Effects of *Paeonia radix* on 5-Hydroxytryptamine Synthesis and Tryptophan Hydroxylase Expression in the Dorsal Raphe of Exercised Rats

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**Paeonia radix** is the root of *Paeonia japonica Miyabe*, a perennial plant classified in the family Paeoniaceae. In the present study, the effects of *Paeonia radix* on performance in treadmill exercise, and 5-hydroxytryptamine (5-HT) synthesis and tryptophan hydroxylase (TPH) expression in the dorsal raphe were investigated. Time to exhaustion in treadmill exercise was increased and exercise-induced increases in 5-HT synthesis and TPH expression in the dorsal raphe were shown to be suppressed by *Paeonia radix* treatment; 5-HT synthesis and TPH expression were inhibited by *Paeonia radix* treatment under resting conditions as well. In sum, treatment with *Paeonia radix*, inhibiting 5-HT synthesis and TPH expression, may bring about reduced fatigue, both during exercise and the resting state.

**Keywords** *Paeonia radix*; treadmill exercise; 5-hydroxytryptamine; tryptophan hydroxylase

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*Paeonia radix* is the root of *Paeonia japonica Miyabe*, which is a perennial plant classified in the family Paeoniaceae. The aqueous extract of *Paeonia radix* has been used in Oriental medicine to treat various illnesses including gastralgia, gynopathy, enterorrhagia, and headache. *Paeoniae radix* possesses various pharmacological properties such as sedative, analgesic, anti-inflammatory, and anti-stress action.1—4) Recently it has been reported that *Paeonia radix* has antimicrobial and immunomodulating action as well.5,4)

In mammals, serotonin (5-hydroxytryptamine, 5-HT) is known to participate in the modulation of body temperature, blood pressure, endocrine activity, appetite, sexual behavior, movement, emesis, and pain.5) 5-HT is one of the central neurotransmitters which regulate the behavioral functions in the vertebrate brain. The activity of serotonergic neural projections is influenced by extrinsic and intrinsic impulses carrying body information.6) Electrophysiological studies in cats indicate that the activity of serotonergic neurons in the dorsal raphe is affected by various forms of metabolic, psychological, and physical stress.7,8,9) This 5-HT system plays an important role in neuromodulation of cognition and behavior.7)

Tryptophan hydroxylase (TPH) catalyzes the rate-limiting step of serotonin biosynthesis in serotonergic neurons of the raphe nuclei. As such, the TPH gene is a likely target in the modulatory pathway for serotonin functions.5,9) It has been reported that TPH expression is modulated by several neurotransmitters which regulate the behavioral functions in the brain resulting from the enhanced activity impairs central nervous system (CNS) functions and brings about deterioration in exercise performance and fatigue.12,13) Several studies have shown that physical exercise increases the synthesis and metabolism of 5-HT in the brain, and increased concentration of 5-HT was shown to impair exercise performance in both rats and humans; in contrast, performance in running was improved significantly by decreased 5-HT concentration.7,13)

**Paeonia japonica Miyabe** is known to nourish and restore the blood in Oriental medicine, and it has been widely used as a component of blood-building decoctions and for reducing fatigue and fatigability.7,14) However, the effect of *Paeonia radix* on endurance exercise has not been investigated in relation to central fatigue to date. In the present study, the effects of *Paeonia radix* on time to exhaustion in treadmill exercise, and on 5-HT synthesis and TPH expression in the dorsal raphe were investigated.

**MATERIALS AND METHODS**

**Animals and Treatments** Adult male Sprague-Dawley rats weighing 300±10 g were obtained from a commercial breeder (Daehan Biolink Co., Chungbuk, Korea). The experimental procedures were performed in accordance with the animal care guidelines of the National Institute of Health (NIH) and the Korean Academy of Medical Sciences. Each animal was housed under controlled temperature (20±2°C) and lighting (08:00 h—20:00 h) conditions with food and water made available *ad libitum*. The animals were divided into eight groups (n=10 in each group): the control group, the 10 mg/kg *Paeonia radix*-treated group, the 50 mg/kg *Paeonia radix*-treated group, the 100 mg/kg *Paeonia radix*-treated group, the exercise group, the exercise 10 mg/kg *Paeonia radix*-treated group, the exercise 30 mg/kg *Paeonia radix*-treated group, and the exercise 100 mg/kg *Paeonia radix*-treated group.

*Paeonia radix* used in this experiment was obtained from Kyung-Dong Market (Seoul, Korea). After washing, *Paeonia radix* was immersed in cold water for 12 h. To obtain the aqueous extract, 200 g of *Paeonia radix* was added to dis-
tilled 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, animals of the *Paeonia radix*-treated groups received the extract of *Paeonia radix* at the respective doses intraperitoneally 30 min before the start of treadmill exercise for 7 consecutive days, and those of the control group received equivalent amounts of saline once a day for the same duration of time.

**Treadmill Exercise Protocols** The physical exercise load applied in the present study took the form of treadmill running on a motor-driven treadmill. Rats of the exercise groups were made to run on treadmills for 30 min once a day for 6 consecutive days, whereas those of the non-exercise groups including the control group were left on the treadmill without running for 30 min. 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 7th 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 setting of the treadmill used for measurement of the time to exhaustion was 18 m/min for 2 min, 21 m/min for 2 min, 24 m/min for 2 min, and then 26 m/min until exhaustion, the presumed equilibrium speed of running for rats. Immediately after determination of the time to exhaustion, the rats were sacrificed.

**Tissue Preparation** To begin the sacrificial process, animals were fully anesthetized using Zoletil 50® (10 mg/kg, i.p.; Vibac Laboratories, Carros, France), transcardially perfused with 50 mm phosphate-buffered saline (PBS), and fixed with 4% paraformaldehyde (PFA) in 100 mm phosphate buffer (PB) 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).

**5-HT and TPH Immunohistochemistry** For detection of 5-HT-positive and TPH-positive cells in the dorsal raphe, immunohistochemistry was performed. To begin the procedure, five sections on average were selected in each brain region spanning from Bregma −7.30 to −8.00 mm. Sections were incubated in PBS for 10 min and washed three times, again with PBS, then incubated in 1% hydrogen peroxide (H₂O₂) for 30 min. Next, the sections were incubated overnight with rabbit anti-5-HT antibody (Oncogene Research Products, Cambridge, U.K.) at a dilution of 1:500 for visualization of 5-HT expression or with mouse monoclonal anti-TPH antibody (Oncogene Research Products, Cambridge, U.K.) at a dilution of 1:1000 for visualization of TPH expression. They were then incubated for 1 h with biotinylated anti-rabbit secondary antibody or with anti-mouse secondary antibody (Vector Laboratories, Burlingame, CA, U.S.A.), and subsequently incubated with avidin-biotin-peroxidase complex (Vector Laboratories, Burlingame, CA, U.S.A.) for 1 h at room temperature. Immunoreactivity was visualized by incubating the sections in a solution consisting of 0.05% 3,3′-diaminobenzidine and 0.01% H₂O₂ in 50 mm Tris–buffer (pH 7.6) for approximately 3 min. The sections were then mounted on gelatin-coated glass slides.

**Data Analysis** The numbers of 5-HT-positive and TPH-positive cells were counted in the dorsal raphe in each of the selected sections using a light microscope (Olympus, Tokyo, Japan). Data was analyzed using SPSS by one-way analysis of variance (ANOVA) followed by Duncan’s post-hoc test, and results are expressed as mean±standard error mean (S.E.M.). Differences were considered significant for *p*<0.05.

**RESULTS**

**Effect of *Paeonia radix* on the Time to Exhaustion in Treadmill Running** The time to exhaustion of the rats in each exercise group is presented in Fig. 1. The mean time to exhaustion for forced treadmill running was 43.15±2.48 min in the only exercise group, 44.17±2.31 min in the exercise and 10 mg/kg *Paeonia radix*-treated group, 51.95±2.04 min in the exercise and 50 mg/kg *Paeonia radix*-treated group, and 48.82±2.21 min in the exercise and 100 mg/kg *Paeonia radix*-treated group. These results suggest that *Paeonia radix* increases the time to exhaustion, although only the increase following treatment with 50 mg/kg *Paeonia radix* is of statistical significance.

**Effect of *Paeonia radix* on the Number of 5-HT-Positive Cells in the Dorsal Raphe** Photomicrographs of 5-HT-positive cells in the dorsal raphe are presented in Fig. 2. The number of 5-HT-positive cells in the dorsal raphe was 92.71±5.74/sec in the control group, 89.57±5.47/sec in the 10 mg/kg *Paeonia radix*-treated group, 71.57±4.36/sec in the 50 mg/kg *Paeonia radix*-treated group, 82.57±2.78/sec in the 100 mg/kg *Paeonia radix*-treated group, 133.29±5.90/sec in the exercise group, 105.00±2.86/sec in the exercise and 10 mg/kg *Paeonia radix*-treated group, 91.71±2.76/sec in the exercise and 50 mg/kg *Paeonia radix*-treated group, and 109.00±2.36/sec in the exercise and 100 mg/kg *Paeonia radix*-treated group (Fig. 2). Treadmill exercise appears to have increased the number of 5-HT-positive cells in the dorsal raphe, and *Paeonia radix* treatment decreased 5-HT synthesis significantly in the dorsal raphe under both resting and exercise conditions.

**Effect of *Paeonia radix* on the Number of TPH-Positive Cells in the Dorsal Raphe** Photomicrographs of TPH-positive cells in the dorsal raphe are presented in Fig. 3. The number of TPH-positive cells in the dorsal raphe was...
141.00±21.10/section in the control group, 117.00±19.89/section in the 10 mg/kg Paeonia radix-treated group, 71.50±8.16/section in the 50 mg/kg Paeonia radix-treated group, 124.67±13.72/section in the 100 mg/kg Paeonia radix-treated group, 161.83±18.90/section in the exercise group, 138.00±14.71/section in the exercise and 10 mg/kg Paeonia radix-treated group, 95.00±13.65/section in the exercise and 50 mg/kg Paeonia radix-treated group, and 139.17±7.56/section in the exercise and 100 mg/kg Paeonia radix-treated group (Fig. 3). Treadmill exercise appears to have increased the number of TPH-positive cells in the dorsal raphe, and Paeonia radix treatment decreased TPH expression significantly in the dorsal raphe under both resting and exercise conditions, although statistical significance was demonstrated only for the decrease following treatment at the dose of 50 mg/kg.

DISCUSSION

In Oriental medicine, Paeonia radix has traditionally been used for lessening fatigue and fatiguability and has been thought to refresh and nourish the blood. Egger and Keil reported that Paeonia arborea possesses anti-inflammatory and anti-stress-induced ulcer effects. It has also been reported that Paeonia japonica Myabe is useful for stress, pain, and a tonic state. In addition, Park et al. showed that Paeonia japonica Myabe improves the immune response of helper T cells, B cells, and macrophages. In the present study, treatment with Paeonia japonica Myabe at a dose of 50 mg/kg was shown to increase the time to exhaustion in treadmill running while similar treatment at doses of 10 mg/kg and 100 mg/kg had no significant effect.

Increased 5-HT concentration is known to result in lethargy, loss of motivation, and decreased power output during sustained exercise. 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 of mental fatigue as well. Newsholme et al. proposed the “central fatigue hypothesis,” which states that maximal exertion or exhaustion may directly affect serotonergic activity via locomotor regulation or stimulation of long-term stress responsiveness. Increase in the level of serotonin during endurance exercise coincides with the onset of fa-
tigue, raising the possibility that differences in serotonin receptor sensitivity may be an important determinant of relative endurance.\textsuperscript{13,15,16} In the present results, treadmill exercise was shown to increase 5-HT synthesis in the dorsal raphe, and \textit{Paeonia radix} administration suppressed the exercise-induced increment in the number of 5-HT-positive cells in the dorsal raphe. This inhibitive effect of \textit{Paeonia japonica} \textsc{miyabe} on 5-HT synthesis differed with the dose applied: at doses of 10 mg/kg and 100 mg/kg, the plant significantly suppressed 5-HT synthesis in the exercise groups, while its effect on 5-HT synthesis visible at these doses was statistically insignificant in the non-exercise groups. The most potent inhibition of 5-HT synthesis was observed at the dose of 50 mg/kg in both non-exercise and exercise groups.

Reduction in TPH activity has been shown from \textit{in vivo} studies to lead to rapid decrease in 5-HT release,\textsuperscript{9} indicating that changes in TPH levels can profoundly influence synaptic 5-HT activity. Increased TPH mRNA expression has been shown to lead to heightened TPH activity and 5-HT metabolism, but the extent of elevation in TPH mRNA levels has been found to be much greater than the change in 5-HT turnover.\textsuperscript{11}

In the present study, \textit{Paeonia radix} treatment was shown to inhibit exercise-induced increase in the number of TPH-positive cells in the dorsal raphe. This inhibitive effect of \textit{Paeonia japonica} \textsc{miyabe} on TPH expression also differed with the dose administrated. The effect on TPH expression was statistically insignificant at doses of 10 mg/kg and 100 mg/kg in both the non-exercise and the exercise groups, while the most potent suppression was observed at the dose of 50 mg/kg in both groups. In sum, these results suggest that treatment with \textit{Paeonia japonica} \textsc{miyabe}, through inhibition of 5-HT synthesis and TPH expression, may bring about reduced fatigue, both during exercise and at rest. The dose of \textit{Paeonia japonica} \textsc{miyabe} at which the reduction of fatigue in rats was most prominent was 50 mg/kg in this study.

\textit{Paeonia radix} contains a multitude of complex organic compounds. It has been suggested that paeoniflorin, one of the numerous families of compounds extracted from \textit{Paeonia radix} so far, may have anti-inflammatory and anti-stress effects and may decrease blood pressure.\textsuperscript{1,2} Galloantin is yet another group of pharmacologically active components of \textit{Paeonia radix}. Goto et al.\textsuperscript{17} reported that \textit{Paeonia radix} exhibits endothelium-dependent vasodilation when applied to isolated rat aorta and that the component chiefly responsible for this effect is galloantin. Sugaya et al.\textsuperscript{18} showed that albiiflorin and galloantin, both of which are found in \textit{Paeonia radix}, have anticonvulsive action in the rat cerebral cortex and that this effect is brought about through inhibition of the seizure-related decrease in extracellular calcium and the consequent increase in intracellular calcium. It has recently been reported that a novel galloantin called Pistafolia A, isolated from the leaf extract of \textit{Pistacia weinmannifolia}, may have reactive oxygen species-scavenging effects and thus prevent oxidative neuronal cell damage.\textsuperscript{19} In addition, Tsuda \textit{et al.}\textsuperscript{20} reported that a combination of crude galloantin and paeoniflorin protects against hippocampal neuronal damage in the cobalt focus epilepsy model; although, paeoniflorin alone was shown to have no effect. Based on these studies, it is suspected that galloantin and paeoniflorin are the components of \textit{Paeonia radix} chiefly responsible for its effect on fatigue during treadmill running. Further study, however, is needed to identify the main active components of \textit{Paeonia radix} in reducing fatigue.

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\textbf{REFERENCES}