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Duration-dependence of the effect of treadmill exercise on hyperactivity in attention deficit hyperactivity disorder rats

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Attention-deficit hyperactivity disorder (ADHD) is a common neurobehavioral disorder, and its symptoms are hyperactivity and deficits in learning and memory. Physical exercise increases dopamine synthesis and neuronal activity in various brain regions. In the present study, we investigate the duration-dependence of the treadmill exercise on hyperactivity in relation with dopamine expression in ADHD. Spontaneously hypertensive rats were used for the ADHD rats and Wistar-Kyoto rats were used for the control rats. The rats in the exercise groups were forced to run on a treadmill for 10 min, 30 min, and 60 min once daily for 28 consecutive days. For this experiment, open field test and immunohistochemistry for tyrosine hydroxylase were conducted. The present results revealed that ADHD rats showed hyperactivity, and tyrosine hydroxylase expression in the striatum and substantia nigra were decreased in ADHD rats. Treadmill exercise alleviated hyperactivity and also increased TH expression in ADHD rats. Treadmill exercise for 30 min per day showed most potent suppressing effect on hyperactivity, and this dose of treadmill exercise also most potently inhibited tyrosine hydroxylase expression. The present study suggests that treadmill exercise for 30 min once a day is the most effective therapeutic intervention for ADHD patients.

Keywords: Attention-deficit hyperactivity disorder, Treadmill exercise, Duration-dependence, Hyperactivity, Dopamine

INTRODUCTION

Attention deficit/hyperactivity disorder (ADHD) is a neurodevelopmental disorder and this disorder affects about 8-12% of children at school age (6-12 yr) (Pastor and Reuben 2008). ADHD is characterized by three major behavioral symptoms: inattention, hyperactivity, and impulsivity. In addition, ADHD patients often exhibit learning difficulty and cognitive impulsiveness (Wilens and Dodson, 2004). Although its etiology is unknown, the most likely mechanism for ADHD is damage to the dopaminergic and noradrenergic pathway (Biederman and Spencer, 1999; Sagvolden and Sergeant, 1998).

It has been widely suggested that dopamine plays an important role for the modulation of neuroendocrine functions, cognition, attention, reward, and behaviors including motor activity. Dysfunction of dopamine signaling is related to the brain disorders such as Parkinson's disease and ADHD (Volkow et al., 2007; Yoon et al., 2007). Previous studies reported that dysfunction of dopamine signaling in the midbrain substantia nigra (SN) is one of the most possible mechanisms of the behavioral symptoms of ADHD (Bowton et al., 2010). SN plays an important role in brain functions, particularly in movement, motor planning, reward seeking, learning, and addiction. Many functions of SN are mediated through the striatum. The nigral dopaminergic input to the striatum via the nigrostriatal pathway is intimately linked with the striatum's function (Nicola et al., 2000). Tyrosine hydroxylase (TH) is the enzyme synthesizing catecholamine neurotransmitters, and it converts L-tyrosine to L-dihydroxyphenylalanine (L-DOPA), which is

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the rate-limiting step in the dopamine synthesis. TH activity is progressively decreased according to the loss of dopamine neurons in the SN (Haavik and Toska, 1998). TH immunohistochemistry is a useful technique detecting injury or death of dopaminergic fibers or cell bodies (Hurley et al., 2004; Kim et al., 2011).

In ADHD children, physical movement improved working speed and social behavioral problems, and diminished hyperactivity (Majorek et al., 2004). Kim et al. (2011) reported that treadmill exercise ameliorated symptoms of ADHD rats. Despite a variety of studies on the effect of treadmill exercise on various neuropsychiatric disorders (Heo et al., 2014; Jee et al., 2008; Kim et al., 2014), there is no available data on the duration-dependence of the effects of treadmill exercise on the symptoms relief in the ADHD patients.

In the present study, we investigated the duration-dependence of the effect of treadmill exercise on the hyperactivity in relation with DA expression in the ADHD rats. For this study, open field test and immunohistochemistry for TH were performed.

MATERIALS AND METHODS

Experimental animals

Adult male spontaneously hypertensive rats (SHR) weighing 210±10 g were used as the ADHD animal model, because SHR displays the major symptoms of ADHD, such as inattention, hyperactivity, and impulsiveness (Sagvolden, 2000). SHR showing hyperactivity in open field test was selected for this experiment. Meanwhile, Wistar-Kyoto rats (WKYR) weighing 210±10 g were used as the control rats in this study, according to the previous study (Sagvolden, 2000). The rats were housed under controlled temperature (20±2°C) and lighting conditions (07:00-19:00), with food and water made available *ad libitum* throughout the experiments. The animals were randomly divided into four groups (n=10 in each group): the control group, the ADHD group, the ADHD and 10 min treadmill exercise group, the ADHD and 30 min treadmill exercise group, and the ADHD and 60 min treadmill exercise group. All animal procedures were performed in accordance with the animal care guidelines of the National Institutes of Health (NIH) and the Korean Academy of Medical Sciences.

Treadmill exercise protocol

The rats in the treadmill exercise groups were subjected to run on a treadmill for 10 min, 30 min, and 60 min once a day, five times a week, continued for 28 days. Exercise load for the exercise groups consisted of running at a speed of 2 meters/min for the first 5 min, at a speed of 5 meters/min for the next 5 min, and then at a speed of 8 meters/min for the last 20 min, with the 0° inclination. This intensity corresponded to the low-intensity treadmill exercise (% maximal oxygen consumption) according to the rats in this age (Bedford et al., 1979).

Open field test

Activity was determined using the open field test. The animals were randomly assigned to an order of testing and placed in a white square open field arena (100 cm×100 cm) made of wood. As the previously reported method (Durand et al., 1999), it was enclosed with 40-cm high walls and placed under strong illumination (200 lux). The arena was divided into 25 squares (each square is 20×20 cm), defined as 9 central and 16 peripheral squares. The animal was placed in the center of the arena and left free to explore the environment for 1 min. After that time, the numbers of squares that the rat crossed were recorded for 5 min.

Tissue preparation

The rats were sacrificed 29 days after the starting of the experiment. The animals were fully anesthetized using Zoletil 50® (10 mg/kg, i.p.; Vibac Laboratories, Carros, France). The anesthetized rats were transcardially perfused with 50 mM phosphate-buffered saline (PBS), and fixed with a freshly prepared solution consisting of 4% paraformaldehyde (PFA) in 100 mM phosphate buffer (PB) at pH 7.4. Brains were dissected, post-fixed in the same fixative overnight, and transferred to 30% sucrose for cryoprotection. Coronal sections of 40 μm thickness were made with a freezing microtome (Leica, Nussloch, Germany).

TH immunohistochemistry

For immunolabeling of TH in the striatum and SN, TH immunohistochemistry was performed as the previously described method (Kim et al., 2011). Free-floating tissue sections were incubated overnight with mouse anti-TH antibody (1:1,000, Santa Cruz Biotechnology, Santa Cruz, CA, USA) and the sections were then incubated for 1 h with biotinylated anti-mouse secondary antibody (1:200, Vector Laboratories, Burlingame, CA, USA). The sections were subsequently incubated with avidin-biotin-peroxidase complex (Vector Laboratories) for 1 h at room temperature. Immunoreactivity was visualized by incubating the sections in a solution consisting of 0.05% 3,3-diaminobenzidine (DAB) and 0.01% H_2O_2 in 50 mM Tris-buffer (pH 7.6) for approximately 3 min. The sections were then washed three times with PBS

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and mounted onto gelatine-coated slides. The slides were air-dried overnight at room temperature, and coverslips were mounted using Permount®.

Data analysis

TH-immunoreactive fiber density in the striatum was measured in 100 μm ×100 μm square images of the striatum using animage analyzer (Multiscan, Fullerton, CA, USA). To estimate TH-staining density, optical densities were corrected for the nonspecific background density, which was measured in the completely denervated parts of the striatum. TH-positive fiber density ratios in the striatum were calculated as follows: optical density in the lesion side/optical density in the intact side. The number of TH-positive cells in the SN was counted hemilaterally through a light microscope (Olympus, Tokyo, Japan).

Statistical analysis was performed using one-way ANOVA followed by Duncan's post-hoc test, and the results are expressed as the mean ± standard error of the mean (SEM). Significance was set as $P < 0.05$.

RESULTS

Effect of treadmill exercise on hyperactivity in the open field test

The open field test was performed 29 days after starting of experiment. The activity score of the open field test are presented in Fig. 1. The activity score was 38.20 ± 2.73 in the control group, 90.20 ± 9.15 in the ADHD group, 89.20 ± 5.53 in the 10 min treadmill exercise group, 53.60 ± 3.31 in the 30 min treadmill exercise group, and 58.60 ± 2.99 in the 60 min treadmill exercise group. These results show that the activity of in the ADHD rats was higher than that of the control rats. Treadmill exercise for 30 min most potently decreased the activity in the rats of the ADHD group.

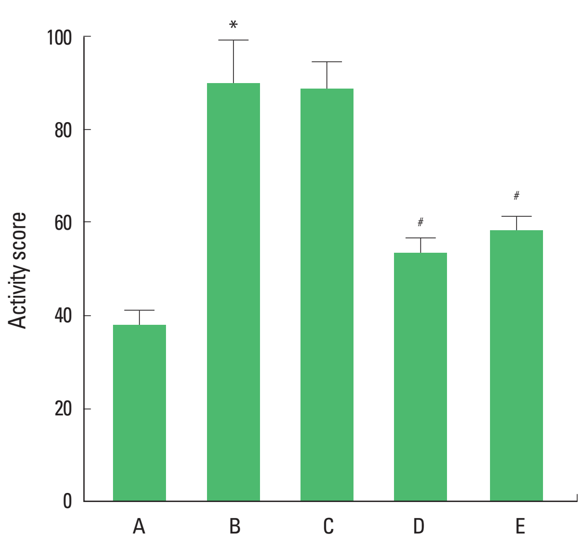

Fig. 1. Effect of treadmill exercise on hyperactivity in the open field test. (A) Control group. (B) attention deficit/hyperactivity disorder (ADHD) group. (C) ADHD and treadmill exercise for 10 min group. (D) ADHD and treadmill exercise for 30 min group. (E) ADHD and treadmill exercise for 60 min group. *Represents $P<0.05$ compared to the control group. #Represents $P<0.05$ compared to the ADHD group.

Effect of treadmill exercise on TH-immunoreactive fibers in the striatum

Photomicrographs of the TH-immunoreactive fibers in the striatum are presented in Fig. 2. The optical density of the TH-immunoreactive fibers in the striatum was 127.18 ± 2.96 in the con-

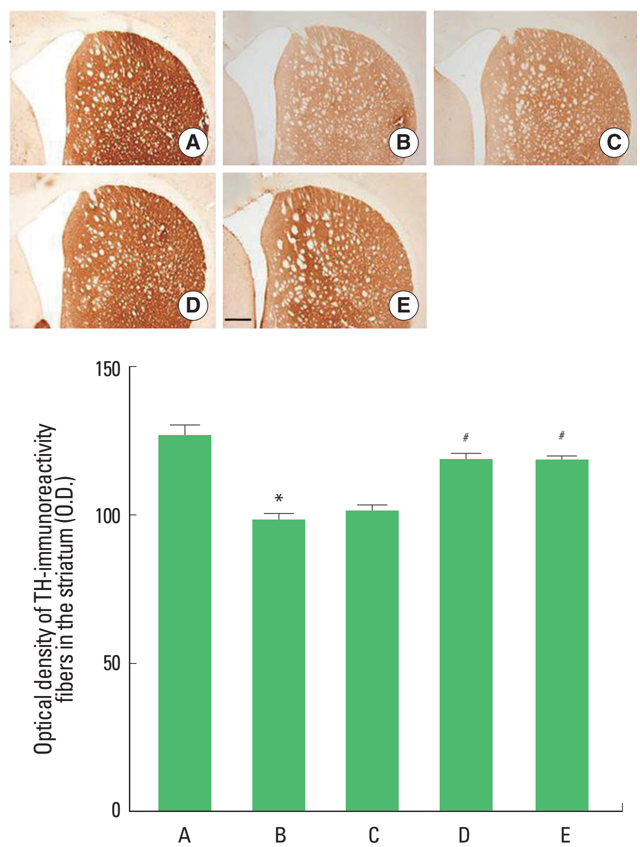

Fig. 2. Effect of treadmill exercise on tyrosine hydroxylase-immunoreactive fibers in the striatum. Upper: Photomicrographs of the tyrosine hydroxylase-positive fibers in the striatum. The scale bar represents 200 μm. (A) Control group. (B) attention deficit/hyperactivity disorder (ADHD) group. (C) ADHD and treadmill exercise for 10 min group. (D) ADHD and treadmill exercise for 30 min group. (E) ADHD and treadmill exercise for 60 min group. *Represents $P<0.05$ compared to the control group. #Represents $P<0.05$ compared to the ADHD group.

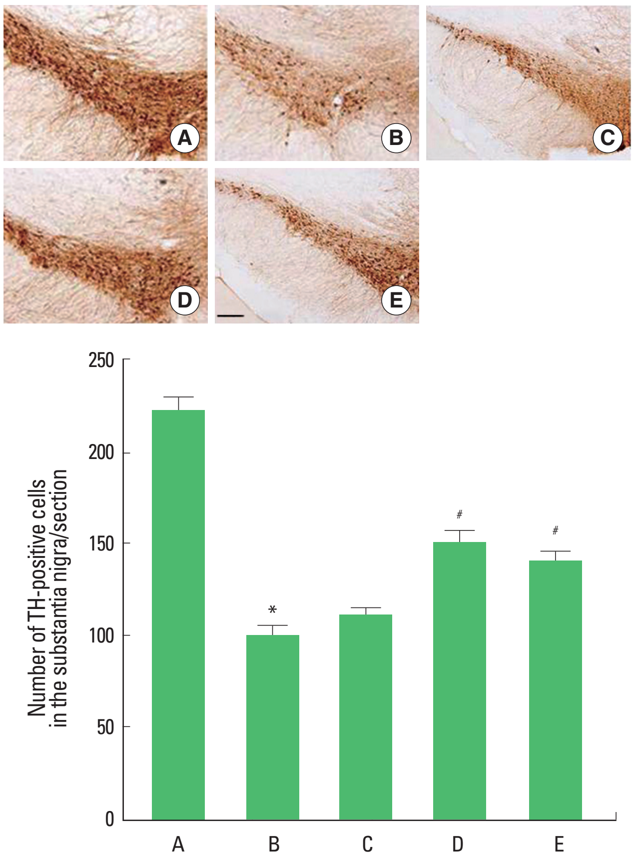

Fig. 3. Effect of treadmill exercise on tyrosine hydroxylase expression in the substantia nigra. Photomicrographs of tyrosine hydroxylase-positive cells in the substantial nigra. The scale bar represents 200 μm. (A) Control group. (B) Attention deficit/hyperactivity disorder (ADHD) group. (C) ADHD and treadmill exercise for 10 min group. (D) ADHD and treadmill exercise for 30 min group. (E) ADHD and treadmill exercise for 60 min group. *Represents $P<0.05$ compared to the control group. #Represents $P<0.05$ compared to the ADHD group.

trol group, 98.70±1.58 in the ADHD group, 101.88±1.57 in the 10 min treadmill exercise group, 119.06±1.60 in the 30 min treadmill exercise group, and 118.53±0.97 in the 60 min treadmill exercise group. The results show that TH fiber expression in the striatum was significantly decreased in the rats of the ADHD group than that of the control group. Treadmill exercise for 30 min most potently increased TH fiber expression in the rats of the ADHD group.

Effect of treadmill exercise on TH expression in the SN

Photomicrographs of the TH-positive cells in the SN are presented in Fig. 3. The number of TH-positive cells was 222.70±6.00 in the control group, 100.90±4.95 in the ADHD group, 112.80±3.11 in the 10 min treadmill exercise group, 151.20±6.11 in the 30 min treadmill exercise group, and 142.10±3.62 in the 60 min treadmill exercise group. The results show that TH expression in the SN was significantly decreased in the rats of the ADHD group than that of the control group. Treadmill exercise for 30 min most potently increased TH expression in the rats of the ADHD group.

DISCUSSION

SHR is the most commonly accepted animal model for ADHD, because SHR strain shows hyperactivity (Sagvolden, 2000). This SHR displayed hyperactivity in the open field test and spatial learning memory deficit in the radial 8-arm maze task, as we expected. WKY rats, used as the control group, did not show any behavior symptoms of ADHD. The symptoms of ADHD, observed in the present study, are thought to be closely associated with hypo-function of dopaminergic system in some brain regions.

Dopamine is a neurotransmitter that is important in regulating brain processes involved with movement. Disease-induced loss of brain dopamine level leads to movement disorders (Servan-Schreiber et al., 1998). Dopamine is also important in regulating pleasurable responses as well as certain aspects of cognitive function, including attention (Sagvolden et al., 2005). These conditions give rise to delay aversion, development of hyperactivity in novel situations, impulsiveness, deficient sustained attention, increased behavioral variability (Sagvolden et al., 2005). Volkow et al. (2007) suggested that suppressed dopamine activity in caudate is associated with inattention in the adults with ADHD. Many studies have suggested the possibility that dysfunction of dopamine signaling in the midbrain is one of the main mechanisms of hyperactivity (Bowton et al., 2010).

In the present results, hyperactivity was observed in the ADHD rats, and expressions of TH in the striatum and SN were significantly decreased in the ADHD rats. The present study suggests that hyperactivity in the ADHD rats is associated with down-regulation of dopamine in the striatum and SN.

Physical movement is known to improve working speed and social behavior problems and to diminishe hyperactivity in ADHD children (Majorek et al., 2004). Kim et al. (2011) reported that treadmill exercise showed alleviating effect on the hyperactivity in the ADHD rats. Increased dopamine synthesis by exercise enhanced the survival of dopaminergic neurons in the SN and treadmill running also alleviated some symptoms of ADHD (Hattori et al., 1994; Kim et al., 2011).

In the present results, treadmill exercise suppressed hyperactiv-

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ity and also inhibited TH expression in the striatum and SN. Treadmill exercise for 30 min showed most potent inhibiting effect on hyperactivity, and this duration of treadmill exercise also most potently inhibited TH expression. Treadmill exercise for 10 min exerted no significant effects on activity and TH expression.

Here in this study, we showed that treadmill exercise can be considered therapeutic strategy for the symptom relief for ADHD. The present study suggests that treadmill exercise for 30 min once a day is the most effective therapeutic intervention for ADHD patients.

CONFLICT OF INEREST

No potential conflict of interest relevant to this article was reported.

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