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Effects of Gypenosides on Acute Stress in Mice

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Abstract – The effects of gypenosides (GPS) on electric footshock (EF)-induced acute stress in mice were investigated. Mice were treated orally with GPS (30 - 400 mg/kg) once a day for 5 days. After 2 days of GPS treatment, mice were exposed to EF stimuli (intensity, 2 mA; interval, 10 s; duration, 3 min) for acute stress for 3 days. Spontaneous locomotor activity was increased by acute EF stress, which was decreased by treatment with GPS (100 and 400 mg/kg). In addition, the increased levels of dopamine and serotonin by acute EF stress in the brain were reduced by treatment with GPS (100 and 400 mg/kg). The serum levels of corticosterone increased by acute EF stress were also reduced by GPS (100 and 400 mg/kg). These results suggest that GPS shows the ameliorating effects on acute EF stress by modulating the activity of dopaminergic and serotonergic neurons, and the serum levels of corticosterone. Clinical trials of GPS need to be conducted further so as to develop promising anti-stress agents.
Keywords – Gypenosides, Acute electric footshock stress, Spontaneous locomotor activity, Dopamine and serotonin, Corticosterone, Mice

Introduction

Various stresses can cause physical changes as well as mental performances. Acute stress can make human and animals exciting to protect against the nociceptive stimuli, which increase the heart rate and blood pressure (Kovacs *et al.,* 2005). In contrast, chronic stress has been associated with the many illnesses, including anxiety disorders and depression (Kendler *et al.,* 1999). Both acute and chronic stresses activate the hypothalamic-pituitary-adrenal (HPA) axis, which are characterized by a sudden rise in adrenocorticotrophic hormone followed by the release of glucocorticoids, such as corticosterone and cortisol (Keeney *et al*., 2006; Rivier and Plotsky, 1986). In addition, the brain levels of dopamine and serotonin are increased under the conditions of acute stress, whereas the repeated and chronic stress leads to decrease in dopamine and serotonin levels in the brain (Sheikh *et al*., 2007).

Many stress models, including electric footshock (EF) stimulus, forced swimming, noise stimulus, restraint and immobilization have been employed to examine the stressful responses in mice and rats (Xie *et al*., 2008). Spontaneous locomotor activity increases after being exposed to acute stress (Katz *et al*., 1981). In contrast, locomotor activity, grip strength, body weight and endurance decrease after exposure to chronic stress (Retana-Márquez *et al*., 2003).

Gynostemma pentaphyllum Makino (Cucurbitaceas, GP) is a traditional medicinal herb that has shown various effects on diabetes, fatigue, hyperlipidemia, immunity, oxidative stress and tumor (Razmovski-Namovski *et al*., 2005). Recently, it has been reported that ethanol extract from GP (GP-EX) had an anti-stress function by improving the loss of body weight and the reduction of grip strength which were induced by chronic EF stress, as well as an immunomodulatory effect in mice (Choi *et al*., 2008; Im *et al*., 2012). GP-EX also had an ameliorating effect on chronic stress-induced anxiety disorders, which were evaluated by the elevated plus-maze and marble burying tests (Choi *et al*., 2013). In addition, GP-EX had a protective effect against neurotoxicity in the 6-hydroxy-dopamine (6-OHDA)-lesioned rat model of Parkinson's disease (PD) (Choi *et al*., 2010). Gypenosides (GPS) are the dammarane-type gynosaponin-enriched components isolated from GP (Razmovski-Namovski *et al*., 2005). GPS shows a neuroprotective effect in the 1-methyl-4-

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phenyl-1,2,3,6-tetrahydropyridine (MPTP)-induced mouse model of PD (Wang *et al*., 2010a).

In this study, the main GPS was obtained from GP (Shang *et al*., 2006; Wang *et al*., 2010b), and the pharmacological effects of GPS on acute EF stress-induced behaviors in mice were investigated in order to further define the anti-stress function of GP-EX. After being exposed to acute stress by electric EF stimuli, we examined the behavioral changes using the spontaneous locomotor test and the biochemical changes on the levels of dopamine, serotonin and corticosterone.

Experimental

Materials – GPS was purchased from Ankang Dongke Maidisen Nature Pharmaceutical Co. (Xi'an, China) (Shang *et al*., 2006; Wang *et al*., 2010b). Dopamine, serotonin, isoproterenol and 5-hydroxyindoleacetic acid (HIAA) were purchased from Sigma Co. (St. Louis, MO, USA). A corticosterone kit was purchased from USCN Life Sci. (E0504m, Wuhan, China). All other chemicals were of HPLC grade.

Animals – Mice (ICR, male, 20 - 25 g) were purchased from Samtako Co. (Animal Breeding Center, Osan, Korea). Animals were housed in a temperature (23 ± 2 ºC) and humidity (50 ± 2%) controlled environment with a 12 h light/dark cycle (lights on at 07:00), and with *ad libitum* access to standard mouse food and water. The present study was performed in accordance with the guidelines for the care and use of laboratory animals of Chungbuk National University Laboratory Animal Research Center (approval number: CBNU-481-12-01).

Experimental design and the exposure to acute EF stress – Mice were randomly divided into the groups containing 8 - 12 animals. The control groups received saline (0.9%). GPS was the groups which were treated orally with GPS (30 - 400 mg/kg) for 5 days once a day including a 2 day-adaptation period. Stress was the groups which, after 2 days of GPS treatment, were exposed to the acute EF stimuli (intensity, 2 mA; interval, 10 s; duration, 3 min) in an electrified shock chamber at 14:00 every day for 3 days using an electric shock generator (Seil Electric Co., Daejeon, Korea). During the periods of acute stress, mice were treated with GPS approximately 2 h before the exposure of EF stress. After the final treatment with GPS and behavioral tests, mice were anaesthetized and sacrificed to obtain brain tissues and serum for biochemical analyses.

The spontaneous locomotor activity test – Spontaneous locomotor activity was measured every day using a tilting-type ambulometer (Model AMB-10, O'Hara, Tokyo, Japan). Each mouse was placed in a round cage (diameter, 20 cm; depth, 18 cm) and the numbers of horizontal movements were detected automatically for 30 min.

Measurement of dopamine and serotonin levels – After the final behavioral tests, the whole brain tissues were homogenized in perchloric acid (1 M, 300 μl) and isoproterenol (100 pmol, internal standard) or HIAA (300 pmol, internal standard) and the homogenates were centrifuged at 12,000 × *g* at 4 ºC for 20 min. The supernatants were filtered using pore filters (Millex-GV, 0.45 μm, Waters, Milford, MA, USA) and the filtrate (100 μl) was injected into an HPLC system (Satoh *et al*., 2008; Yanagisa *et al*., 1982).

Measurement of corticosterone – After the final behavioral tests, blood was collected from the heart of sacrificed mice and centrifuged at 12,000 × *g* at 4 ºC for 15 min to obtain serum. The serum levels of corticosterone were assessed using an enzyme-linked immunosorbent assay kit.

Statistical analysis – Data were analyzed using a one-way analysis of variance (ANOVA) followed by a Tukey's test for evaluating the dose-dependent effects of GPS. Two-way ANOVA followed by Tukey's test was also employed to evaluate the effects of GPS on acute EF stress. All data were expressed as means ± S.E.M. with p values < 0.05 being considered statistically significant.

Results

Effects of GPS on spontaneous locomotor activity – Treatment with GPS (30 - 400 mg/kg) did not alter the counts of spontaneous locomotor activity at day-3, compared with the control groups (Fig. 1). In contrast, the counts of spontaneous locomotor activity after being exposed to acute EF stress were increased by 16.8% ($p < 0.05$) at day-3, compared with the control groups (n = 12). However, the counts of spontaneous locomotor activity were reduced by 3.2%, 5.0%, 10.9% ($p < 0.05$), 12.3% ($p < 0.05$) and 13.1% ($p < 0.05$) by treatment with GPS (30, 50, 100, 200 and 400 mg/kg) respectively for 5 days, compared with the acute EF-stressed groups (n = 12) (Fig. 1). In addition, at day-2, the counts of spontaneous locomotor activity were increased by 7.9% by acute EF stress, and they were reduced by 0.7 - 6.1% by treatment with GPS (30 - 400 mg/kg), but it was not significant (data not shown).

Effects of GPS on the levels of dopamine and serotonin in the brain – Treatment with GPS (30 - 400 mg/kg) for 5 days did not alter the levels of dopamine and serotonin in the brain, compared with the control groups