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ORIGINAL ARTICLE

Exercise-Induced Neuroprotection in the 6-Hydroxydopamine Parkinson's Disease Model

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Abstract

Exercise exerts helpful effects in Parkinson's disease. In this study, the 6-hydroxydopamine (6-OHDA) injection was used to investigate the effect of exercise on apomorphine-induced rotation and neurorestoration. Rats ($n = 32$) were divided into four groups: (1) Saline+Noexercise (Sham); (2) 6-OHDA+Noexercise (6-OHDA); (3) Saline+Exercise (S+EXE), and (4) 6-OHDA+Exercise (6-OHDA+EXE). The rats were administered 8 μg 6-OHDA by injection into the right medial forebrain bundle. After 2 weeks, the exercise group was run (14 consecutive days, 30 min per day). One month after the surgery, following the injection of apomorphine, the 6-OHDA group displayed a significant increase in rotation and the 6-OHDA+EXE group showed a significant reduction of rotational asymmetry ($P < 0.001$). 6-OHDA injection reduced the mRNA and protein expression of the AMP-activated protein kinase, brain-derived neurotropic factor, and tyrosine hydroxylase in relation to the Sham group and exercise increased these levels. Expression of the silent information regulator 2 homolog 1 and peroxisome proliferator-activated receptor gamma coactivator 1-alpha was unexpectedly enhanced in the 6-OHDA groups in relation to the Sham group. These findings suggest that the 6-OHDA injection increased the neurodegeneration and mitochondrial and behavioral dysfunctions and the treadmill running attenuated these disorders in the ipsilateral striatum of the 6-OHDA+EXE group.

Keywords Parkinson's disease · Treadmill running · 6-OHDA · Tyrosine hydroxylase · Apomorphine

Introduction

Parkinson's disease (PD) is a neurodegenerative disorder that causes suffering of millions of people, especially in the growing aging population. Patients with PD are typically debilitated with movement disorder symptoms, with increased reactive oxygen species production and mitochondrial apoptotic susceptibility, as well as decreased transcriptional drive for mitochondrial biogenesis (Oliveira et al. 2014; Petzinger et al. 2015; Corona and Duchen 2015). However, the precise underlying mechanisms of these processes remain unclear (Oliveira et al. 2014) and currently, there is no cure for PD (Petzinger et al. 2015). The major pathology of PD involves the deletion or downregulation of mitochondrial genes that are essential for supporting mitochondrial biogenesis, leading to mitochondrial dysfunction and contributing to progressive degeneration of the dopaminergic neuron (Gerecke et al. 2010; Patki and Lau 2011). A common neurotoxin substance in animal models of PD is 6-hydroxydopamine (6-OHDA) that destructs dopamine neurons (Aguiar Jr et al. 2016; Garcia et al. 2017; Rezaee et al. 2019a; b).

A set of enzymes fine-tunes the mitochondrial function in a cell. AMP-activated protein kinase (*Ampk*) is an enzyme that rapidly regulates metabolic and mitochondrial enzymes by their direct phosphorylation, and also affects the transcription of specific genes to adapt gene expression to cellular energy demands (Cantó and Auwerx 2009). The silent information regulator 2 homolog 1 (*Sirt1*) may influence the aging process and many age-associated diseases. It is downregulated in aging cells, suggesting that *Sirt1* may function to extend the life span (Oliveira et al. 2014), and it plays an intricate role in the pathology of multiple diseases (Chong et al. 2012). *Ampk* and

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Sirt1, through phosphorylation and deacetylation, respectively (Kang et al. 2013), modulate the activity of peroxisome proliferator-activated receptor gamma coactivator 1-alpha (*Pgc1α*), which is a key factor activating mitochondrial biogenesis and its expression can be used as a marker of this process (Steiner et al. 2011; Oliveira et al. 2014). *Sirt1* appears to contribute to the regulation of metabolism by acting in a pathway whereby it deacetylates and activates *Pgc1α* (Cantó and Auwerx 2009; Kang et al. 2013). Brain-derived neurotrophic factor (*Bdnf*) is an essential vital protein in learning and memory, neuronal plasticity, neuroprotection, and mitochondrial function (Miranda et al. 2019; Rezaee et al. 2019b). The trophic effect of *Bdnf* on dopamine neurons is evaluated as potential neuroprotective (Razgado-Hernandez et al. 2015). Finally, tyrosine hydroxylase (*Th*) is a rate-limiting enzyme involved in the synthesis of catecholamine neurotransmitters, such as dopamine, and it is used as a marker of neuronal degeneration (Yoon et al. 2007; Tuon et al. 2014). One of the important ways to evaluate the efficacy of therapeutic methods of neurodegenerative disease is an assessment of the *Th* level (Tuon et al. 2014; Aguiar Jr et al. 2016). According to previous studies, *Th* is positively regulated by *Bdnf*, and *Pgc1α* is effective in regulating *Bdnf* (Hsueh et al. 2018; Rezaee et al. 2019b).

Severe motor, mental, functional, and mitochondrial disability following the progressive neuronal degeneration in PD suggests that a therapeutic approach preventing neurodegeneration and promoting neuroprotection would be a valuable strategy to control the disease. Exercise is one intervention that has been shown to reduce the production of free radicals and mitochondrial dysfunction (Gerecke et al. 2010). Implications for an exercise-induced enhancement of neuroprotective effects and cognitive functions are vast and contradictory (Steiner et al. 2011; Chen et al. 2017; Costa et al. 2017). The data differ depending on the exercise program, age of animals, and type and amount of injection of neurotoxin (Choe et al. 2012; Aguiar Jr et al. 2016; Real et al. 2017).

Accordingly, the present study investigates the therapeutic effect of exercise on neuroprotection after the unilateral injection of 6-OHDA.

Materials and Methods

Animals

For the study, 32 male Wister rats (weighing 270 ± 20 g, 6 months old, from the Pasteur Institute of Iran) were used. The animals were housed ($n = 4$ per cage) in a temperature-controlled room (22 ± 2 °C) with a 12-h light/12-h dark cycle, and free access to food and water. One week before the surgery, to familiarize the rats with the experimental conditions, the animals were placed on a treadmill, for 10 min/day at 5 m/min, to ensure that all animals performed similarly prior to 6-OHDA lesioning. After the familiarization, the rats were randomly assigned to four groups ($n = 8$ animals/group), as follows: (1) Saline+Noexercise (Sham); (2) 6-OHDA+Noexercise (6-OHDA); (3) Saline+Exercise (S+EXE); and (4) 6-OHDA+Exercise (6-OHDA+EXE) (as explained below).

Ethical Standards

All experiments were performed in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals, and have been approved by the Ethic Committee for Animal Experiments at the University of Isfahan (IR.UI.REC.1396,008).

Surgical Procedures

Rats were anesthetized using xylazine (0.67 mg/kg, intraperitoneal injection, i.p.) and ketamine (0.33 mg/kg, i.p.), and then received unilateral stereotaxic injections of 8 μg of 6-OHDA (Sigma-Aldrich; 4 μL of 2 μg/μL solution prepared in 0.2% ascorbic acid and 0.9% NaCl) or saline (4 μL of 0.2% ascorbic acid and 0.9% NaCl) (Tuon et al. 2015), into the right medial forebrain bundle anteroposterior (AP), − 1.8 mm; lateral (LAT), 4.7 mm from the bregma; and vertical (DV), − 8.2 mm from the skull surface (Mabandla et al. 2004; Yoon et al. 2007; Carvalho et al. 2013), by a 5-μL Hamilton syringe attached to an infusion pump (BI Insight 2000), at a rate of 0.5 μL/min for 8 min for each rat. The cannula was left in place for an additional 5 min after the injection before being slowly retracted. Briefly, 5 days following the 6-OHDA or saline administration, a time point to complete the cell death process, the rotations following the intraperitoneal injection of apomorphine were calculated (with a similar way to Behavioral test section). Finally, 6-OHDA-induced rats that showed significant rotations were selected to continue the study and the others were excluded for further investigation (Tuon et al. 2014).

Exercise Protocols

Two weeks after the induction of lesions, exercise groups began following a light treadmill exercise protocol for 30 min, once a day, for 14 consecutive days (Cho et al. 2013). The exercise load was started at a velocity of 5 m/min for the first 5 min, 8 m/min for the next 5 min, and 15 m/min for the last 20 min, without electrical shock and at 0° of inclination. The noexercise groups were placed on an unmoving treadmill for the same period of time.

Behavioral Test (Asymmetry Rotational)

Forty-eight hours after the last training session, rats are placed in a plastic container (25-cm high and 28-cm diameter) for 10 min to habituate. This was followed by a challenge with the dopamine receptor agonist R(−)-apomorphine (0.5 mg/kg, i.p.). The rotation number under apomorphine (Sigma) is one test that used to quantify the degree of lesion in the PD model and is related to the extent of dopamine depletion, after the unilateral 6-hydroxydopamine lesion. The contralateral rotations (opposite to the lesioned right side) induced by apomorphine are related to the unbalance in the nigrostriatal dopaminergic pathways between the right (lesioned) and left (unlesioned) brain hemispheres (Tuon et al. 2012; 2014; Aguiar Jr et al. 2016; Costa et al. 2017; Real et al. 2017). This test was monitored for 60 min.

Euthanasia and Tissue Collection

One day after the behavioral test, the rats were anesthetized with ketamine (5 mg/100 g of body weight, i.p.) and xylazine (1 mg/100 g of body weight, i.p.), and the right striatum was surgically removed. The extracted tissues were deep-frozen in liquid nitrogen, and then stored at − 80 °C. Tissue homogenates were centrifuged at 11,000×*g* (40 min, 4 °C) for 40 min to remove insoluble material (Tuon et al. 2014; 2015).

Isolation of Total RNA and Real-Time Quantitative PCR

Total RNA was isolated from tissues ($n = 8$ per group) using the RNeasy mini kit (Qiagen Inc., Valencia, CA, USA), as recommended by the manufacturer. The concentration and purity of RNA were assessed using NanoDrop spectrophotometer (NanoDrop Technologies Wilmington, DE, USA) by reading sample absorbance at 230 nm, 260 nm, and 280 nm. Then, 1 μg of total RNA was used for the synthesis of cDNA (cDNA synthesis kit, Fermentas, Lithuania) by utilizing oligo dT primers. RT-qPCR was performed using SYBR Green PCR master mix (TaKaRa, Japan) and a Step One Plus thermocycler (ABI Applied Biosystems, USA). The forward and reverse primer sequences are provided in Table 1. The Ct value is the fractional cycle number at which sample fluorescence exceeds a fixed threshold. The fold-change in expression was calculated by using the ΔΔCt method, and the data are presented as the percentage of fold-change of expression in treated groups compared with the corresponding control group after normalization to *Gapdh* endogenous control (Patki and Lau 2011).

Table 1 Primers used in RT-qPCR experiments (5′–3′)

Locus	bp	Sequence
Ampk-Fw	131	GCCAAATCAGGGACTGCTACTC
Ampk-Rv		GAGGTCACGGATGAGGTAAG AGA
Sirt1-Fw	132	TGACGCCTTATCCTCTAGTTCCT
Sirt1-Rv		TCAGCATCATCTTCCAAGCCATT
Pgc1α-Fw	169	ACAACCGCAGTCGCAACA
Pgc1α-Rv		AGGAGTCGTGGGAGGAGTTAG
Bdnf-Fw	83	ACTCGCAATGCCGAACTACC
Bdnf-Rv		CCTTATGAACCGCCAGCCAAT
Th-Fw	125	TCATCACCTGGTCACCAAGTT
Th-Rv		GGTCGCCGTGCCTGTACT
Gapdh-Fw	181	CTAGAGACAGCCGCATCTTCTTG
Gapdh-Rv		AATCCGTTCACACCGACCTTC

Gapdh was used as a housekeeping gene for data normalization

Protein Extraction and Western Blotting

Proteins were extracted from the striatum samples ($n = 5$ per group) in the RIPA buffer (Sigma) according to the manufacturer's protocol. As previously described by our group (Rezaee et al. 2019a), briefly, 1 mL of RIPA buffer was added to 100 mg of brain tissue in a 1.5-mL tube and homogenized (five times, 5 min each, at 4 °C). Insoluble material was removed by centrifugation (12,000×*g* for 20 min). Soluble protein concentration in sample supernatants was determined using a protein assay kit (Bio-Rad, Hercules, CA). Equal amounts of protein (0.2 mg per sample) were resolved on 10% SDS-PAGE and transferred on to polyvinylidene difluoride membranes (Bio-Rad Laboratories, USA). The membranes were blocked overnight in 10% (*w*/*v*) non-fat dried milk (Merk, Germany) in phosphate-buffered saline, incubated with antibodies, and the signals developed as described elsewhere (Tuon et al. 2012). The antibodies used were antiphospho (Thr172) *Ampk* (1:200; sc-33524), anti-*Sirt1* (1:200; sc-15404), anti-*Pgc1α* (1:200, sc-55476), anti-brain-derived neurotrophic factor (1:200, sc-65514), anti-*Th* (1:200; sc-25269) and anti–β-actin antibodies (1:200; sc-47778), from Santa Cruz Biotechnology (Santa Cruz, USA). Chemiluminescent detection was enabled by using a secondary antibody conjugated with a horseradish peroxidase (1:2500; Bio-Rad, 170-6516), and the bands were visualized using an Amersham ECL advanced western blotting detection kit. NIH ImageJ program was used to compare the density of bands on Western blot.

Data Analysis

All data are presented as the mean + standard error of the mean (SEM). Data for the treatment groups, 1 month after the

surgery, were compared using GraphPad Prism 8 Software, La Jolla, CA, USA and one-way analysis of variance (ANOVA) (version 23.0), followed by Tukey's post hoc test. $P < 0.05$ was considered to be statistically significant.

Results

Apomorphine-Induced Rotations

Apomorphine-induced RT was performed 30 days after 6-OHDA injection. Statistical analysis indicated differences in apomorphine-induced rotation behavior of animals in different groups [$F(3,28) = 2174.7$, $P < 0.001$], with post hoc analysis revealing a significantly higher rotational asymmetry in the 6-OHDA group than in other groups. As shown in Fig. 1, the treadmill exercise significantly reduced the rotational asymmetry in the 6-OHDA+EXE group (318.7 ± 6.7 turns/h) compared with the sedentary parkinsonian group (363.6 ± 22.2 turns/h) ($P < 0.001$). This analysis was complemented by the assessment of *Th* level (Figs. 2, 3e).

Assessment of *Ampk*/*Sirt1*/*Pgc1a*, *Bdnf*, and *Th* mRNA Levels

Forty-eight hours after the behavioral test, the effect of exercise on expression of specific genes in animals was investigated. One-way ANOVA analysis revealed that the interaction between groups was significantly different in the case of *Ampk* [$F(3,28) = 26.04$, $P < 0.001$], *Bdnf* [$F(3,28) = 203$, $P < 0.001$], and *Th* [$F(3,28) = 56.42$, $P < 0.001$]. We found that the 6-OHDA administration led to a significant reduction of these mRNA levels (Fig. 2a–e) compared with the Sham group.

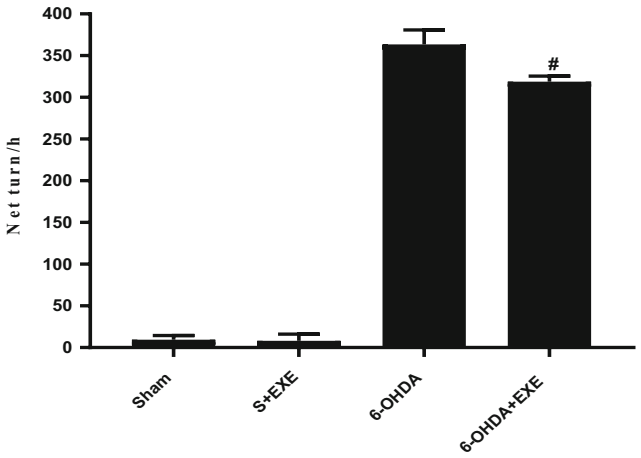

Fig. 1 The effect of treadmill exercise on apomorphine-induced rotation in rats. Values are expressed as the mean + SEM ($n = 8$ per group); # $P < 0.001$ in relation to 6-OHDA group, according to one-way ANOVA followed by Tukey's post hoc test. Sham, Saline+Noexercise; S+EXE, Saline+Exercise; 6-OHDA, 6-OHDA+Noexercise; and 6-OHDA+EXE, 6-OHDA+Exercise

Exercise resulted in increased mRNA levels in the 6-OHDA+EXE group up to Sham group for every three genes and there were significant increases compared with the 6-OHDA group (respectively 80%, 73%, and 71%). Furthermore, analysis of the interaction between all experimental groups by one-way ANOVA revealed a significant difference in *Sirt1* levels [$F(3,28) = 13.6$, $P < 0.001$] (Fig. 2b) and *Pgc1a* expression [$F(3,28) = 10.08$, $P < 0.001$] (Fig. 2c). Unexpectedly, and contrary to previous mRNA levels, the striatal expression of *Sirt1* (30%) and *Pgc1a* (27%) in the 6-OHDA group was significantly higher than in the Sham group. The striatal levels for later genes in 6-OHDA and 6-OHDA+EXE groups were similar and there were no significant differences between them (Fig. 2b, c).

Protein Analysis by Western Blotting

The striatal *Ampk*, *Bdnf*, and *Th* protein levels were shown in Fig. 3 a, d, and e, respectively. A significant difference was evident by interaction analysis of all groups in *Ampk* [$F(3,16) = 352.8$, $P < 0.001$], *Bdnf* [$F(3,16) = 231.9$, $P < 0.001$], and *Th* [$F(3,16) = 265.9$, $P < 0.001$] levels. These protein levels in animals administered 6-OHDA were significantly reduced compared with the Sham group. Exercise in the 6-OHDA+EXE group significantly increased the *Ampk* (60%), *Bdnf* (72%), and *Th* (66%) levels compared with sedentary 6-OHDA animals, but these levels significantly were lower than the Sham group ($P < 0.001$).

Surprisingly, the *Sirt1* protein level in all of groups increased significantly (~ 30%) compared with the Sham group [$F(3,16) = 269.3$, $P < 0.001$] (Fig. 3b), also *Pgc1α* protein level in the Sham group was significantly lower (~ 20%) than the other groups [$F(3,16) = 38.7$, $P < 0.001$] (Fig. 3c). Here were no statistically significant differences between 6-OHDA and 6-OHDA+EXE groups in *Sirt1* and *Pgc1α* protein levels (Fig. 3b, c).

Discussion

The aim of the current study was to examine the effect of exercise on the neuroprotection of dopaminergic neuron, rotational behavior and some mitochondrial factors following a 6-OHDA–induced cell death. Using the rat model of PD, we showed that 6-OHDA injection resulted in behavioral and mitochondrial disorders. Furthermore, treadmill exercise ameliorated the behavioral impairment in rats with a significant effect on *Bdnf* and *Th* expression levels (Figs. 2d, e and 3d, e). We observed that regular treadmill exercise resulted in increase of mRNAs and proteins in the striatum of S+EXE rats (Figs. 2, 3). This was different to what has been reported previously by Tillerson et al. (2003). One difference between the current study and that was the age of the

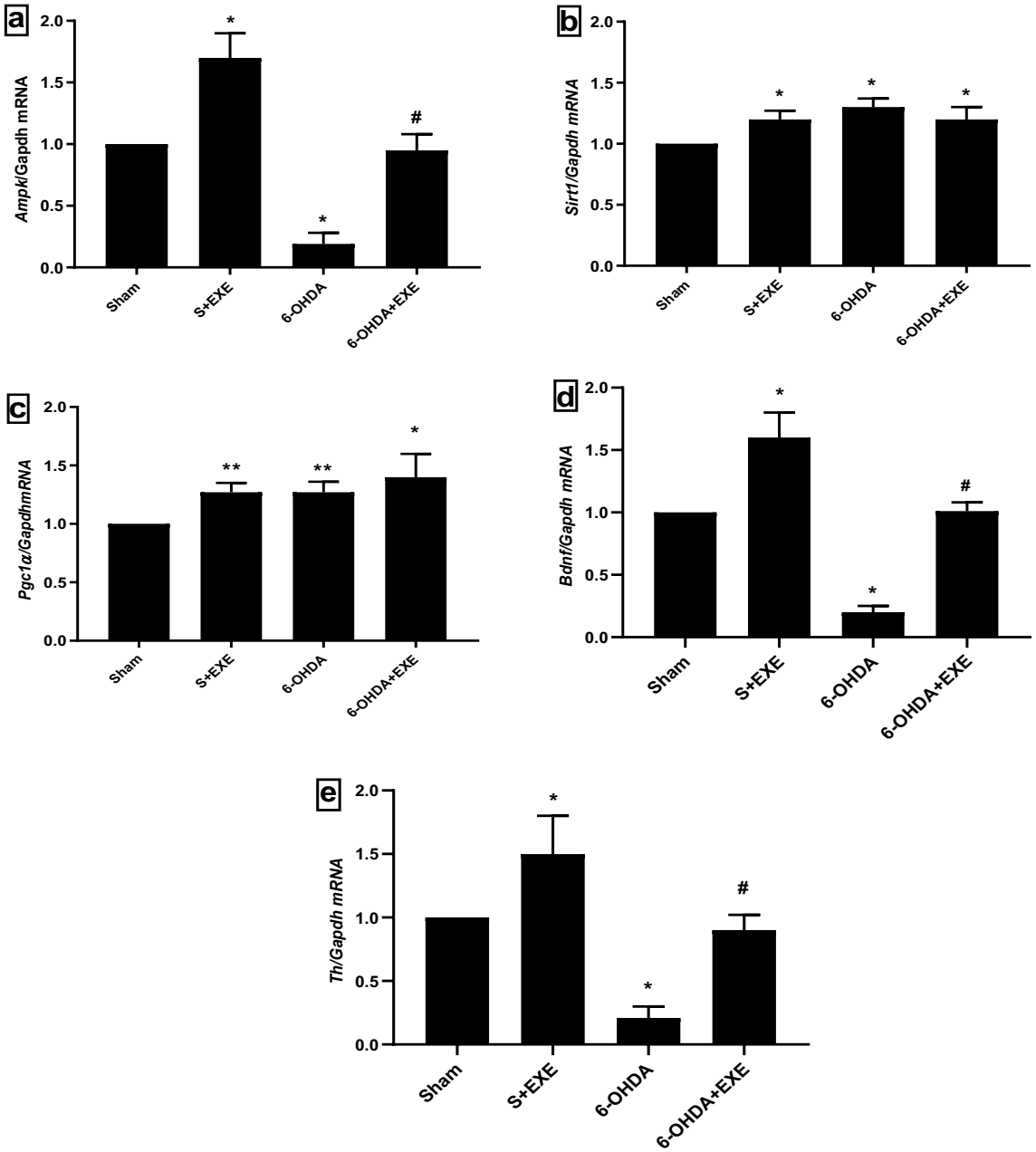

Fig. 2 Effects of treadmill exercise on *Ampk*, *Sirt1*, *Pgc1a*, *Bdnf*, and *Th* mRNA levels. RT-qPCR was performed to detect changes in the expression of *Ampk* (**a**), *Sirt1* (**b**), *Pgc1a* (**c**), *Bdnf* (**d**), and *Th* (**e**) genes in the striatum of animals administered 6-OHDA before the exercise. Values are expressed as the mean + SEM ($n = 8$ per group); *$P < 0.001$ and **$P < 0.01$ compared with the Sham group; #$P < 0.001$ compared with the 6-OHDA group, according to one-way ANOVA followed by Tukey's post hoc test. Sham, Saline+Noexercise; S+EXE, Saline+Exercise; 6-OHDA, 6-OHDA+Noexercise; and 6-OHDA+EXE, 6-OHDA+Exercise

experimental animals (aged vs. young). In the present study, the rats were young. Thus, age is an important factor affecting the expression of biochemical factors following exercise, even in the healthy rat. Similar results with our observations, on the heart and liver, were reported by previous studies (Bayod et al. 2012; Oliveira et al. 2014). Furthermore, Bayod et al. (2012) demonstrated that *Ampk*, *Sirt1*, and *Pgc1a* genes are expressed in young human after exercise, indicating that mitochondrial biogenesis-related genes are readily activated in young individuals, and that these responses are attenuated in older individuals. Muscle heat generation during exercise can play a role in the activation of the *Ampk–Sirt1–Pgc1a* pathway and mitochondrial biogenesis (Bayod et al. 2012). Mitochondrial abnormality, mainly in the brain, plays a vital role in the development of PD (Chong et al. 2012). Many studies reported that exercise is an effective intervention that upregulates important factors of mitochondrial biogenesis and neurogenesis, e.g., *Pgc-1α*

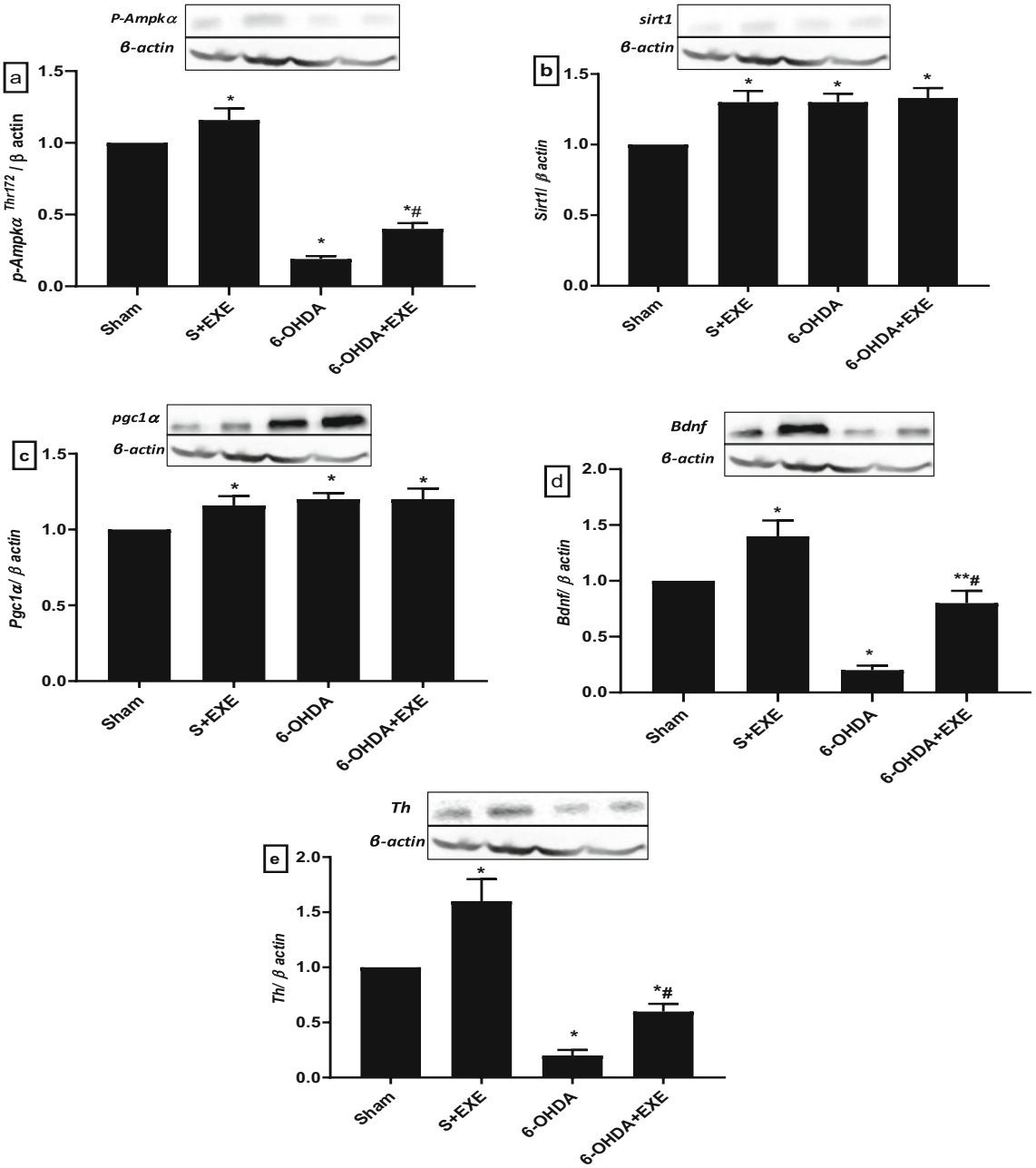

Fig. 3 Effects of treadmill exercise on *Ampk* (**a**), *Sirt1* (**b**), *Pgc1a* (**c**), *Bdnf* (**d**), and *Th* (**e**) levels in the striatum of animals administered 6-OHDA before the exercise, as assessed by western blotting. Values are expressed as the mean + SEM ($n = 5$ per group); *$P < 0.001$ and **$P < 0.01$ compared with the Sham group, #$P < 0.001$ compared with the 6-OHDA group, according to one-way ANOVA followed by Tukey's post hoc test. Sham, Saline+Noexercise; S+EXE, Saline+Exercise; 6-OHDA, 6-OHDA+Noexercise; and 6-OHDA+EXE, 6-OHDA+Exercise

(Patki and Lau 2011; Kang et al. 2013; Oliveira et al. 2014; LaHue et al. 2016). In the present study, the exercise after the 6-OHDA injection resulted in attenuation of behavioral abnormality and an increase of the genes expression, especially *Th* level, that confirm neuroprotective effects of exercise on the dopaminergic system. This finding is supported by the previous study that assessed preventive exercise before 6-OHDA injection (Carvalho et al. 2013; Rezaee et al. 2019b). In contrast, Real et al. (2017) indicated exercise-induced stress causes neurodegeneration and increases rotations in exercised rats compare with the control group.

It has been also demonstrated that exercise leads to increased expression of neurotrophic factors especially *Bdnf*, resulting in neuroprotection and increase of *Th* protein level (Pothakos et al. 2009) that has been confirmed in the present study (Figs. 2d, e and 3d, e). Indeed, the efficiency of every proposed experimental protocol on PD is confirmed by measuring the *Th* levels (Phillipson 2014; Real et al. 2017).

Consistent with what has been previously reported (Aguiar Jr et al. 2016; Garcia et al. 2017), and contrary to what has been anticipated, we observed 6-OHDA injection leading to increased *Sirt1* and *Pgc1a* mRNA and protein level 30 days after the 6-OHDA injection (Figs. 2b, c and 3b, c). This indicated continuous mitochondrial transcription and activity of metabolic factors, probably to compensate for the cellular damage caused by 6-OHDA (Patki and Lau 2011). However, these observations suggested that increasing expression of these factors were not sufficient to prevent the reduction of *Th* protein level and behavioral disorder in the 6-OHDA sedentary group (Figs. 1, 2, 3e). Since in this group, regardless of compensatory increase in expression of these genes, disorders of behavioral and neurodegeneration are not attenuated. On the other hand, 14-day light exercise after the lesion reduced these abnormalities (Figs. 1, 2, 3). It is not clear why exercise can alleviate disorders in PD. Probably, exercise increases blood flow for the removal of the neurotoxin from the various brain regions (Mabandla et al. 2004). Furthermore, exercise by enhancing the expression of *Ampk*, *Sirt1*, and *Pgc1a*, as upstream of *Bdnf* gene, enhances mitochondrial biogenesis, brain plasticity, and neuroprotective effects due to neurotrophins and reduces of behavioral disorders and brain insults (McMurphy et al. 2019; Miranda et al. 2019; Rezaee et al. 2019b).

Several explanations can account for the differences in conclusions of studies involving animal models of PD. These include the induction method used and severity of lesion, the type and intensity of the exercise regimen, and the time of starting the exercise after the induction of lesion (Gerecke et al. 2010; Garcia et al. 2017). Inconsistent with previous studies (Fisher et al. 2004; Yoon et al. 2007; Pothakos et al. 2009; Gerecke et al. 2010), in the current study, we observed that the exercise exerted a protective effect against behavioral disorder (asymmetry rotation) (Fig. 1). The rotational test produces a useful parameter for evaluating behavioral deficits and the imbalances of dopamine in striatum of the unilateral rat model of PD (Tuon et al. 2012). Loss of *Th* expression and increase of apomorphine-induced turning behavior displayed that our model of 6-OHDA MFB injections caused dopaminergic degeneration. We found that the exercised animals with 6-OHDA injection when compared with the 6-OHDA sedentary group showed a reduction in the number of turns that reflects a protective effect of exercise on dopaminergic neurons. This result is reinforced by larger *Th* expression in the striatum of this group that occurred during the 14-day training. *Bdnf* as upstream gene of *Th* is upregulated also in the 6-OHDA+EXE group (Figs. 2, 3e). One of the few studies published that the exercise exerts neuroprotective effects on the dopaminergic system, partly through the upregulation of *Bdnf* (Tajiri et al. 2010). *Bdnf* is an essential vital protein that is involved in learning and memory and can improve brain plasticity. Moreover, the expression of *Bdnf* is a protective mechanism against toxicity (Wrann Christiane et al. 2013; Sleiman et al. 2016; Xia et al. 2017). On the other hand, regardless of increase of *Th* level in the 6-OHDA+EXE group compare with the 6-OHDA sedentary group, its level was significantly lower than control rats in the Sham group (Figs. 2, 3e). It is important to note that there was a 14-day delay before the beginning of exercise (after the surgery) in the present study. Since depletion of dopamine in the striatum is reportedly maximal 1 week after 6-OHDA infusion into the medial forebrain bundle (Yoon et al. 2007), in this study, starting training protocol after the short time of surgery might have better results. Garcia et al. (2017) also reported only in the third month after injection that the changes in *Th* level were similar to control animals and its level in closer assessments to injection was significantly lower than the Sham group. Hence, probably longer times after surgery, more than 1 month, are necessary to the rehabilitation of the deficits up to normal level.

In the current study, our lesion protocol was successful in causing a rat model of PD. As shown, the 6-OHDA injection increased apomorphine-induced rotation behavior and dopamine degeneration that is confirmed by loss of *Th* expression in response to 6-OHDA. On the other hand, the exercise following the surgery could reduce behavioral (apomorphine-induced rotation) and non-behavioral impairments and increase neuroprotection. However, a compensatory increase of *Sirt1* and *Pgc1a* expression in the sedentary 6-OHDA group were not lead to the attenuation of behavioral disorder and dopamine loss. Therefore, based on the findings, the compensatory regulation of *Sirt1* and *Pgc-1α* is not a useful strategy for treating PD. It seems that exercise triggers mechanisms on CNS resulted in neuroprotective effects and mitochondrial recovery in the 6-OHDA animal models. Hence, light treadmill exercise provides a therapeutic response for the treatment of PD.

Compliance with Ethical Standards All experiments were performed in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals, and have been approved by the Ethic Committee for Animal Experiments at the University of Isfahan (IR.UI.REC.1396,008).

Conflict of Interest The authors declare that they have no conflict of interest.

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