Effects of *Phellinus linteus* Administration on Serotonin Synthesis in the Brain and Expression of Monocarboxylate Transporters in the Muscle during Exhaustive Exercise in Rats

Jin-Hee SEO¹, Yun-Hee SUNG¹, Ki-Jeong KIM¹, Mal-Soon SHIN¹,
Eun-Kyu LEE^{1,2} and Chang-Ju KIM^{1,*}

¹Department of Physiology, College of Medicine, Kyung Hee University, #1 Hoigi-dong,
Dongdaemoon-gu, Seoul 130–701, Republic of Korea

²Department of Internal Medicine, Andong Medical Group, #574–2 Susang-dong, Andong,
Kyungbuk 760–410, Republic of Korea

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Summary This study was conducted to determine the effects of *Phellinus linteus* (PL) on serotonin synthesis in the brain and on the expression of monocarboxylate transporters (MCTs) in muscles during exhaustive exercise in rats. In this study, 60 male Sprague-Dawley rats were divided into the following 6 groups: control; exercise; exercise and 50 mg/kg of PL treatment; exercise and 100 mg/kg PL treatment; exercise and 200 mg/kg of PL treatment; and exercise and 100 mg/kg of caffeine treatment. Treatment with 200 mg/kg of PL led to a significant increase in the time to exhaustion in response to running on a treadmill and a significant decrease in 5-hydroxytryptamine synthesis and tryptophan hydroxylase expression in the dorsal raphe of rats. MCT1 and MCT4 expression of the gastrocnemius muscles was also increased in response to treatment with 200 mg/kg of PL. The results of the present study demonstrated that the administration of PL increased endurance exercise performance through inhibition of serotonin production in the brain and increased the expression of MCT1 and MCT4 in muscles. These results suggest that PL exerts an ergogenic effect.

Key Words *Phellinus linteus*, exhaustive performance, 5-hydroxytryptamine, tryptophan hydroxylase, monocarboxylate transporters

The neurotransmitter serotonin (5-hydroxytryptamine [5-HT]) has a variety of effects on respiratory neuronal activity (1, 2). For example, 5-HT is causally involved in multiple central nervous facets of mood control, as well as the regulation of sleep, anxiety, alcohol intake, drug abuse, food intake, and sexual behavior (3). The variability in the effects of exogenous and endogenous 5-HT may be due to the subtypes of 5-HT receptors that are preferentially expressed (1). In the first step in the biosynthesis of serotonin, tryptophan hydroxylase (TPH) catalyzes the formation of 5-hydroxytryptophan (4). TPH is also the rate-limiting enzyme in serotonin production, and its expression has been used as an indicator of serotonin synthesis (5).

Prolonged exhausting exercise inevitably leads to fatigue, which is a complex multifactorial phenomenon that involves peripheral and central components. The induction of fatigue in response to an increase in the serotonin concentration in the brain has been described in experimental animals (6–8). Additionally, an increase in serotonin levels has been found to inhibit descending motoneurons in animals (9). Fatigue caused by an increase in the concentration of brain

serotonin has been referred to as central fatigue (10). This type of fatigue is known to limit endurance exercise performance via deterioration in brain function (11, 12).

The monocarboxylate transporters (MCTs) are a family of proteins that are expressed in tissue-specific patterns in human and animal muscles (13), and MCT isoforms (MCT1–14) have been identified (14). It has been suggested that MCT1 and MCT4 are the key transporters involved in the regulation of the lactate flux across the plasma membrane (15). In human and rat skeletal muscles, MCT1 expression is highly correlated with indices of oxidative capacities of muscles (16, 17). Additionally, MCT1 plays a major role in the influx of lactic acid for oxidation (13, 18, 19). MCT4 is primarily expressed in fast-twitch fibers, and its expression is associated with indices of glycolytic capacity (20). It has also been suggested that MCT4 plays a primary role in the efflux of lactate from muscle fibers, especially in white muscle fibers, during intense exercise (18, 21). MCT4 protein expression is less easily perturbed by muscle activity, apparently requiring more intense exercise to be up-regulated (17, 20, 22). MCT1 and MCT4 are co-expressed in muscles; however, MCT1 is more prevalent in type I fibers, while MCT4 is more abundant in type II fibers (17). As a result, there is good agree-

*To whom correspondence should be addressed.

E-mail: changju@khu.ac.kr

ment among alterations in muscle activity patterns, the levels of expression of MCT1 and MCT4, and the rates of lactate transport (15).

Phellinus linteus (PL) is a medicinal mushroom that has long been used to treat various diseases, such as tumors, gastroenteric disorders, inflammation, and lymphatic diseases (23, 24). PL is rich in polysaccharides and contains several aromatic compounds, including *p*-hydroxybenzaldehyde, caffeic acid, and hispidin, which can be isolated from cultured mycelia (23). Many studies have demonstrated that polysaccharides from PL are remarkably effective at inhibiting the growth of tumors without inducing severe toxic side effects (23, 25). The therapeutic effects of PL can be attributed to its ability to induce antioxidant enzyme activities, which suggests that some components of the PL preparation may afford protection against reactive oxygen species (26). In addition to immune modulating activity, β -glucan originating from PL is known to modulate inflammation (27, 28) and hyperplasia (29). It is believed that polysaccharides and proteoglycans are responsible for the biological effects of these medicinal mushrooms, and there is an extensive body of literature regarding the immunologic response to β -glucans isolated from these medicinal mushrooms (30).

Many pharmacologic effects of PL have been reported; however, the effects of PL on endurance exercise in relation to central fatigue have not been clarified. Thus, this study was conducted to determine the effects of PL administration on serotonin expression in the dorsal raphe of the brain and on the expression of MCTs in gastrocnemius muscles during exhaustive exercise in rats.

MATERIALS AND METHODS

Plant material. The *Phellinus linteus* used in the experiments was obtained from the Kyung-Dong Market (Seoul, Korea). The plants were identified by Dr. E.-H. Kim, a professor of Semyung University College of Oriental Medicine in Korea, and the voucher specimen was deposited at the herbarium of the Institute (NL0198).

Preparation of the aqueous extract of *Phellinus linteus*. To obtain the aqueous extract of PL, 300 g of PL was heat-extracted with distilled water, then concentrated with a rotary evaporator (Eyela, Tokyo, Japan). After being concentrated, the aqueous extract of PL was lyophilized and 10.9 g of powder was obtained. The fine powder of PL was then diluted in distilled water and passed through a 0.45- μ m syringe filter prior to use.

Animals and treatments. Male Sprague-Dawley rats weighing 200 $\pm$ 10 g (7 wk old) were obtained from a commercial breeder (Daehan Biolink, Co., Chungbuk, Korea). All experimental procedures were performed in accordance with the animal care guidelines of the National Institutes of Health (NIH) and the Korean Academy of Medical Sciences. The animals were housed under laboratory conditions for 1 wk prior to experiments under controlled temperature (20 $\pm$ 2°C) and light (07:00–19:00 h) conditions with food and water

provided ad libitum.

The animals were equally divided into the following 6 groups ($n=10$ in each group): control; exercise; exercise and 50 mg/kg of PL treatment; exercise and 100 mg/kg of PL treatment; exercise and 200 mg/kg of PL treatment; and exercise and caffeine treatment. The rats in the PL-treated groups received the indicated doses of aqueous extracts of PL orally once a day for 7 d.

The treatment was administered 60 min prior to the start of exercise, which was conducted as described below. The rats in the control and exercise groups received normal saline and the rats in the caffeine-treated group received 100 mg/kg of caffeine orally once a day for 7 d.

Treadmill exercise protocols. The physical exercise load applied in the present study consisted of running on a motor-driven treadmill without inclination. The rats in the exercise groups were forced to run on a treadmill for 30 min once a day during 6 consecutive days, whereas the rats in the control group were left on the treadmill without running for 30 min. The exercise load consisted of forced running at a speed of 10 m/min for 10 min, followed by 16 m/min for 10 min, then 21 m/min for the last 10 min.

On the 7th day of the experiments, the time to exhaustion during treadmill running was determined for the exercise groups. The time to exhaustion was defined as the time between the start of exercise and the first occurrence of failing to keep up with the treadmill for a period of 3 min or more. The speeds used for determination of the exhaustion time were 10 m/min for 5 min, followed by 16, 18, 21, 24, 26, 29, 32, 34, and 37 m/min for 3 min each, then 40 m/min until exhaustion, as described in a previous study (31).

Biochemical assays. Blood from each rat was collected by cardiac puncture and the serum was separated. The concentrations of total cholesterol, glucose, and triglycerides were estimated.

Brain preparation. The animals were sacrificed on the 7th day of the experiments immediately after determination of the exhaustion time. One-half of the animals in each group were used for the brain preparation ($n=5$ in each group). To prepare the brains, the animals were fully anesthetized with Zoletil 50® (10 mg/kg, i.p.; Vibac, Carros, France), after which the rats were transcardially perfused with 50 mM phosphate-buffered saline (PBS), then fixed with a freshly prepared solution of 4% paraformaldehyde in 100 mM phosphate buffer (PB, pH 7.4). The brains were then removed, post-fixed in the same fixative overnight, and transferred into a 30% sucrose solution for cryoprotection. Coronal sections with a thickness of 40 μ m were made using a freezing microtome (Leica, Nussloch, Germany).

Immunohistochemistry for 5-HT and TPH. Immunohistochemistry was conducted to evaluate the 5-HT-positive and TPH-positive cells in the dorsal raphe. An average of 10 sections within the dorsal raphe region spanning from Bregma -7.30 mm to -8.00 mm were obtained from each brain. To begin the procedure, the sections were incubated in PBS for 10 min, then

washed 3 times in the same buffer. The sections were then incubated in 1% hydrogen peroxide (H_2O_2) for 30 min. Next, the sections were incubated overnight with rabbit anti-5-HT antibody (1 : 500; Immuno Star, Hudson, WI, USA) or mouse monoclonal anti-TPH antibody (1 : 1,000; Oncogene Research Products, Cambridge, UK). The sections were then incubated for 1 h with anti-rabbit secondary antibody (1 : 200; Vector Laboratories, Burlingame, CA, USA) for 5-HT immunohistochemistry or with anti-mouse secondary antibody (1 : 200; Vector Laboratories) for TPH immunohistochemistry. Next, the sections were incubated with avidin-biotin-peroxidase complex (1 : 100; Vector Laboratories) for 1 h at room temperature. For staining, the sections were incubated in a solution consisting of 0.02% 3,3'-diaminobenzidine tetrahydrochloride (DAB) and 0.03% H_2O_2 in 50 mM Tris-HCl (pH 7.6) for approximately 5 min, after which they were then washed with PBS and mounted on gelatin-coated slides. The number of 5-HT- and TPH-positive cells was counted in the dorsal raphe of the selected sections using a light microscope (Olympus, Tokyo, Japan).

Western blot analysis. The remaining animals in each group were used for muscle preparation ($n=5$ in each group). The left gastrocnemius muscles were dissected on an ice-cold plate and subsequently homogenized in an ice-cold whole cell lysate buffer containing 50 mM HEPES (pH 7.5), 150 mM NaCl, 10% glycerol, 1% Triton X-100, 1.5 mM magnesium chloride hexahydrate, 1 mM ethyleneglycol-bis-(β -aminoethyl ether)- N,N' -tetraacetic acid (EGTA), 1 mM phenylmethylsulfonyl fluoride (PMSF), 2 μ g/mL leupeptin, 1 μ g/mL pepstatin, 1 mM sodium ortho vanadate, and 100 mM sodium fluoride, after which the mixture was incubated on ice for 30 min. The mixture was then centrifuged at $14,000 \times g$ for 15 min at $4^\circ C$. The supernatants were then used to analyze the expression of the MCT1 and MCT4 proteins. The protein concentration was measured using a Bio-Rad colorimetric protein assay kit (Bio-Rad, Hercules, CA, USA). Next, 40 μ g of protein were separated on SDS-polyacrylamide gels and transferred onto a nitrocellulose membrane (Schleicher and Schuell GmbH, Dassel, Germany). Goat MCT1 (1 : 1,000; Santa Cruz Biotechnology, Inc., Santa Cruz, CA, USA) and rabbit MCT4 antibodies (1 : 1,000; Santa Cruz Biotechnology, Inc.) were used as primary antibodies. In addition, horseradish peroxidase-conjugated anti-goat antibody against MCT4 (1 : 4,000; Santa Cruz Biotechnology, Inc.) was used to probe for MCT1, while anti-rabbit antibody (1 : 2,000; Santa Cruz Biotechnology, Inc.) was used as a secondary antibody. Band detection was performed using an enhanced chemiluminescence (ECL) detection system (Santa Cruz Biotechnology, Inc.).

Statistical analysis. The results are presented as the mean $\pm$ the standard error (SE). The data were analyzed by one-way analysis of variance (ANOVA), followed by Duncan's post-hoc test using SPSS. Differences were considered significant at a $p < 0.05$.

Fig. 1. The treadmill running time to exhaustion in response to treatment with different doses of *Phellinus linteus* (PL). A: exercise group, B: exercise and 50 mg/kg of PL-treated group, C: exercise and 100 mg/kg of PL-treated group, D: exercise and 200 mg/kg PL-treated group, E: exercise and caffeine-treated group. [#] $p < 0.05$ when compared to the exercise group. Values shown represent the mean $\pm$ SE.

Table 1. Serum concentrations of total cholesterol, glucose, triglyceride (mg/dL).

Group	Total cholesterol	Glucose	Triglyceride
A	47.62 $\pm$ 2.78	202.62 $\pm$ 29.47	132.25 $\pm$ 16.69
B	48.50 $\pm$ 2.19	183.87 $\pm$ 8.63	60.37 $\pm$ 6.52 [*]
C	55.42 $\pm$ 5.12	171.42 $\pm$ 16.63	40.80 $\pm$ 7.62 ^{*,#}
D	67.42 $\pm$ 5.47 ^{*,#}	148.71 $\pm$ 12.94 ^{*,#}	20.14 $\pm$ 5.96 ^{*,#}
E	64.57 $\pm$ 4.73 ^{*,#}	108.57 $\pm$ 14.85 ^{*,#}	11.14 $\pm$ 3.16 ^{*,#}

A: control group, B: exercise group, C: exercise and 50 mg/kg *Phellinus linteus* (PL)-treated group, D: exercise and 100 mg/kg PL-treated group, E: exercise and 200 mg/kg PL-treated group. ^{*} $p < 0.05$ when compared to the control group. [#] $p < 0.05$ when compared to the exercise group. Values shown represent the mean $\pm$ SE.

RESULTS

Effect of *Phellinus linteus* on the time to exhaustion during treadmill running

The effects of PL on the time to exhaustion are presented in Fig. 1. These findings indicate that the time to exhaustion in response to treadmill running was increased in response to PL treatment. Specifically, treatment with PL at 200 mg/kg and caffeine at 100 mg/kg statistically significantly increased the time to exhaustion by treadmill running when compared to the exercise group ($p < 0.05$). The results show that PL was just as effective as caffeine for increasing the time to exhaustion by treadmill running.

Effect of *Phellinus linteus* on total cholesterol, glucose, and triglyceride concentrations

The effects of PL on serum total cholesterol, glucose, and triglyceride concentrations are presented in Table 1. The serum total cholesterol was significantly increased by treatment with PL compared to the exercise group ($p < 0.05$). However, the serum glucose and triglycerides were significantly decreased by treatment

Fig. 2. Dose-dependent effect of *Phellinus linteus* (PL) on 5-hydroxytryptamine (5-HT) expression in the dorsal raphe. (Upper) Photomicrographs of 5-HT-positive cells in the dorsal raphe. The scale bar represents 250 μ m. (Lower) Number of 5-HT-positive cells in the dorsal raphe in each group. A: control group, B: exercise group, C: exercise and 50 mg/kg of PL-treated group, D: exercise and 100 mg/kg of PL-treated group, E: exercise and 200 mg/kg of PL-treated group, F: exercise and caffeine-treated group. * $p < 0.05$ when compared to the control group. # $p < 0.05$ when compared to the exercise group. Values shown represent the mean $\pm$ SE.

Fig. 3. Dose-dependent effect of *Phellinus linteus* (PL) on tryptophan hydroxylase (TPH) expression in the dorsal raphe. (Upper) Photomicrographs of TPH-positive cells in the dorsal raphe. The scale bar represents 250 μ m. (Lower) Number of TPH-positive cells in the dorsal raphe in each group. A: control group, B: exercise group, C: exercise and 50 mg/kg of PL-treated group, D: exercise and 100 mg/kg of PL-treated group, E: exercise and 200 mg/kg of PL-treated group, F: exercise and caffeine-treated group. * $p < 0.05$ when compared to the control group. # $p < 0.05$ when compared to the exercise group. Values shown represent the mean $\pm$ SE.

Fig. 4. Results of Western blot analysis of the protein levels of monocarboxylate transporter 1 and 4. A: control group, B: exercise group, C: exercise and 50 mg/kg of *Phellinus linteus* (PL)-treated group, D: exercise and 100 mg/kg of PL-treated group, E: exercise and 200 mg/kg of PL-treated group, F: exercise and caffeine-treated group. Actin was used as the internal control. * $p < 0.05$ when compared to the control group. # $p < 0.05$ when compared to the exercise group. Values shown represent the mean $\pm$ SE.

with PL compared to the exercise group ($p < 0.05$).

Effect of *Phellinus linteus* on 5-HT expression in the dorsal raphe

Photomicrographs of 5-HT-positive cells in the dorsal raphe are presented in Fig. 2. These results demonstrate that treadmill exercise led to increased synthesis of 5-HT in the dorsal raphe ($p < 0.05$) and that PL-treatment suppressed the exercise-induced 5-HT synthesis. Specifically, treatment with PL at 200 mg/kg and with caffeine at 100 mg/kg statistically significantly suppressed 5-HT synthesis in the dorsal raphe when compared to the exercise group ($p < 0.05$).

Effect of *Phellinus linteus* on TPH expression in the dorsal raphe

Photomicrographs of TPH-positive cells in the dorsal raphe are presented in Fig. 3. These results demonstrate that treadmill exercise increased the expression of TPH in the dorsal raphe ($p < 0.05$) and that PL treatment suppressed exercise-induced TPH expression. Specifically, treatment with PL at 100 and 200 mg/kg and caf-

feine at 100 mg/kg statistically significantly suppressed TPH expression in the dorsal raphe when compared to the exercise group ($p < 0.05$).

Effect of Phellinus linteus on MCT1 and MCT4 expression in gastrocnemius muscles

The expression of MCT1 and MCT4 protein was analyzed to provide an estimate of the relative level of expression. In the present study, the expression of MCT1 and MCT4 protein in the control was set at 1.00 (Fig. 4).

These results demonstrate that treadmill exercise increased the expression of MCT1 and MCT4 in the gastrocnemius muscle ($p < 0.05$), and that treatment with PL led to an increase in exercise-induced MCT1 and MCT4 expression. Specifically, treatment with PL at 200 mg/kg and caffeine at 100 mg/kg statistically significantly increased MCT1 and MCT4 expression in the gastrocnemius muscles when compared to the exercise group ($p < 0.05$).

DISCUSSION

In the present study, the effect of PL on exhaustion time was determined using rats. The time to exhaustion in response to treadmill running was significantly prolonged in response to treatment with 200 mg/kg PL or 100 mg/kg caffeine. Ingestion of caffeine is known to increase endurance performance, particularly during prolonged periods of exercise (32–34). Indeed, caffeine may play an ergogenic role in exercise performance by altering both neural perception of effort and substrate availability (35). The ergogenic action of caffeine has been well-documented (12, 32–34), and administration of caffeine increased the exhaustion time by treadmill running in the current study. Caffeine has an issue with respect to doping tests. In addition, a high concentration of caffeine is known to induce many medical problems. Developing new ergogenic aids free from doping tests is an important strategy for athletes (36). In the present study, treatment with PL increased a running time similar to caffeine. Based on our previous studies, we tried to identify herbs, including PL, which can be developed as new ergogenic aids without the complications associated with caffeine.

Physical fatigue is defined as the inability to continue exercise performance (37–39). The mechanisms of fatigue during exercise are not yet clear. Muscle fiber composition, physical fitness, and the intensity, type, and duration of the muscle contraction, are related to physical fatigue (40). The rapid depletion of glycogen and glucose stores (41, 42), the accumulation of catabolites, such as lactate (43), the expression of brain serotonin (44, 45), the increase in H^+ and intracellular Pi (46–50), increased oxygen consumption causing oxidative stress and tissue damage (41, 51, 52), and failure of Ca^{2+} release by the sarcoplasmic reticulum (53, 54) have been suggested as underlying factors inducing physical fatigue. These factors, alone or in combination, seem to diminish the ability to perform exercise.

Our research on the central fatigue hypothesis is that over-expression of the level of serotonin in the brain

induced by exhaustive exercise disturbs exercise performance. It is possible that exercise performance can be continued if the level of serotonin in the brain is suppressed. As shown in this study, PL and caffeine suppressed serotonin expression in the brain with a corresponding increase in exhaustive running time. These results suggest that PL and caffeine overcame central fatigue, and then extended the time of treadmill running.

We also evaluated the peripheral factors concerned with stopping exercise. As shown in Table 1, the serum cholesterol concentration was not matched with the running time (distance). The serum glucose and triglycerides concentrations were decreased in relation with running time. These results showed that long-term exercise depleted the levels of glucose and triglycerides; however, these data did not show that depletion of glucose or triglycerides is the main factor underlying fatigue-induced cessation of exercise.

To elucidate the ergogenic mechanism of PL, we evaluated the serotonin levels in the dorsal raphe. Most of the cell bodies of the serotonergic neurons in the brain arise from the dorsal raphe nuclei, which send projections to diverse target regions, such as the limbic system, hypothalamus, striatum, and cerebral cortex (8). The central fatigue hypothesis states that increases in the concentration of 5-HT in the brain during prolonged exercise impairs CNS function, thereby inducing a deterioration of exercise performance (55). An increase in 5-HT depends on the exercise types or the endurance running capacity. Wheel running significantly elevates 5-HT_{1A} autoreceptor mRNA in the dorsal raphe compared to sedentary controls (56, 57). A number of experiments support the hypothesis that constraints of the 5-HT system during physical activity could be involved in the onset of exercise-induced fatigue observed in animals (58). It has also been suggested that an increase or decrease in 5-HT activity in the brain during prolonged exercise hastens or delays the onset of fatigue, respectively (59), while blocking 5-HT activity delays the onset time of fatigue (6, 60). Furthermore, there are indications that increased 5-HT concentrations in the brain and the overall serotonergic activity that occur during endurance exercise play a role in physical and mental fatigue (61). TPH was not saturated under normal physiologic conditions (62); a change in rate-limiting TPH2 availability in the presence of adequate substrate (i.e., tryptophan) could potentially alter the amount of 5-HT synthesized during physical activity. Increased TPH mRNA expression is a good predictor of increased TPH activity, which leads to enhanced 5-HT synthesis (63). Fatigue during prolonged exercise may be influenced by the activity of the brain serotonergic system, and this is referred as the “central fatigue hypothesis” (64). It has been suggested that elevated central tryptophan availability increases 5-HT activity during prolonged exercise, which causes fatigue by increasing lethargy and loss of central motivation (64, 65).

Several studies have demonstrated that excessive

exercise increases TPH-immunoreactivity and 5-HT synthesis in the dorsal raphe of rats, which in turn leads to exhaustion during running (11, 12, 66–68). Treatment with *Red ginseng* and *Acanthopanax senticosus* (100 mg/kg for each) inhibits the exercise-induced increase in the number of 5-HT- and TPH-positive cells in the dorsal raphe (68, 69). Treatment with *Paeonia radix* (50 mg/kg) reduces fatigue, both during exercise and at rest, through inhibition of 5-HT synthesis and TPH expression (11). *Acanthopanax senticosus* contains an abundance of organic compounds. Chlorogenic acid (CHA) and syringaresinol di-*O*- β -D-glucoside (SYG) are the main components of the *Acanthopanax senticosus* (70, 71). SYG was suggested as the main compound exerting a pharmacologic effect on swimming time in rats (70). 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: ginsenosides Rb1 and Rg3 protect neurons against excitotoxicity and oxidative stress (72). *Paeonia radix* contains a multitude of complex organic compounds. Paeoniflorin, one of the compounds extracted from *Paeonia radix*, is known to have anti-inflammatory and anti-stress effects, and decreases blood pressure (73). All of these above-mentioned herbs, including PL, increase the time to exhaustion through inhibition of serotonin production in the brain. These suppressing effects of PL appear at higher doses compared to *Red ginseng*, *Acanthopanax senticosus*, and *Paeonia radix*. The ergogenic action of PL is not as potent; however, the polysaccharides and aromatic compounds in PL exert antitumor and antioxidant activities (23, 25). Our previous results showed that any substances, one substance or the combined action of several substances, from these herbs may exert ergogenic actions, although they have not been clarified.

In the present study, exhaustive treadmill exercise led to increased 5-HT synthesis and TPH expression in the dorsal raphe, but treatment with PL attenuated these effects. The most potent inhibition of PL on 5-HT synthesis and TPH expression was observed in response to treatment with 200 mg/kg. We also compared the effects of PL on the synthesis of 5-HT and the expression of TPH with the effects of caffeine. Treatment with PL at a dose of 200 mg/kg and treatment with caffeine at 100 mg/kg were equally effective at suppressing 5-HT synthesis and TPH expression in the dorsal raphe. A reduction in TPH activity in vivo rapidly leads to a decrease in the release of 5-HT (74), indicating that TPH can profoundly influence the synaptic function of 5-HT. The present results suggest that the time to exhaustion was increased through a decrease in 5-HT synthesis and TPH expression in the dorsal raphe.

Thomas et al. (75) demonstrated that increased levels of MCT1 in human skeletal muscles were associated with an improved ability to remove blood lactate and fatigue resistance following a single bout of all-out exercise. An increase in MCT1 was demonstrated 2 h after exhaustive running, which may be indicative of increased lactate removal (76). It has also been sug-

gested that increased MCT1 protein content was associated with training-induced improvement of lactate shuttling (77, 78). The extent of adaptation of MCT1 and MCT4 expression to exercise was related to the intensity of training (20). Taken together, these isoform-specific differences suggest that lactate is primarily taken up into the muscle cells via MCT1, whereas MCT4 is involved in the removal of lactate so that oxidation can occur in inactive muscle fibers (16, 20, 77).

In the present study, treadmill exercise led to increased MCT1 and MCT4 expression in the gastrocnemius muscles, and treatment with 200 mg/kg PL further enhanced the expression of MCT1 and MCT4 when compared with the untreated exercise group. The rats treated with caffeine (the positive control group) also showed an increase in MCT1 and MCT4 expression compared to the untreated exercise group. Gastrocnemius muscle has both type I and type II fibers (e.g., red and white gastrocnemius; 79, 80). White and red muscles were not separated in the current experiments; however, we analyzed the effects of PL on MCT1 and MCT4 expression in the mixed fiber gastrocnemius muscles. It is possible that the exhaustion exercise applied in this study was sufficient to induce an increase in the expression of MCT1 and MCT4 protein. An increase in lactate transport capacity was also reported following exhaustive exercise (81, 82). It has previously been reported that the rate of lactate flux into and out of the muscles is correlated with the content of MCT1 and MCT4 in the muscles (19, 20, 83). Chronic skeletal muscle activity is also known to have a strong influence on the expression of MCTs. Additionally, inactivity has been shown to reduce the expression of MCT1 and MCT4 (21), while training increased the expression (17, 84). Thus, the increase in MCT1 and MCT4 in response to PL treatment indicates that PL may possess the ability to increase the rate of lactate removal from cells.

In the present study, PL treatment led to increased endurance exercise performance via the inhibition of 5-HT synthesis and TPH expression in the dorsal raphe. The treatment with caffeine also decreased 5-HT synthesis and TPH expression compared to the untreated exercise group. These results suggest that PL effectively suppresses endurance exercise-induced central fatigue through inhibition of serotonin production in the brain. PL also enhanced the expression of MCT1 and MCT4 in gastrocnemius muscles, which suggests that PL is effective at suppressing endurance exercise-induced peripheral fatigue through increased lactate removal in the muscles. In this study, we verified the effect of treatment of PL or caffeine for 7 d. The present results were obtained from repeated administration of PL in rats with cumulative training. However, there is a possibility that a single treatment with PL might exert similar results. Additional studies are needed to verify the acute effectiveness of PL. Nevertheless, the results of this study clearly demonstrate that PL exerts an ergogenic effect.

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