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Effect of *Acanthopanax senticosus* on 5-hydroxytryptamine synthesis and tryptophan hydroxylase expression in the dorsal raphe of exercised rats

Yong-Taek Rhim [a], Hong Kim [c], Sung-Jin Yoon [a], Sung-Soo Kim [a], Hyun-Kyung Chang [b], Taeck-Hyun Lee [b], Hee-Hyuk Lee [d], Min-Chul Shin [b], Mal-Soon Shin [b], Chang-Ju Kim [b,*]

[a] *Department of Exercise Physiology, Graduate School, Korea University, #1 5-Ka Anam-dong, Sungbuk-gu, Seoul 136-701, South Korea*
[b] *Department of Physiology, College of Medicine, Kyung Hee University, #1 Hoigi-dong, Dongdaemoon-gu, Seoul 130-701, South Korea*
[c] *Department of Oriental Sports Medicine, College of Herbal Bio-industry, Daegu Haany University, #290 Yugok-dong, Gyeongsan-si, Gyeongsangbuk-do 712-715, South Korea*
[d] *Department of Sports Science, College of Natural Sciences, Hannam University, #133 Ujung-dong, Daeduk-gu, Daejeon 306-791, South Korea*

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Abstract

Acanthopanax senticosus Harms (AS) is classified into the family of Araliaceae. The plant has been used as an analeptic aid, which improves weakened physical status and strength. Serotonin (5-hydroxytryptamine, 5-HT) is an important neurotransmitter and tryptophan hydroxylase (TPH) catalyzes the rate-f the raphe nuclei. These are associated with "central fatigue hypotheses" in the brain.

In the present study, the effects of *Acanthopanax senticosus* on the time to exhaustion by treadmill exercise and on 5-HT synthesis and TPH expression in the dorsal raphe were investigated by immunohistochemistry. In the present results, *Acanthopanax senticosus* increased the time to exhaustion by treadmill running and it suppressed the exercise-induced increase of 5-HT synthesis and TPH expression. *Acanthopanax senticosus* was effective as caffeine for increasing the exhaustion time in treadmill running and for reducing the exercise-induced increase of 5-HT synthesis and TPH expression in the dorsal raphe. The present study shows that *Acanthopanax senticosus* reduces fatigue during exercise by the inhibition of exercise-induced 5-HT synthesis and TPH expression in the dorsal raphe.

Keywords: *Acanthopanax senticosus*; 5-Hydroxytryptamine; Tryptophan hydroxylase; Dorsal raphe

1. Introduction

In mammals, serotonin (5-hydroxytryptamine, 5-HT) is an important neurotransmitter in the brain and it is known to regulate behavioral functions including body temperature, blood pressure, endocrine activity, appetite, sexual behavior, movement, emesis, and pain (Jacobs and Azmitia, 1992; Strüder and Weicker, 2001; Zhou et al., 2001). The activity of serotonergic neural projections is influenced by extrinsic and intrinsic impulses carrying body information (Jacobs and Azmitia, 1992). 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 stresses (Jacobs and Azmitia, 1992). In addition, the 5-HT system is known to regulate cognition and behavior (Strüder and Weicker, 2001).

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 serotonergic functions (Singh and Corley, 1990; Gartside et al., 1992). It has been reported that TPH expression is modulated by several forms of stress, such as immobilization and noise (Culman et al., 1984; Singh and Corley, 1990; Gartside et al., 1992). Increased TPH mRNA expression has been shown to enhance TPH activity and 5-HT metabolism; however, the extent of elevation of the TPH mRNA level was found to be much large than the change in 5-HT turnover (Chamas et al., 1999).

The central fatigue hypothesis states that maximal exertion or exhaustion may directly enhance serotonergic activity *via* locomotor regulation or the stimulation of long-term stress

* Corresponding author. Tel.: +82 2 961 0407; fax: +82 2 964 2195.
E-mail address: changju@khu.ac.kr (C.-J. Kim).

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responsiveness. The increase of 5-HT concentration in the brain induced by enhanced physical activity impairs the central nervous system (CNS) functions, resulting in deterioration of exercise performance and fatigue (Newsholme et al., 1992). Several studies have shown that physical exercise increases the synthesis and metabolism of 5-HT in the brain. The increase of 5-HT impaired exercise performance in both rats and humans, and in contrast running performance was improved significantly by decreasing the 5-HT concentration (Blomstrand et al., 1988; Strüder and Weicker, 2001).

Acanthopanax senticosus Harms (AS) is a medicinal herb that is classified into the family of Araliaceae and it is also known botanically as *Eleutherococcus senticosus*. Several parts of this plant have been used for the treatment of a various diseases such as rheumatism, hypertension, gastric ulcer, ischemic heart disease, and hepatitis (Nishibe et al., 1990; Fujikawa et al., 1996; Lin and Huang, 2000; Yi et al., 2001). It has also been used as analeptic aid to reduce fatigue and enhance physical strength (Nishibe et al., 1990; Deyama et al., 2001).

However, the effects of *Acanthopanax senticosus* on the endurance exercise in relation to CNS fatigue have not been yet clarified. In the present study, the effects of *Acanthopanax senticosus* on the time to exhaustion by treadmill exercise and on 5-HT synthesis and TPH expression in the dorsal raphe were investigated.

2. Materials and methods

2.1. Animals and treatments

Adult male Sprague-Dawley rats weighing 200 ± 10 g (6 weeks of age) 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 (08:00–20:00 h) conditions with food and water made available *ad libitum*.

In the first part of the experiment, the dose-dependent effect of *Acanthopanax senticosus* (AS) on the time to exhaustion by treadmill exercise and on 5-HT synthesis and TPH expression in the dorsal raphe was investigated. The animals were divided into six groups ($n = 8$ in each group): the control group, the exercise group, the exercise and AS10 (mg/kg)-treated group, the exercise and AS50-treated group, the exercise and AS100-treated group, and the exercise and caffeine-treated group. Rats of the AS-treated groups were injected intraperitoneally with an aqueous extract of *Acanthopanax senticosus* at the respective one time dose at the 30 min before the determination of exhaustion time. Rats of the caffeine-treated group received 100 mg/kg caffeine intraperitoneally by the same method.

In the second part of the experiment, the dose frequency-dependent effect of *Acanthopanax senticosus* on the time to exhaustion by treadmill exercise and on 5-HT synthesis and TPH expression in the dorsal raphe was investigated. The animals were divided into five groups ($n = 8$ in each group): the control group, the exercise group, the exercise and 1-day AS100-treated group, the exercise and 3-day AS100-treated group, and the exercise and 7-day AS100-treated group. *Acanthopanax senticosus* at a dose of 100 mg/kg was given intraperitoneally to each animal of the AS-treated groups once a day over the respective period of time at 30 min before the start of treadmill exercise.

2.2. Preparation of water extract of Acanthopanax senticosus

The fresh stem barks of *Acanthopanax senticosus* used in this study were originally collected in North Korea, obtained from Kyung-Dong Market (Seoul, South Korea), The fresh stem barks of *Acanthopanax senticosus* used in this study were identified by Prof. Ee-Hwa Kim, College of Oriental Medicine, Semyung University (Jechon, South Korea). It has been well documented that *Acanthopanax senticosus* contains eight kinds of glucoside such as elutheroside A, B, B1, C, D, E, F, and E. The stem bark of *Acanthopanax senticosus* used in this experiment has plenty of elutheroside A, B, C, D, and E. It contains a high percentage of elutheroside E. Total glucoside in dried stem bark is about 1.6–1.5% and elutheroside D and E, especially, exist 5–10% of them.

After washing, *Acanthopanax senticosus* was immersed in cold water for 12 h. To obtain the aqueous extract of *Acanthopanax senticosus*, 200 g of *Acanthopanax senticosus* was added to distilled water and heat-extracted at 80 °C, concentrated using a rotary evaporator and then lyophilized. The resulting powder, weighing 25 g, was diluted with saline. After filtering through a 0.45 μm syringe filter, animals of the *Acanthopanax senticosus*-treated groups were intraperitoneally injected with the respective dose of *Acanthopanax senticosus* at 30 min before the start of treadmill exercise during the respective days.

2.3. 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 forced to run on a treadmill for 30 min once a day for 6 consecutive days, whereas the control group was 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, at 13 m/min for another 10 min, and at 16 m/min for the last 10 min with 0° of inclination.

On the 7th day of the experiment, the 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 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, which was the presumed equilibrium speed of running for rats. Immediately after determination of the time to exhaustion, the rats were sacrificed.

2.4. Tissue preparation

To begin the sacrificial process, the animals were fully anesthetized using Zoletil 50® (10 mg/kg, i.p.; Vibac Laboratories, Carros, France), then 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).

2.5. Immunohistochemistry for 5-HT and TPH

For the detection of 5-HT-positive and TPH-positive cells in the dorsal raphe, immunohistochemistry was performed. Briefly, an average of five sections were selected from each brain region spanning from the bregma −7.30 mm to −8.00 mm. The sections were then incubated in PBS for 10 min and washed three times, again with PBS and then incubated in 1% hydrogen peroxide (H_2O_2) for 30 min. Next, the sections were incubated overnight with rabbit anti-5-HT antibody (Oncogene Research Product, Cambridge, UK) at a dilution of 1:500 for visualization of 5-HT expression or with mouse monoclonal anti-TPH antibody (Oncogene Research Products) at a dilution of 1:1000 for visualization of TPH expression. 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). The sections were subsequently incubated with avidin–biotin–peroxidase complex (Vector Laboratories, Burlingame) 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% H_2O_2 in 50 mM Tris-buffer (pH 7.6) for approximately 3 min. The sections were then mounted on gelatin-coated glass slides.

2.6. Data analysis

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

3. Results

3.1. Dose-dependent effect of Acanthopanax senticosus on the time to exhaustion by treadmill running

The impact of the dose of *Acanthopanax senticosus* on the time to exhaustion of the rats in each exercise group is presented in Fig. 1. The mean time to exhaustion by forced treadmill running was 27.41 ± 1.10 min in the exercise group, the exercise and AS10-treated group was 27.21 ± 0.74 min, the exercise and AS50-treated group was 39.25 ± 3.71 min, and the exercise and AS100-treated group was 44.10 ± 2.44 min. The results show that *Acanthopanax senticosus* increased the time to exhaustion by treadmill running in a dose-dependent manner.

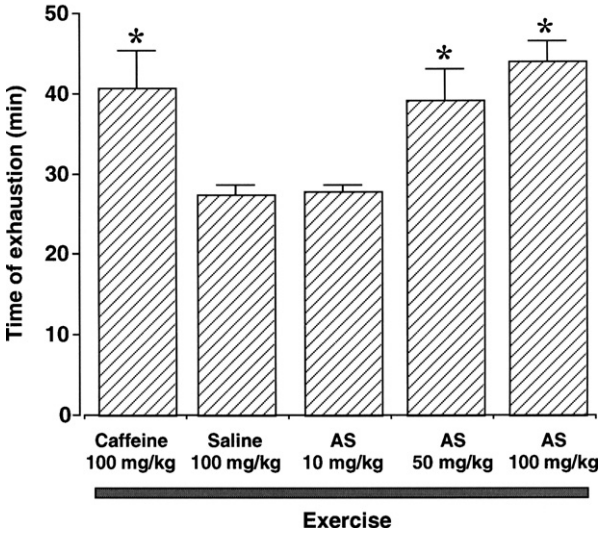

Fig. 1. The mean treadmill running time to exhaustion depending on the dose of *Acanthopanax senticosus* (AS) treatment in the exercise groups. *$P < 0.05$ compared to the exercise group. Values are represented as the mean ± S.E.M.

The time to exhaustion in treadmill running between the AS-treated group and caffeine-treated group was compared. The mean time to exhaustion by forced treadmill running was 44.10 ± 2.44 min in the exercise and AS100-treated group and it was 40.08 ± 6.85 min for the exercise and caffeine100-treated group. The results show that *Acanthopanax senticosus* was just as effective as caffeine for increasing the exhaustion time by treadmill running.

3.2. Dose-dependent effect of Acanthopanax senticosus on the number of 5-HT- and TPH-positive cells in the dorsal raphe

Photomicrographs of 5-HT- and TPH-positive cells in the dorsal raphe are presented in Figs. 2 and 3. The number of 5-HT-positive cells in the dorsal raphe was 92.05 ± 10.04/section in the control group, the exercise group was 285.07 ± 57.32/section, the exercise and AS10-treated group was 283.28 ± 26.68/section, the exercise and AS50-treated group was 225.71 ± 23.61/section, and the exercise and AS100-treated group was 170.06 ± 7.62/section.

The number of TPH-positive cells in the dorsal raphe was 128.25 ± 6.89/section in the control group, the exercise group was 320.80 ± 31.00/section, the exercise and AS10-treated group was 305.25 ± 32.53/section, the exercise and AS50-treated group was 260.13 ± 33.93/section, and the exercise and AS100-treated group was 195.90 ± 10.80/section.

The results show that treadmill exercise increased the synthesis of 5-HT and the expression of TPH in the dorsal raphe, and *Acanthopanax senticosus* suppressed the exercise-induced increase of 5-HT synthesis and TPH expression in a dose-dependent manner.

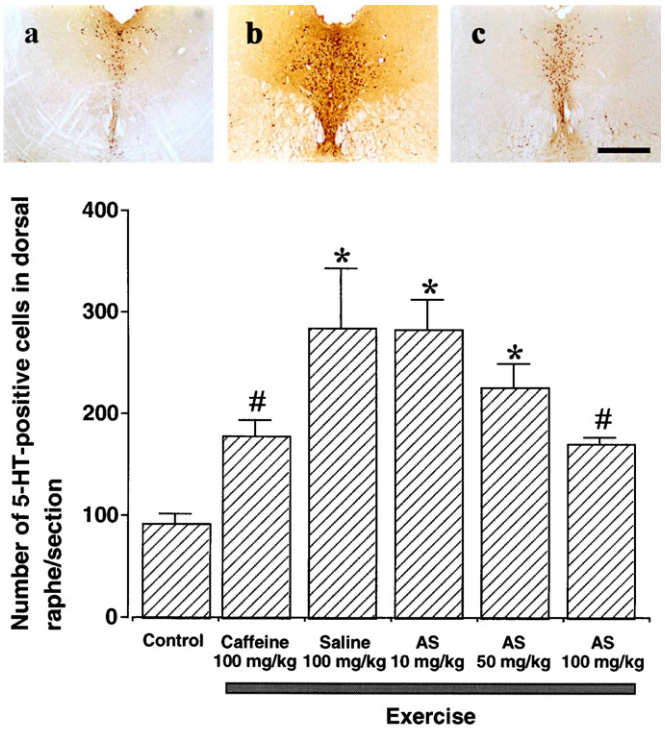

Fig. 2. Dose-dependent effect of *Acanthopanax senticosus* (AS) on 5-hydroxytryptamine (5-HT) expression in the dorsal raphe. (Upper) Photomicrographs of 5-HT-positive cells in the dorsal raphe. Sections were stained for 5-HT-like immunoreactivity (brown dots). (a) Control group, (b) exercise group, and (c) exercise and AS100-treated group. A scale bar represents 250 μm. (Lower) Number of 5-HT positive cells in dorsal raphe in each group depending on dose of *Acanthopanax senticosus* treatment. $*P < 0.05$ compared to the control group. $\#P < 0.05$ compared to the exercise group. Values are represented as the mean ± S.E.M. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of the article.)

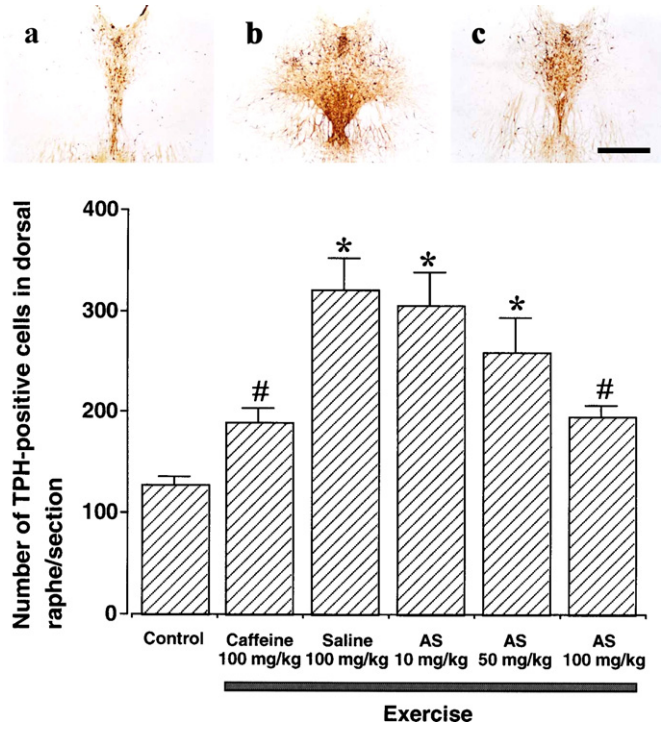

Fig. 3. Dose-dependent effect of *Acanthopanax senticosus* (AS) on tryptophan hydroxylase (TPH) expression in the dorsal raphe. (Upper) Photomicrographs of TPH-positive cells in the dorsal raphe. Sections were stained for TPH-like immunoreactivity (brown dots). (a) Control group, (b) exercise group, and (c) exercise and AS100-treated group. A scale bar represents 250 μm. (Lower) Number of TPH positive cells in dorsal raphe in each group depending on dose of *Acanthopanax senticosus* treatment. $*P < 0.05$ compared to the control group. $\#P < 0.05$ compared to the exercise group. Values are represented as the mean ± S.E.M. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of the article.)

The number of 5-HT- and TPH-positive cells for the AS-treated group and caffeine-treated group was compared. The number of 5-HT-positive cells in the dorsal raphe was 170.06 ± 7.62/section in the exercise and AS100-treated group and it was 178.16 ± 15.52/section in the exercise and caffeine100-treated group. The number of TPH-positive cells in the dorsal raphe was 195.90 ± 10.80/section in the exercise and AS100-treated group and it was 189.41 ± 13.70/section in the exercise and caffeine100-treated group. The results show that *Acanthopanax senticosus* was effective as caffeine for reducing the exercise-induced synthesis of 5-HT and expression of TPH in the dorsal raphe.

3.3. Dose frequency-dependent effect of Acanthopanax senticosus on the time to exhaustion by treadmill running

The impact of the frequency of dosage of *Acanthopanax senticosus* on the time to exhaustion of the rats is presented in Fig. 4.

The mean time to exhaustion by forced treadmill running was 27.41 ± 66.02 min in the exercise group, the exercise and 1-day AS100-treated group was 44.10 ± 146.67 min, the exercise and 3-day AS100-treated group was 48.08 ± 540.64 min,

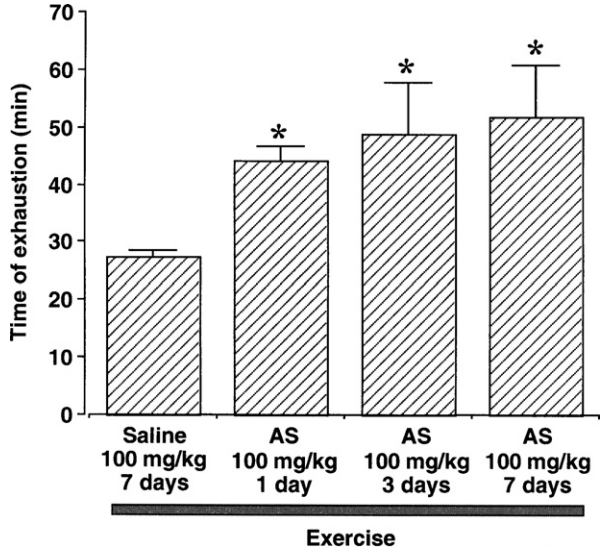

Fig. 4. The mean treadmill running time to exhaustion depending on the frequency of *Acanthopanax senticosus* (AS) treatment in the exercise groups. $*P < 0.05$ compared to the exercise group. Values are represented as the mean ± S.E.M.

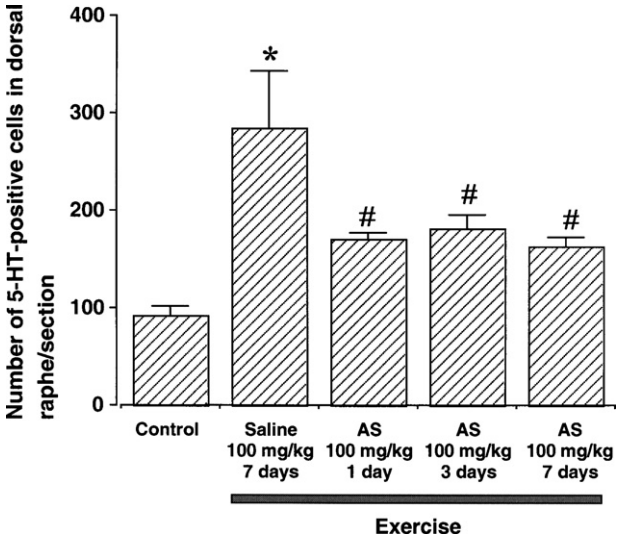

Fig. 5. Frequency-dependent effect of *Acanthopanax senticosus* (AS) on 5-hydroxytryptamine (5-HT) expression in the dorsal raphe. $*P < 0.05$ compared to the control group. $\#P < 0.05$ compared to the exercise group. Values are represented as the mean ± S.E.M.

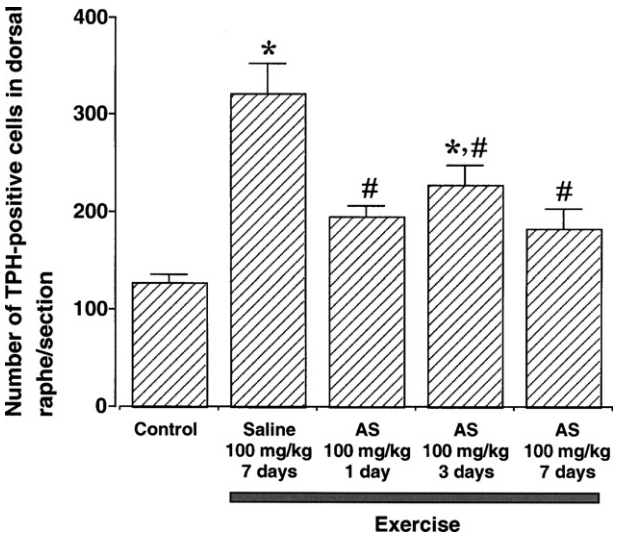

Fig. 6. Frequency-dependent effect of *Acanthopanax senticosus* (AS) on tryptophan hydroxylase (TPH) expression in the dorsal raphe. $*P < 0.05$ compared to the control group. $\#P < 0.05$ compared to the exercise group. Values are represented as the mean ± S.E.M.

and the exercise and 7-day AS100-treated group was 51.09 ± 536.49 min. The results show that *Acanthopanax senticosus* increased the time to exhaustion by treadmill running. However, there was no statistical significance among the dose frequencies of *Acanthopanax senticosus* administration.

3.4. Dose frequency-dependent effect of Acanthopanax senticosus on the number of 5-HT- and TPH-positive cells in the dorsal raphe

The number of 5-HT-positive cells in the dorsal raphe was 92.05 ± 10.04/section in the control group, the exercise group was 285.07 ± 57.32/section, the exercise and 1-day AS100-treated group was 170.06 ± 7.62/section, the exercise and 3-day AS100-treated group was 181.40 ± 14.59/section, and the exercise and 7-day AS100-treated group was 162.91 ± 9.46/section Fig. 5.

The number of TPH-positive cells in the dorsal raphe was 128.25 ± 6.89/section in the control group, the exercise group was 320.80 ± 31.00/section, the exercise and 1-day AS100-treated group was 195.90 ± 10.80/section, the exercise and 3-day AS100-treated group was 228.06 ± 20.30/section, and the exercise and 7-day AS100-treated group was 183.74 ± 20.11/section Fig. 6.

The results show that treadmill exercise increased the synthesis of 5-HT and the expression of TPH in the dorsal raphe and that *Acanthopanax senticosus* reduced the exercise-induced increase of 5-HT synthesis and TPH expression in the dorsal raphe. However, there was no statistical significance among the dose frequencies of *Acanthopanax senticosus* administration.

4. Discussion

In the present study, treatment with *Acanthopanax senticosus* was shown to increase the time to exhaustion by treadmill running in a dose-dependent manner. It has been previously reported that an aqueous extract of the family of *Acanthopanax senticosus* affected the stress-induced physiological and physical changes in mice (Fujikawa et al., 2002). *Acanthopanax senticosus* prolonged the swimming time of a forced swimming test for rats (Nishibe et al., 1990). The present results also show that *Acanthopanax senticosus* enhanced the endurance ability for treadmill exercise for rats. The precise mechanism of the *Acanthopanax senticosus* increasing the time till exhaustion during exercise has been poorly understood. Many neurotransmitters, especially 5-HT, have been suggested to be involved in fatigue and the fatigability induced by hard exercise.

Increased 5-HT concentration is known to induce lethargy, loss of motivation, and decrease power during sustained exercise. It has been suggested that the increase of 5-HT concentration in the brain and the overall serotonergic activity taking place during endurance exercise are relevant to the increase in the level of physical fatigue and perhaps of mental fatigue as well (Davis et al., 2000). Newsholme et al. (1992) 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. The increase in the level of serotonin during endurance exercise coincides with the onset of fatigue, and this raises the possibility that differences in serotonin receptor sensitivity may be an important determinant of relative endurance (Blomstrand et al., 1988; Dwyer and Browning, 2000; Lim et al., 2001).

In the present results, treadmill exercise increased 5-HT synthesis in the dorsal raphe and *Acanthopanax senticosus* suppressed the exercise-induced increase of 5-HT synthesis in the dorsal raphe. The most potent inhibition of *Acanthopanax senticosus* on 5-HT synthesis was observed at the dose of 100 mg/kg of *Acanthopanax senticosus*. The effect of *Acanthopanax sen-*

ticosus inhibition on 5-HT synthesis was not shown to be dose frequency-dependent in our experiment.

Reduction in TPH activity has been shown to lead to rapid decrease in 5-HT release (Gartside et al., 1992), indicating that change in the TPH level can profoundly influence the synaptic 5-HT activity. The increase of TPH mRNA expression heightened TPH activity and 5-HT metabolism, but the extent of the elevation for TPH mRNA level was much larger than the change in 5-HT turnover (Chamas et al., 1999).

In the present study, *Acanthopanax senticosus* inhibited exercise-induced increase of TPH expression in the dorsal raphe. Similar to the results we achieved for 5-HT, the most potent suppression of TPH expression was observed at the dose of 100 mg/kg of *Acanthopanax senticosus*. The inhibition of *Acanthopanax senticosus* on TPH expression did not show dose frequency-dependency in our experiments.

In addition, the effect of *Acanthopanax senticosus* was compared to that of caffeine, which is well known as an ergogenic aid (Lim et al., 2001). *Acanthopanax senticosus* at 100 mg/kg was just as effective as caffeine at 100 mg/kg on the exhaustion-time by treadmill running, the 5-HT synthesis, and the TPH expression in the dorsal raphe.

Acanthopanax senticosus contains quite a lot of organic compounds. Chlorogenic acid (CHA) and syringaresinol di-*O*-β-D-glucoside (SYG) are main components of the *Acanthopanax senticosus*. CHA and SYG are known to prevent the occurrence of gastric ulcer induced by restraint stress in rats (Nishibe et al., 1990; Fujikawa et al., 1996). In particular, SYG was suggested to be the main compound exerting a pharmacological effect on swimming time for rats (Nishibe et al., 1990). The present study shows that *Acanthopanax senticosus* reduces fatigue during exercise by inhibition of 5-HT synthesis and TPH expression. Further study is needed to identify the main active components of *Acanthopanax senticosus* that are responsible for the reducing of fatigue.

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