

Treadmill exercise activates PI3K/Akt signaling pathway leading to GSK-3 β inhibition in the social isolated rat pups

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Social isolation is known to precipitate depression-like symptoms in rodents and has emerged as a dependable paradigm to screen the behavioral and neurobiological changes observed in humans. In the present study, the underlying mechanisms of treadmill exercise on social isolation-induced depression was evaluated. The rat pups in the social isolation groups were housed individually. The social isolation procedures started on the postnatal day 14. The rat pups in the exercise groups were forced to run on treadmill for 30 min once a day from postnatal day 21 to postnatal day 34. The expression of phosphoinositide 3 kinase (PI3K), tyrosine kinase B (Akt), and glycogen synthase kinase 3 β (GSK-3 β) in the hippocampus were determined by using western blot analysis. The ratio phosphorylated PI3K (p-PI3K)/PI3K and phosphorylated

Akt (p-Akt)/Akt were decreased and the ratio of phosphorylated-GSK-3 (p-GSK-3 β)/GSK-3 β was increased by social isolation. Treadmill exercise increased the ratio p-PI3K/PI3K and p-Akt/Akt and suppressed the ratio of p-GSK-3 β /GSK-3 β in the hippocampus of social isolated rat pups. Treadmill exercise activates PI3K/Akt signaling pathway leading to GSK-3 β inhibition in social isolated rats. These results suggested that treadmill exercise may improve depressive symptoms via activation of PI3K/Akt signaling pathway.

Keywords: Social isolation, Treadmill exercise, Depression, PI3K, Akt, GSK-3 β

INTRODUCTION

Exposure to stress during the early stage of development exerts negative influence on brain development and stress is associated with psychological disorders, such as depression and anxiety (Fone and Porkess, 2008; Ladd et al., 2000). Among stressors, social isolation is known to precipitate depression-like symptoms (Baek et al., 2012; Dandekar et al., 2009).

The effectiveness of exercise for stress-related disorders, especially depression, is well documented (Lee and Baek, 2017). Physical exercise has an antidepressant action in animals and humans (Lawlor and Hopker, 2001; Santarelli et al., 2003). Cho et al. (2017) reported that treadmill exercise alleviated depression caused by social isolation by increasing hippocampal neurogenesis through serotonin upregulation.

Protein kinase B (Akt) is a member of a class of serine or threonine protein kinases, and is a major effector in the phosphoinos-

itide 3 kinase (PI3K) signaling pathway (Brazil and Hemmings, 2001; Shiojima and Walsh, 2002). Akt has a significant role in multiple cellular processes, including cell survival, metabolism, growth, proliferation and mobility (Brazil and Hemmings, 2001). Akt also regulates vascular homeostasis and angiogenesis (Shiojima and Walsh, 2002).

Glycogen synthase kinase 3 (GSK-3) has two isoforms, GSK-3 α and GSK-3 β , that are inactivated by Akt phosphorylation on Ser21 and Ser9, respectively (Cross et al., 1995). Inhibition of GSK-3 is known to protect apoptosis in many situations (Pap and Cooper, 1998). GSK-3 β is a downstream target of Akt and activation of Akt inhibits GSK-3 β by inducing its phosphorylation (Wu et al., 2007).

In the present study, the underlying mechanisms of treadmill exercise on social isolation-induced depression was evaluated. We hypothesize that antidepressive effect of treadmill exercise might be mediated by GSK-3 β inactivation through activation of the

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PI3K/Akt signaling pathway.

MATERIALS AND METHODS

Animals and treatments

On the postnatal day 14, the rat pups were divided into one of the four groups: the control group, the exercise group, the social isolation group, and the social isolation and exercise group ($n=8$ in each group). The rat pups in the control group and exercise groups were housed with their respective mothers under standard conditions, while the rat pups in the social isolation groups were housed individually. The social isolation procedures started on the postnatal day 14. The rat pups in the exercise groups were forced to run on treadmill for 30 min once a day from postnatal day 21 to postnatal day 34. The exercise load consisted of running at a speed of 2 m/min for the first 5 min, at a speed of 5 m/min for the next 5 min, and then at a speed of 8 m/min for the last 20 min with 0% grade of inclination.

Tissue preparation

After anesthetizing with Zoletil 50 (10 mg/kg intraperitoneally; Vibac Laboratories, Carros, France), the rat pups were transcar-

dially perfused with 50 mM phosphate-buffered saline and subsequently fixed with freshly prepared 500 mM phosphate buffer (pH, 7.4) containing 4% paraformaldehyde. The brains of the rat pups were removed and fixed in the same fixative overnight and then transferred into a 30% sucrose solution for cryoprotection. Serial coronal sections of 40- μ m thickness were obtained using a freezing microtome (Leica, Nussloch, Germany).

Western blot analysis

Western blot analysis was performed according to the previous method (Cho et al., 2017; Kim et al., 2010). Protein separation was performed using 10% polyacrylamide with 0.05% bis-acrylamide. Proteins were then transferred to nitrocellulose and the blots were probed with anti-PI3K mouse monoclonal antibody (1:1,000; Santa Cruz Biotechnology, Santa Cruz, CA, USA), anti-p-PI3K rabbit polyclonal antibody (1:1,000; Santa Cruz Biotechnology), anti-Akt rabbit polyclonal antibody (1:1,000; Cell Signaling Technology Inc., Beverly, Massachusetts, USA), anti-p-Akt rabbit polyclonal antibody (1:1,000; Cell Signaling Technology), anti-GSK-3 β rabbit polyclonal antibody (1:1,000; Santa Cruz Biotechnology), and anti-p-GSK-3 β rabbit polyclonal antibody (1:1,000; Santa Cruz Biotechnology). Peroxidase anti-rabbit IgG

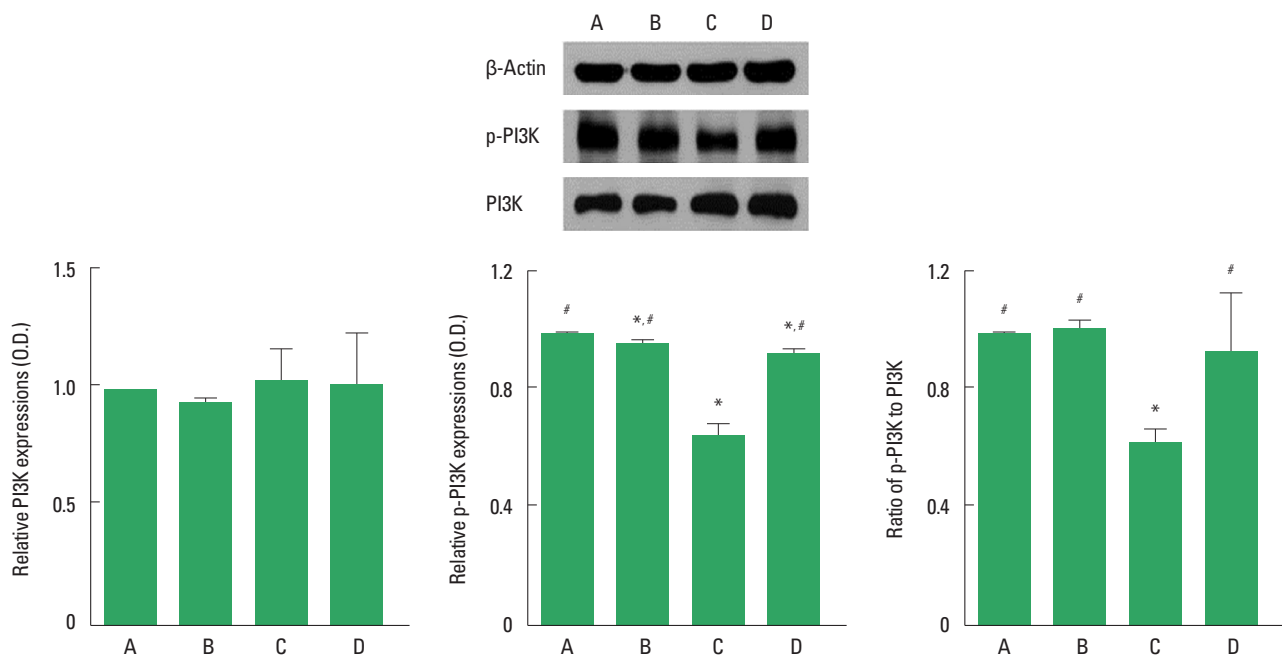


Fig. 1. Effect of treadmill exercise on phosphoinositide 3 kinase (PI3K) and phosphorylated PI3K (p-PI3K) expression in the hippocampus. Upper panel: representative expression of PI3K, p-PI3K, and β -actin. Lower panel: relative expression of PI3K, p-PI3K, and p-PI3K/PI3K ratio. Left panel: relative PI3K expression. Middle panel: relative p-PI3K expression. Right panel: relative ratio of p-PI3K/PI3K. The data are represented as the mean \pm standard error of the mean. A, control group; B, exercise group; C, social isolation group; D, social isolation and exercise group. * $P < 0.05$ compared to the control group. # $P < 0.05$ compared to the social isolation group.

(1:5,000; Vector Laboratories) and peroxidase anti-mouse IgG (1:10,000; Vector Laboratories) were used as the secondary antibodies. Immunoreactivity was detected by enhanced chemiluminescence (ECL) detection kit (Santa Cruz Biotechnology).

Data analysis

Differences among the groups were evaluated using IBM SPSS Statistics ver. 21.0 (IBM Co., Armonk, NY, USA) by the one-way analysis of variance followed by Duncan *post hoc* test. All values are expressed as the mean \pm standard error of the mean. Statistically significant differences were established at $P < 0.05$.

RESULTS

PI3K and p-PI3K expression in the hippocampus

Western blot analysis for the expression of PI3K and p-PI3K in the hippocampus was performed (Fig. 1). Social isolation and treadmill exercise did not significantly affect PI3K expression in rat pups of all groups (Fig. 1, left panel). The expression of p-PI3K was decreased by social isolation. Treadmill exercise increased p-PI3K expression in the rat pups of social isolation group (Fig. 1, middle panel). The ratio of p-PI3K/PI3K was decreased by social isolation. Treadmill exercise increased the ratio of p-PI3K/PI3K in the rat pups of social isolation group by enhancing the expres-

sion of p-PI3K (Fig. 1, right panel).

Akt and p-Akt expression in the hippocampus

Western blot analysis for the expression of Akt and p-Akt in the hippocampus was performed (Fig. 2). The expression of Akt was decreased by social isolation. Treadmill exercise increased Akt expression in the rat pups of social isolation group (Fig. 2, left panel). The expression of p-Akt was decreased by social isolation. Treadmill exercise increased p-Akt expression in the rat pups of social isolation group (Fig. 2, middle panel). The ratio of p-Akt/Akt was decreased by social isolation. Treadmill exercise increased the ratio of p-Akt/Akt in the rat pups of social isolation group by enhancing the expression of p-Akt (Fig. 2, right panel).

GSK-3 β and p-GSK-3 β expression in the hippocampus

Western blot analysis for the expression of GSK-3 β and p-GSK-3 β in the hippocampus was performed (Fig. 3). The expression of GSK-3 β was decreased by treadmill exercise in both normal and social isolated group (Fig. 3, left panel). The expression of p-GSK-3 β was increased by social isolation. Treadmill exercise decreased p-GSK-3 β expression in the rat pups of social isolation group (Fig. 3, middle panel). The ratio of p-GSK-3 β /GSK-3 β was increased by social isolation. Treadmill exercise decreased the ratio of p-GSK-3 β /GSK-3 β in the rat pups of social isolation group by suppress-

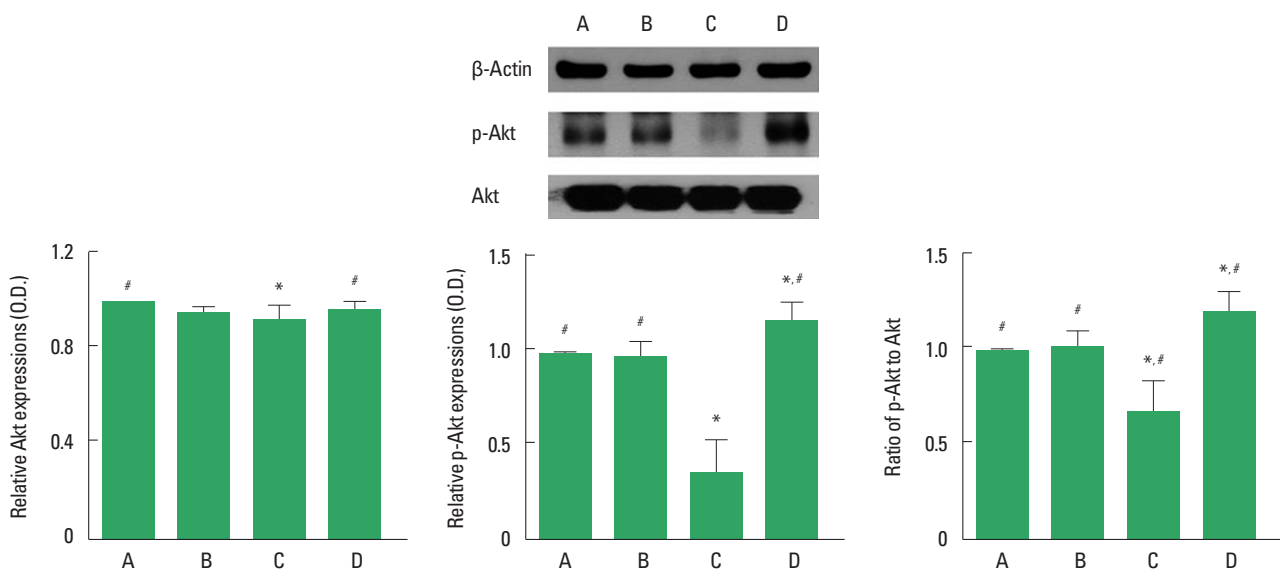


Fig. 2. Effect of treadmill exercise on protein kinase B (Akt) and phosphorylated Akt (p-Akt) expression in the hippocampus. Upper panel: representative expression of Akt, p-Akt, and β -actin. Lower panel: relative expression of Akt, p-Akt, and p-Akt/Akt ratio. Left panel: relative Akt expression. Middle panel: relative p-Akt expression. Right panel: relative ratio of p-Akt/Akt. The data are represented as the mean \pm standard error of the mean. A, control group; B, exercise group; C, social isolation group; D, social isolation and exercise group. * $P < 0.05$ compared to the control group. # $P < 0.05$ compared to the social isolation group.

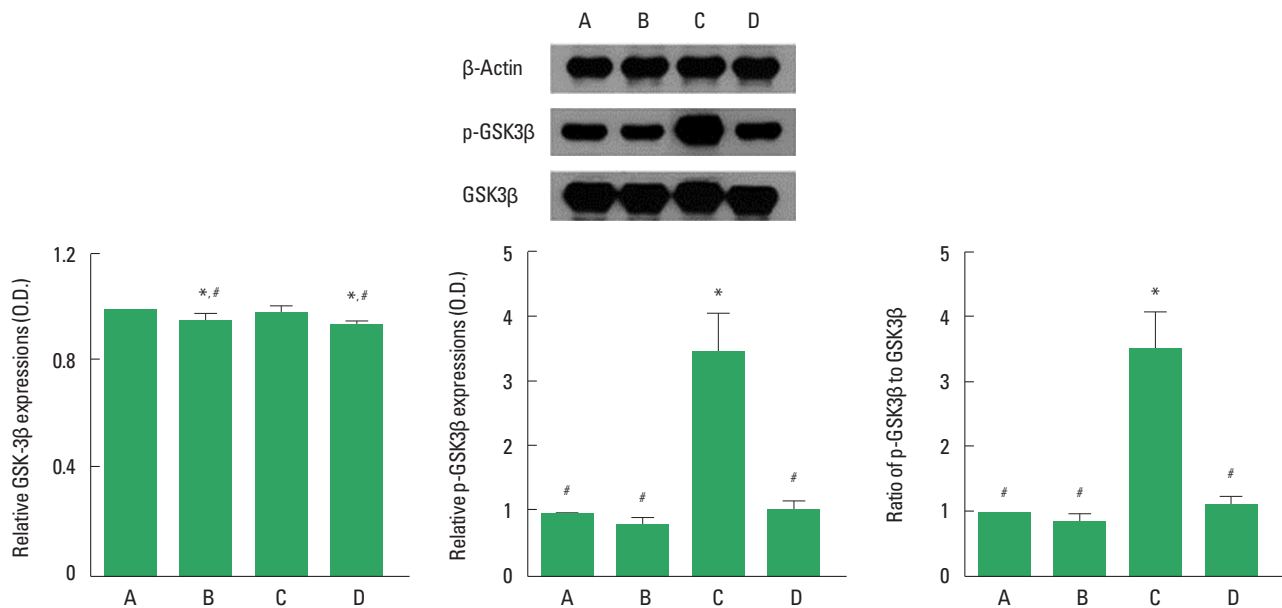


Fig. 3. Effect of treadmill exercise on glycogen synthase kinase 3 β (GSK-3 β) and phosphorylated GSK-3 β (p-GSK-3 β) expression in the hippocampus. Upper panel: representative expression of GSK-3 β , p-GSK-3 β , and β -actins. Lower panel: relative expression of GSK-3 β , p-GSK-3 β , and p-GSK-3 β /GSK-3 β ratio. Left panel: relative GSK-3 β expression. Middle panel: relative p-GSK-3 β expression. Right panel: relative ratio of p-GSK-3 β /GSK-3 β . The data are represented as the mean \pm standard error of the mean. A, control group; B, exercise group; C, social isolation group; D, social isolation and exercise group. * P <0.05 compared to the control group. # P <0.05 compared to the social isolation group.

ing the expression of p-GSK-3 β (Fig. 3, right panel).

DISCUSSION

Activation of PI3K is the most important mechanism of promoting cell survival by neurotrophins (Vaillant et al., 1999). PI3K/Akt pathway is implicated in the cell proliferation and migration during neurogenesis (Zhang et al., 2011). Activation of PI3K/Akt is a hallmark of cell survival/proliferation, and PI3K/Akt pathway is implicated in the protective role in many neuropathological conditions (Chavali et al., 2011). In the present study, the ratio of p-PI3K/PI3K in the hippocampus was decreased by social isolation. However, treadmill exercise restored the social isolation-induced decreased p-PI3K/PI3K ratio by enhancing the expression of p-PI3K. The present result showed that treadmill exercise exerted ameliorating effect on depression through the phosphorylation of PI3K.

Akt promote neuronal differentiation (Vojtek et al., 2003), and neural progenitor cell proliferation is mediated by phosphorylation of Akt (Wu et al., 2009). Activation of Akt signaling in the endothelial cells stimulates endothelial cell bioactivity and angiogenesis (Kureishi et al., 2000). Phosphorylation of Akt is predominantly expressed in the diabetic retina (Wang et al., 2010). Akt

phosphorylation is related to neurogenesis, and nuclear translocation of Akt is required for cell cycle progression (Parcellier et al., 2008). In the present study, the ratio of p-Akt/Akt in the hippocampus was decreased by social isolation, however, treadmill exercise increased the ratio of p-Akt/Akt by enhancing the expression of p-Akt. The present result showed that treadmill exercise exerted ameliorating effect on depression through the phosphorylation of Akt.

Akt is one of the kinases that inactivate GSK-3 β via its phosphorylation. PI3K/Akt activation is linked to inhibition of GSK-3 β (Shaw et al., 1998). GSK-3 β is one of the key targets of the well-established apoptotic signaling mediated by the PI3K/Akt pathway (Pap and Cooper, 1998). GSK-3 β is a serine/threonine kinase mediating cellular signaling and cell death (Grimes and Jope, 2001). Selective inhibitors for GSK-3 β showed antidepressant effect in the depression model (Kaidanovich-Beilin et al., 2004). GSK-3 β is also important factor in both mood disorder and suicide behavior (Liu et al., 2012). In the present study, the expression of GSK-3 β in the hippocampus was increased by social isolation. However, treadmill exercise decreased GSK-3 β expression. The present results suggest that GSK-3 β expression was suppressed by treadmill exercise through activation of PI3K/Akt signaling pathway.

Lee and Baek (2017) suggested that exercise may preserve brain function by increasing neurogenesis in the psychiatric disorders, such as depression. Cho et al. (2017) also showed that treadmill exercise may ameliorates social isolation-induced depression through increasing neuronal generation.

Here in the present study, we have shown that treadmill exercise activates PI3K/Akt signaling pathway leading to GSK-3 β inhibition in social isolated rats. These results suggest that treadmill exercise may improve depressive symptoms via activation of PI3K/Akt signaling pathway.

CONFLICT OF INTEREST

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

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