These limitations of the current available medications make the research and development of new antidepressants urgent. Plant products are frequently considered to be less toxic and have fewer side effects than synthetic agents. There is an increasing trend to use alternative therapies including herbal medicines[2,3].

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The HPA system receives and integrates various inputs indicative of depression, converging in the PVN of the hypothalamus. Neurons of the PVN synthesize corticotropin-releasing hormone (CRH or CRF), which released to the hypophyseal portal blood reaches the anterior pituitary[4]. Evidence from pre-clinical and clinical studies support impairment of neuro-endocrinological function associated with depression[5]. The most frequently occurring neuroendocrinological abnormality in patients with depression is hyperactivity of the HPA axis characterized by hypersecretion of CRF[6]. It is well known that CRF is a neurotransmitter or neuromodulator in the brain and acts within the central nervous system to modulate a number of behavioral, neuroendocrine and autonomic responses to environmental stimuli through its actions on the HPA axis[7]. Interactions of the CRF system might be central to the pathophysiology of depression[8].

Glycyrrhizae radix (GR) is an herbal medicine that has been most frequently prescribed for the treatment of a variety of diseases including stomach disorders[9]. It has also been described in classical Asian medicine as an agent with the ability to 'improve the tone of the "middle-jiao" and replenish "qi", to remove "heat" and toxic substances and arrest coughing as well as the relief of spasms and pain[10]. In addition, it is

widely used as a flavoring adjuvant in drug preparations and an ingredient of cigarettes for its taste and properties that reduce irritation[10,11]. It has also been reported that an aqueous extract of G. Glara L. Showed significant antidepressant-like activity in mouse tail suspention test[12]. Zhao et al., repoted that the antidepressant-like effect of liquiritin from Glycyrrhiza uralensis in chronic variable stress induced depression model rats[13].

The goal of this study was to determine the effects of GR on depression in a rat model. The c-Fos expression and corticotropinergic mechanisms associated with the antidepressant-like effects of GR were evaluated in rats on the forced swimming test.

Materials & Methods

1. Subjects

Male Sprague-Dawley rats (Orient, Kyunggi-do, Korea), weighing between 200-220 g at the start of the experiment were used in the experiments. The rats were kept on a 12:12 h light: dark cycle in individual home cages with food and water available ad libitum. The animals were allowed at least 1 week to adapt to their environment before the experiments. The rats were randomly divided into three groups (n=6 per group): the forced swimming test (FST) group (Control), and the FST and Glycyrrhizae radix treatment group (GR). The GR groups was treated daily with GR extract (100 mg/kg or 400 mg/kg, p.o.) through FST procedures, and the control group was given sterile saline.

2. Preparation of GR extracts

The Glycyrrhizae radix (GR) was purchased from an oriental drug store (HMAX, Inc. Chungcheongbuk-do, Korea). The boucher specimens were deposited at the herbarium located in the College of Oriental Medicine, Kyung won University. The dried GR samples (200 g) were immersed in a 10-fold volume of dH_2O, boiled at 80℃for 1h, and then the water extract was collected. The process was repeated once, and the extracts were combined and concentrated with a rotary evaporator and vacuum-dried to yield 30.5% (w/w) extract.

3. Behavioral test

The FST was originally described by Porsolt et al. (1977)[14-16] and now is the most widely used pharmacological model for assessing antidepressant activity[17]. The development of immobility when the rodents are placed in an inescapable cylinder of water reflects the cessation of persistent escape-directed behavior[18]. The apparatus consisted of a transparent Plexiglas cylinder (50 cm high x 20 cm wide) filled to a 30 cm depth with water at room temperature. During the pre-test, the rats were placed in the cylinder for 15 min, 24 h prior to the 5-min swimming test. GR extract (100, 400 mg/kg) or saline was administrated p.o. three times: immediately after the initial 15 min pre-test, 6 and 0.5 h prior to the swimming test. During the 5-min swimming test, the following behavioral responses were recorded by a trained observer: climbing behavior, defined as upward-directed movements of the forepaws along the side of the swim chamber; swimming behavior, defined as movement throughout the swim chamber, including crossing into another quadrant. Immobilitywas considered when the rat made no further attempts to escape but made the movement necessary to keep its head above the water. Increases in active responses, such as climbing or swimming, and reduction in immobility, were considered behaviors consistent with antidepressant-like activity[4].

4. Immonohistochemistry of c-Fos and corticotropin-releasing factor (CRF)

After the behavioral tests were completed, the animals were anesthetized with sodium pentobarbital (100 mg/kg, i.p.) and then perfused transcardially with 100 ml of saline followed by 500 ml of a 4% solution of formaldehyde prepared in phosphate buffer. The brains were then removed, postfixed in the same fixative for two to three hours at 4℃ and then placed overnight at 4℃ in PBS containing 20% sucrose. The following day, the brain was cut into coronal sections that were sliced to 30 ㎛-thicknesses.Sections were processed for c-Fos immunoreactivity using rabbit c-Fos polyclonal antibody (c-Fos, concentration 1:2000; Santacruz biotechnology, Delaware Avenue Santa Cruz, CA, U.S.A.) or corticotropin-releasing factor (CRF) immunoreactivity using goat-CRF polyclonal antibody (CRF, concentration 1:100; Santacruz biotechnology, Delaware Avenue Santa Cruz, CA, U.S.A.). The sections were then processed by a conventional avidin-biotin-peroxidase method (Vector Laboratories, Burlingame, CA, U.S.A.). The tissue was developed using diaminobenzadine (Sigma, St. Louis, CA, U.S.A.) as the chromogen. The sections were mounted on gelatin-coated slides, air-dried and coverslipped for microscopic analysis. For measuring the cells, a microrectangular grid (200×200 ㎛) was placed on paraventricular nucleus area according to the atlas of Paxinos and Watson[19], under the light microscope (×100 magnification).

5. Statistical analysis

Statistical comparisons were performed for the behavioral

and histochemical studies using the one-way ANOVA and Tukey post hoc test. All of the results are presented as the means±S.E.M; SPSS 15.0 for Windows was used for the statistical analysis. The significance level was set at $P<0.05$.

Results

1. Effects of GR on forced swimming test

The effects of the GR on the active behaviors in the FST of rats are shown in Fig. 1 (a) and (b). The ANOVA showed that the effects of the GR treatment on immobility were significant, $F_{2,53}=5.285$, $P<0.01$; swimming behavior, $F_{2,53}=2.370$, $P=0.103$; and climbing behavior, $F_{2,53}=2.112$, $P=0.131$. Post hoc analysis demonstrated that 400 mg/kg of GR in the treatment group significantly shortened the time the rats were immobile compared to the controls ($P<0.01$). There was a slight trend for a significant interaction effect on the increases swimming behavior, after 400 mg/kg. These results show that higher doses (400 mg/kg) of the extract resulted in more effective responses than GR at 100 mg/kg in reducing the immobile time.

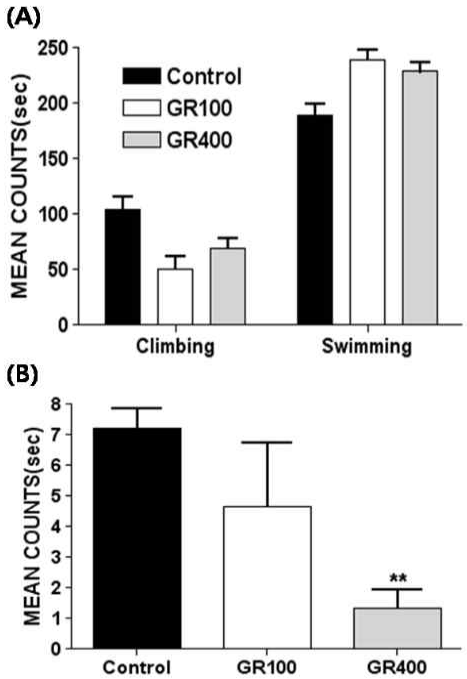

Fig. 1. (a)The effects of Glycyrrhizae radix (GR) extract on the forced swimming test in the rats. Data represent means±SEM of the duration of climbibing, swimming during the 5-min test session. (b) The effects of Glycyrrhizae radix (GR) extract on the forced swimming test (FST) in the rats. Data represent means±SEM of the duration of immobility during the 5-min test session. The results of FST were analyzed by performing separate one-way ANOVA of mean counts among the groups. Each value represents the mean±S.E.M. **p<0.01 compared to control.

2. Effects of GR on c-Fos

The results of c-Fos expression in the paraventricular area of the rats are shown in Fig. 2(a) and (b). The number of c-Fos neurons in the PVN area was 34.0±3.5 in the control group, 34.9±4.3 in the GR100 group and 18.9±0.9 in the GR 400 group [$F_{2,23}=6.714$, $P<0.01$]. The number of neurons was significantly decreased to 55.6% of the controls in the GR400group ($P<0.05$). This result showed that the administration of GR (400 mg/kg) downregulated the increase of c-Fos positive neurons in the PVN.

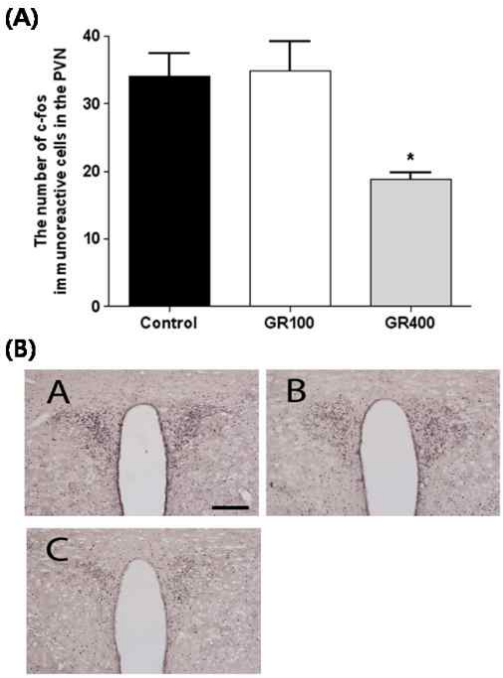

Fig. 2. (a)The mean (±S.E.M.) values of quantities of c-Fos immunostained nuclei in the paraventriular nucleus (PVN) of the experimental groups after forced swimming test. The results of c-Fos reactivity were analyzed by performing one-way ANOVA of neurons among the groups. Each value represents the mean±S.E.M. *p<0.05 compared to control. (b)Photographs showing the distribution of c-Fos immunoreactive cells in the paraventriular nucleus (PVN) of Control group (A), GR 100 group (B), GR 400 group (C). Rats after forced swimming test. Sections were cut coronally at 30㎛ and the scale bar represents 50㎛ (100x100)

3. Effects of GR on the corticotrophinergic system

The evaluation of the CRF immunoractive cells per section of the paraventricular area are shown in Fig. 3(a) and (b). The number of c-fos positive neurons in the PVN area was 7.0±0.7 in the control group, 2.2±0.6 in the GR100 group and 1.4±0.4 in the GR 400 group [$F_{2,12}=25.0$, $P<0.001$]. The number of neurons was significantly decreased to 20.0% of the controls in the GR400 group ($P<0.001$). The LSD post-hoc test revealed that the number of CRF neurons in the PVN was significantly decreased in the GR treated groups compared to the control group. Therefore, the administration of GR (400 mg/kg) suppressed the increase of CRF positive neurons in the PVN.

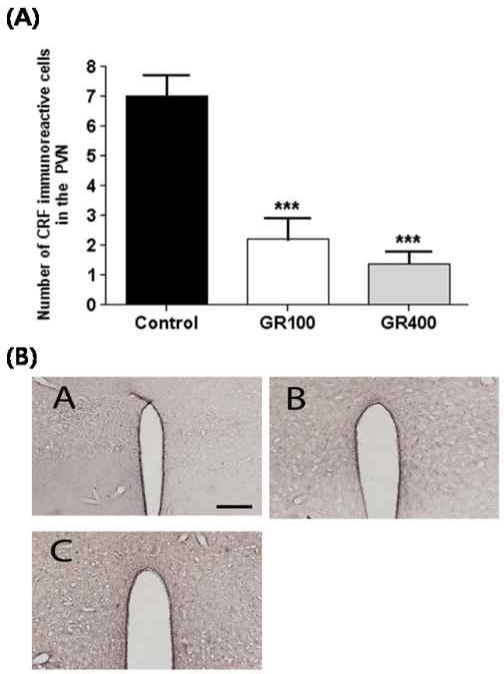

Fig. 3. (a)The mean (±S.E.M.) values of quantities of corticotropin releasing factor (CRF)immunostained nuclei in the PVN of the experimental groups after forced swimming test. The results of CRF reactivity were analyzed by performing separate one-way ANOVA of neurons among the groups. Each value represents the mean±S.E.M. ***p<0.001 compared to control. (b)Photographs showing the distribution of CRF immunoreactive cells in the PVN of Control group (A), GR 100 group (B), GR 400 group (C). Rats after forced swimming test. Sections were cut coronally at 30㎛ and the scale bar represents 50㎛ (100x100)

Discussion

There is an increasing interest in the study of the antidepressant effects of herbal medicines, since the treatment of depression with conventional antidepressant medications (monoamine oxidase inhibitors, tricyclics, selective serotonin reuptake inhibitors, selective noradrenaline reuptake inhibitors) provides a complete remission in only 50% of treated patients[20]. Reports on studies have indicated that herbal extracts and their components including liquiritin apioside, liquiritin, liquiritigenin, and glycyrrhiza acid exert antidepressant-like effects in animal models of depression[21].

The FST is widely used for screening potential antidepressants. Antidepressants reduce the time that the animal is immobile in the FST. The immobility behavior displayed by rodents when subjected to an unavoidable and inescapable stress has been hypothesized to reflect behavioral despair, which in turn may represent depressive disorders in humans. There is, indeed, a significant correlation between the clinical potency and effectiveness of antidepressants in both models[15,17]. In the present study, our results demonstrate that acute treatment with the hydrolic extract of GR produced a significant antidepressant-like response in rats subjected to the FST. At a dose of 400 mg/kg the immobile time was reduced and active behaviors such as climbing were simultaneously increased. Thus, the results showed that the GR extract acted like an antidepressant drug in the rats.

Immunohistochemical localization of c-Fos protein expression was used in the present study as a marker of neuronal activity to demonstrate the brain regions involved in the regulation of behavior associated with the forced swimming test. Expression of the c-Fos protein is thought to reflect an increase in the neuronal activity induced by a variety of physiologically relevant stimuli, suggesting that Fos may participate in the process of trans-synaptic regulation of gene expression by neurotransmitters or neuromodulators. Performance of avoidance or escape tasks in the elevated T-maze, motivated by fear/anxiety, has been found to promote an increase Fos-like immunoreactivity in different brain structures[22]. Our results also showed that performance on the forced swimming test was associated with increased Fos-like immunoreactivity in hypothalamic brain regions. However, treatment with GR significantly downregulated the neuronal activation (c-Fos expression) in the paraventricular nucleus.

Normalization of these systems is hypothesized to play an important role in mediating antidepressant activity[23-25]. Mice exposed to FST have been found to elicit HPA axis dysfunction[18,26,27], including elevations of CRF in serum[17,26,27]. However, the treatmemt of antidepressat (SSRI, MAOI) in rats downregulated the elevation of CRF in serum and brain regions. Also, they in duced inactivation of HPA axis. In the present study, administration of GR significantly attenuated the swim stress-induced increases of CRF-ir neurons in the PVN, suggesting that GR might have altered the dysregulated function of the HPA axis induced by the FST.

In conclusion, the results of the present study suggest that administration of the GR extract resulted in a specific antidepressant-like effect in an animal model. Moreover, the antidepressant-like effects appear to be mediated by corticotropinergic systems. However, further studies are necessary to confirm and extend these results. These findings may have implications for clinical trials on the effects of GR in clinical depression.

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