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Short Communication

Red Ginseng Inhibits Exercise-Induced Increase in 5-Hydroxytryptamine Synthesis and Tryptophan Hydroxylase Expression in Dorsal Raphe of Rats

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Abstract. Red ginseng has been used as an ergogenic aid for endurance exercise. In this study, the effect of aqueous extract of Red ginseng on the endurance in treadmill exercise and 5-hydroxytryptamine (serotonin) synthesis and tryptophan hydroxylase expression in the dorsal raphe of rats were studied. Rats receiving Red ginseng showed increased time to exhaustion for treadmill running, and Red ginseng treatment inhibited exercise-induced increases in 5-hydroxytryptamine synthesis and tryptophan hydroxylase expression in the dorsal raphe. These results suggest that the suppressive effect of Red ginseng on serotonin level during exercise is a possible ergogenic mechanism of Red ginseng.

Keywords: Red ginseng, treadmill exercise, serotonin

Ginseng radix is the root of *Panax Ginseng* C.A. MEYER (Araliaceae, *Ginseng radix*). The aqueous extract of *Ginseng radix* has been used to treat anemia, diabetes mellitus, insomnia, gastritis, abnormalities in blood pressure, dyspepsia, overstrain, and fatigue (1); it contains several triterpene glycosides, referred to as ginsenosides (1, 2). *Ginseng radix* possesses various pharmacological effects including hypotensive, cardiotonic, sedative, aphrodisiac, anti-aging, and anti-oxidant actions (3).

Davis and Bailey (4) reported that multiple neurological factors influence fatigability during prolonged exercise. In the mammalian central nervous system (CNS), serotonin (5-hydroxytryptamine, 5-HT) is known to modulate body temperature, blood pressure, endocrine activity, appetite, sexual behavior, movement, emesis, and pain (5). The central fatigue hypothesis states that increases in the concentration of 5-HT in the brain during prolonged exercise impairs CNS functions and thus bring about a deterioration in exercise performance (6). It was also suggested that increases or decreases in 5-HT activity in the brain during prolonged exercise hasten or delay the onset of fatigue, respectively (4). Most of the cell bodies of the serotonergic neurons in the brain arise from the dorsal raphe nuclei; they send projections to diverse target regions including the limbic system, hypothalamus, striatum, and cerebral cortex (7).

Tryptophan hydroxylase (TPH) catalyzes the rate-limiting step of serotonin biosynthesis in serotonergic neurons. As such, the TPH gene is a likely target in the modulatory pathway for serotonergic functions (8, 9). It has been reported that TPH expression is modulated by several factors, such as immobilization and noise (9, 10).

Red ginseng is produced from *Ginseng radix* by steaming. The aqueous extract of Red ginseng has traditionally been used as an ergogenic aid in endurance exercise. In order to clarify the ergogenic mechanism of Red ginseng, its effect on 5-HT synthesis and TPH expression in the dorsal raphe was investigated via immunohistochemistry.

Male Sprague-Dawley rats weighing 150 ± 10 g (5 weeks of age) were used in the present study. The experiment was conducted in accordance with the

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Guiding Principles for the Care and Use of Laboratory Animals approved by The Japanese Pharmacological Society and the Korean Academy of Medical Sciences. The animals were divided into seven groups (n = 10 in each group): the control group, the exercise group, the exercise and 10 mg/kg Red ginseng-treated group, the exercise and 25 mg/kg Red ginseng-treated group, the exercise and 50 mg/kg Red ginseng-treated group, the exercise and 100 mg/kg Red ginseng-treated group, and the exercise and 10 mg/kg caffeine-treated group (Caffeine was used as a reference drug). Animals of the Red ginseng-treated groups received intraperitoneal injection of the aqueous extract of Red ginseng 30 min prior to the commencement of treadmill exercise over 4 consecutive days, while those of the control group received an equivalent amount of saline. In addition, animals of the caffeine-treated group were injected intraperitoneally with caffeine.

Red ginseng used in this experiment was obtained from Haonar mart (Seoul, Korea). After washing, Red ginseng was immersed in cold water for 12 h. To obtain the aqueous extract of Red ginseng, 200 g of Red ginseng was added to distilled water, heat-extracted at 80°C, concentrated using a rotary evaporator, and lyophilized. The resulting powder, weighing 25 g, was diluted with saline. After filtering through a 0.45-μm syringe filter, it was administered intraperitionally at the respective doses of the groups once a day for 5 consecutive days.

Rats of the exercise groups were made to run on the treadmill for 30 min each day for 4 consecutive days. The exercise load consisted of running at a speed of 10 m/min for 10 min, at 13 m/min for another 10 min, and at 16 m/min for the last 10 min, with 0 degree of inclination. On the 5th day of the experiment, time to exhaustion for treadmill running was determined for the exercise groups. Time to exhaustion is defined as the time between the commencement of exercise and the first occurrence of the experimental animal failing to keep up with the treadmill machine for a period of 3 min or more. The speed of the treadmill used for measurement of the time to exhaustion was 20 m/min. Immediate after determination of the time to exhaustion, rats were sacrificed.

Animals were fully anesthetized using Zoletil 50® (10 mg/kg, i.p.; Vibac Laboratories, Carros, France), transcardially perfused with 50 mM phosphate-buffered saline, and fixed with 4% paraformaldehyde in 100 mM phosphate buffer at pH 7.4. The brains were removed, postfixed in the same fixative overnight, and transferred into a 30% sucrose solution for cryoprotection. Coronal sections of 40-μm thickness were made using a freezing microtome (Leica, Nussloch, Germany). For detection of 5-HT-positive and TPH-positive cells in the dorsal raphe, 10 average sections were selected in each brain from the region spanning from Bregma −7.30 to −8.30 mm. The free-floating tissue sections were incubated overnight with rabbit anti-5-HT antibody (1:500; Oncogene Reserch Product, Cambridge, UK) or with mouse monoclonal anti-TPH antibody (1:1000; Oncogene Research Product, Cambridge, UK). The sections were then incubated for 1 h with biotinylated anti-rabbit secondary antibody or with anti-mouse secondary antibody (Vector Laboratories, Burlingame, CA, USA). After incubation for another 1 h with avidin-biotin-peroxidase complex, the sections were put in a reaction solution consisting of 0.02% 3,3'-diaminobenzidine and 0.03% H_2O_2 in 50 mM Tris-buffer (pH 7.6); afterwards, the sections were mounted on gelatin-coated glass slides.

The numbers of 5-HT-positive and TPH-positive cells in the dorsal raphe were counted using a light microscope (Olympus, Tokyo). Data were analyzed using SPSS by one-way analysis of variance (ANOVA) followed by Scheffé's Post-hoc test, and results are expressed as the mean ± S.E.M. Differences were considered significant for $P<0.05$.

The mean time to exhaustion for forced treadmill running was 56.88 ± 3.44 min for the exercise group; the figure was increased to 59.44 ± 5.76 min for the exercise and 10 mg/kg Red ginseng-treated group, 56.55 ± 3.80 min for the exercise and 25 mg/kg Red ginseng-treated group, 79.43 ± 8.74 min for the exercise and 50 mg/kg Red ginseng-treated group, 98.96 ± 5.72 min for the exercise and 100 mg/kg Red ginseng-treated group, and 120.93 ± 1.37 min for the exercise and 10 mg/kg caffeine-treated group.

The number of 5-HT-positive cells in the dorsal raphe was 54.40 ± 5.19 per section in the control group, 94.50 ± 7.42 per section in the exercise group, 88.61 ± 7.69 per section in the exercise and 10 mg/kg Red ginseng-treated group, 72.04 ± 5.72 per section in the exercise and 25 mg/kg Red ginseng-treated group, 52.81 ± 4.96 per section in the exercise and 50 mg/kg Red ginseng-treated group, 46.00 ± 3.60 per section in the exercise and 100 mg/kg Red ginseng-treated group, and 41.10 ± 4.03 per section in the exercise and 10 mg /kg caffeine-treated group (Fig. 1).

The number of TPH-positive cells in the dorsal raphe was 201.73 ± 13.67 per section in the control group, 263.18 ± 14.23 per section in the exercise group, 225.60 ± 17.89 per section in the exercise and 10 mg/kg Red ginseng-treated group, 216.53 ± 16.93 per section in the exercise and 25 mg/kg Red ginseng-treated group, 181.90 ± 11.98 per section in the exercise and 50 mg/kg Red ginseng-treated group, 178.52 ± 10.45 per section in the exercise and 100 mg/kg Red ginseng-treated group,

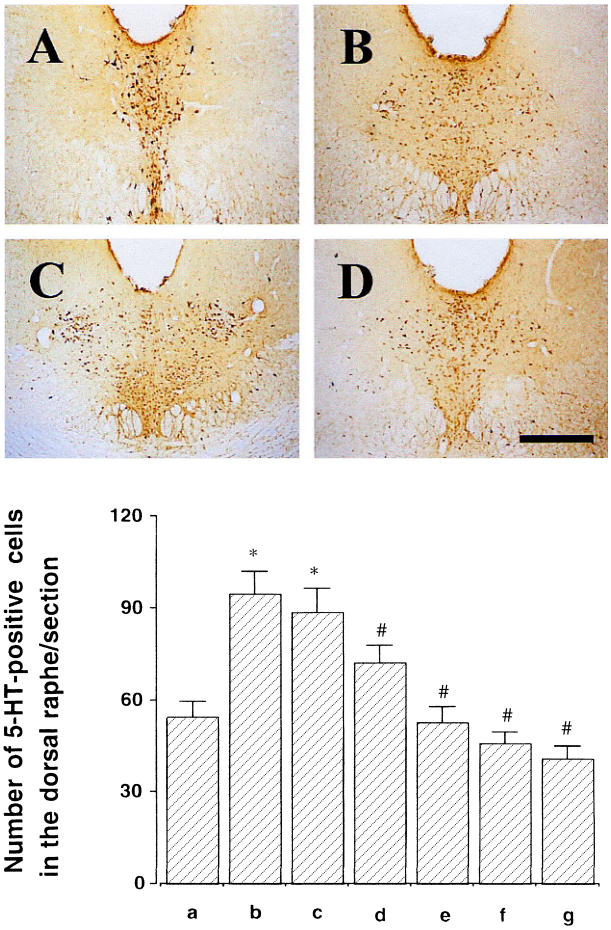

Fig. 1. Effect of Red ginseng on 5-hydroxytryptamine (5-HT, serotonin) expression in the dorsal raphe. Above: Photomicrographs of 5-HT-positive cells in the dorsal raphe. A, control group; B, exercise group; C, exercise and 10 mg/kg Red ginseng-treated group; D, exercise and 50 mg/kg Red ginseng-treated group. Sections were stained for 5-HT-like immunoreactivity (brown). Scale bar represents 250 μm. Below: Number of 5-HT-positive cells in the dorsal raphe of each group. a, control group; b, exercise group; c, exercise and 10 mg/kg Red ginseng-treated group; d, exercise and 25 mg/kg Red ginseng-treated group; e, exercise and 50 mg/kg Red ginseng-treated group; f, exercise and 100 mg/kg Red ginseng-treated group; g, exercise and 10 mg/kg caffeine-treated group. Values are represented as the mean ± S.E.M. * represents $P<0.05$, compared to the control group. # represents $P<0.05$, compared to the exercise group.

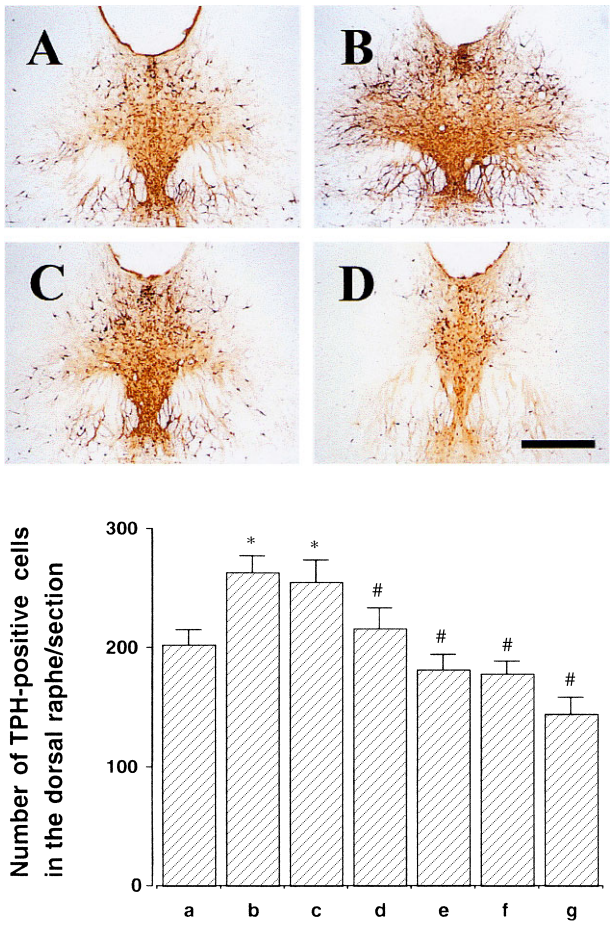

Fig. 2. Effect of Red ginseng on tryptophan hydroxylase (TPH) expression in the dorsal raphe. Above: Photomicrographs of TPH-positive cells in dorsal raphe. A, control group; B, exercise group; C, exercise and 10 mg/kg Red ginseng-treated group; D, exercise and 50 mg/kg Red ginseng-treated group. Sections were stained for TPH-like immunoreactivity (brown). Scale bar represents 250 μm. Below: Mean number of TPH-positive cells in the dorsal raphe of each group. a, control group; b, exercise group; c, exercise and 10 mg/kg Red ginseng-treated group; d, exercise and 25 mg/kg Red ginseng-treated group; e, exercise and 50 mg/kg Red ginseng-treated group; f, exercise and 100 mg/kg Red ginseng-treated group; g, exercise and 10 mg/kg caffeine-treated group. Values are represented as the mean ± S.E.M. * represents $P<0.05$, compared to the control group. # represents $P<0.05$, compared to the exercise group.

and 145.31 ± 12.84 per section in the exercise and 10 mg/kg caffeine-treated group (Fig. 2).

It has been reported that *Ginseng radix* enhances exercise performance during exhaustive exercise in mice (11). However, in yet another study, *Ginseng radix* was shown to have no significant ergogenic effect on performance in graded maximal aerobic exercise (12). Saponin is the main component of *Ginseng radix*. Ginsenosides are known to have an anti-stress effect and attenuate neuronal cell damage induced by glutamate and kainic acid: gisenosides Rb1 and Rg3 protect neurons against excitotoxicity and oxidative stress (13).

In the present study, Red ginseng treatment increased the time to exhaustion for treadmill running in a dose-dependent manner. Newsholme et al. (6) have proposed the "central fatigue hypothesis" which states that maximal exertion or exhaustion may directly affect serotonergic activity via locomotor regulation or stimulation of longer-term stress responsiveness. It has been suggested that increases in 5-HT concentration in the brain and

in overall serotonergic activity taking place during endurance exercise are of relevance to the increase in the level of physical fatigue and perhaps to that of mental fatigue as well (14). Increase in the level of serotonin during endurance exercise coincides with the onset of fatigue (15), raising the possibility that differences in serotonin receptor sensitivity may be an important determinant of relative endurance.

In the present study, Red ginseng treatment was shown to inhibit exercise-induced increase in the numbers of 5-HT positive and TPH-positive cells in the dorsal raphe. Based on the results, it can be suggested that the suppressive effect of Red ginseng on 5-HT synthesis and TPH expression during exercise is a possible ergogenic mechanism of Red ginseng.

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