Effect of treadmill exercise on the BDNF-mediated pathway in the hippocampus of stressed rats

Zheng Huan Fang b, Chan Hong Lee a, Mi Kyoung Seo a, HyeYeon Cho a, Jung Goo Lee a,b, Sung Ju Lee a, Sung Woo Park a,b,c,∗

a Paik Institute for Clinical Research, Inje University, Busan, Republic of Korea
b Institute for Brain Science and Technology, Inje University, Busan, Republic of Korea
c Department of Psychiatry, School of Medicine, Haeundae Paik Hospital, Inje University, Busan, Republic of Korea

1. Introduction

Physical exercise has been shown to improve depressive symptoms (Babyak et al., 2000; Blumenthal et al., 2007). Accumulating evidence suggests that in humans, exercise enhances mood and coping capacity in response to stress (Dischman, 1985) and decreases anxiety (Raglin and Morgan, 1987). Furthermore, exercise has been shown to improve learning, memory, and cognition, suggesting a hippocampal-dependent function (Vaynman et al., 2004; Lambert et al., 2005). However, the intracellular signaling mechanisms underlying these effects of exercise are not well understood.

Brain-derived neurotrophic factor (BDNF), the most abundant neurotrophin in the brain, regulates cell survival, neurogenesis, and neuroplasticity (Pencea et al., 2001; Bramham and Messioudi, 2005). Clinical findings have suggested that depression is associated with a reduction in BDNF levels in the hippocampus and serum (Laske et al., 2010). Studies performed during various types of exercise have demonstrated that in normal rats, physical activity increases hippocampal BDNF expression to a level similar to that induced by antidepressants (Russo-Neustadt et al., 1999; Adlard and Cotman, 2004). However, it is not known whether treadmill exercise increases BDNF expression in the hippocampus of stressed rats.

BDNF and its receptor, TrkB, have been extensively studied in association with depression and antidepressant treatment. BDNF-induced TrkB activation stimulates the mitogen-activated protein kinase (MAPK) and phosphatidylinositol-3 kinase (PI3K)/Akt
signaling pathways, which play a principal role in promoting neuronal survival and synaptic plasticity (Pizzorusso et al., 2000; Rodgers and Theibert, 2002). Moreover, voluntary exercise has been reported to activate these pathways (Chen and Russo-Neustadt, 2005; Lista and Sorrentino, 2010). Particularly, major components of the PI3K/Akt pathway, including glycosynthese kinase-3β (GSK-3β) and the mammalian target of rapamycin (mTOR), have been implicated in depression and the antidepressant response (Duman and Voleti, 2012).

GSK-3β, a ubiquitous cellular serine/threonine protein kinase, was originally thought to regulate glycosyntese synthesis in response to insulin but is now recognized as a downstream regulator that mediates multiple signaling pathways coupled to receptors (e.g., neurotransmitter and neurotrophic factor receptors) (Grimes and Jope, 2001). Activation of PI3K/Akt signaling inhibits GSK-3β activity via phosphorylation at serine6 (Li et al., 2007). Inhibition of GSK-3 activity produced antidepressant-like effects in the forced-swimming test (FST) in rodents (Gould et al., 2004; Kaidanovich-Belin et al., 2004; Rosa et al., 2008), suggesting that GSK-3 inhibition may be involved in antidepressant action. Despite growing interest in the possible involvement of GSK-3β, few studies of GSK-3 regulation in exercise have been reported.

mTOR is an ubiquitously expressed serine/threonine kinase that regulates the initiation of protein translation (Hoeffer and Klaun, 2010). Activated mTOR phosphorylates p70 ribosomal protein S6 kinase (p70S6K) and eukaryotic initiation factor 4E-binding protein 1 (4E-BP1), leading to the promotion of translation initiation for synaptic protein synthesis (Hoeffer and Klaun, 2010); mTOR activity increases via phosphorylation at serine2448 by PI3K/Akt activation (Chiang and Abraham, 2005). Recent studies have shown that a low dose of ketamine (10 mg/kg), which is reported to have antidepressant actions in behavioral models of depression (Maeng et al., 2008), rapidly activated mTOR signaling, leading to an increase in synaptic proteins and the number of spine synapses in the prefrontal cortex of rats (Li et al., 2010). Moreover, the mTOR inhibitor, rapamycin, reversed these effects and blocked the antidepressant effects of ketamine in FST (Li et al., 2010). Thus, these findings suggest new targets for rapid-acting antidepressants. We sought to investigate whether treadmill exercise affects mTOR signaling in stressed rats.

Furthermore, both exercise and antidepressants have been reported to increase several pre- and postsynaptic proteins involved in synapse formation in the rat hippocampus (Hu et al., 2009; Ferreira et al., 2011). Synaptic formation is induced by interactions between presynaptic and postsynaptic neurons that involve cell-adhesion molecules, scaffolding proteins, and proteins associated with synaptic vesicle machinery (Kim and Sheng, 2004; Washbourne et al., 2004). Neurelin 1 (NLG 1), a postsynaptic adhesion molecule, binds with high affinity to the presynaptic adhesion molecule β-neurexin (Dean et al., 2003). This interaction promotes synapse assembly and presynaptic and postsynaptic differentiation (Rao et al., 2000; Washbourne et al., 2004). NLG 1 binds to postsynaptic density protein-95 (PSD-95) via its PSD-95/DisC large/zona accludens 1 binding domain (Ehrlich et al., 2007). In this way, the β-neurexin-NLG 1–PSD-95 complex can establish a link between presynaptic and postsynaptic cells. The postsynaptic density protein PSD-95 is located exclusively in dendritic spines and plays a critical role in regulating dendritic spine size and shape (Ehrlich et al., 2007; Han and Kim, 2008) by acting as a scaffolding protein that regulates the clustering of glutamate receptors in the dendritic spine (Han and Kim, 2008). Synaptophysin (SYP), the major integral membrane protein of presynaptic vesicles, is required for vesicle formation and exocytosis and is widely used as a marker for synapse activity (Valtorta et al., 2004). SYP plays a regulatory role in activity-dependent synapse formation in cultures of hippocampal neurons (Tarsa and Goda, 2002). Loss of SYP in the hippocampus is correlated with the cognitive decline associated with Alzheimer’s disease, highlighting its role in cognitive functioning (Sze et al., 1997).

In humans, the recommended daily exercise is at least 30 min at moderate intensity most days of the week (Hillman et al., 2008). To mimic this in an animal model, we used a short-term treadmill exercise protocol previously reported to show a positive effect on hippocampal plasticity in rats (Ferreira et al., 2011). The aim of the present study was to investigate the mechanisms underlying the beneficial effects of exercise on immobilization stress, a rat model for stress. We investigated whether treadmill exercise affected the PI3K/Akt signaling pathway that includes GSK-3β and mTOR in the hippocampus of stressed rats. Furthermore, we investigated the effect of exercise on the synaptic proteins, synaptophysin, PSD-95 and β-neurexin, and neureolin 1.

2. Materials and methods

2.1. Animals

Experiments were conducted using male 8-week-old Sprague-Dawley rats (Orient Bio, Gyeonggi, Korea) weighing 200–250 g. The animals were maintained in a temperature-controlled room (21 °C) with a 12-h light cycle (lights on 7:00 a.m.) and free access to food and water. The procedures used in the present study complied with the animal care guidelines in the “Principles of Laboratory Animal Care” (NIH publication no. 23-85, 1996). All experiments involving animals were approved by the Committee for Animal Experimentation and the Institutional Animal Laboratory Review Board of Inje Medical College (approval no. 2011-031).

2.2. Experimental design

After 7 days of acclimatization, the rats were randomly divided into four groups of six rats each (control sedentary; control exercise; stressed sedentary; stressed exercise). The treadmill is equipped with black boxes at the forward direction of belt; these act to motivate the rats to run continuously black boxes since the rats prefer a dark place. Familiarization involved exposure to the running treadmill for 15–30 min on three consecutive days. Belt speed was gradually increased from 5–15 m/min during familiarization and rats belonging to the untrained group were excluded. The control-sedentary group had no exercise or stress. In the control-exercise group, rats were allowed to run on the treadmill (Model SC 2061; SECO, Tianjin, China) at a speed of 15 m/min for 30 min (3 × 10 min bouts at 15 m/min with 30 min break between bouts) per day for 5 days. In the stressed-sedentary group, rats were subjected to 2 h/day immobilization stress for 7 days in a specially designed plastic restraint tube (dimensions: 20 cm high, 7 cm diameter). After 2 days of immobilization stress, rats in the stressed-exercise group were trained to run on the treadmill after 2 h of stress; this procedure was repeated once daily for 5 days.

2.3. Protein extraction and western blot

Rats were sacrificed 24 h after the last exercise session. The brain was removed, and the hippocampus dissected out. Whole hippocampal samples were homogenized in ice-cold lysis buffer containing 20 mM Tris–HCl, 137 mM NaCl, 10% glycerol, 1% Nonidet p-40, 0.1% sodium dodecyl sulfate (SDS), 0.5% sodium deoxycholate, 2 mM EDTA, 1 tablet complete protease inhibitor (Roche, Laval, Quebec, Canada), 20 mM NaF, and 1 mM Na3VO4. The homogenates were centrifuged at 13,000 rpm for 20–30 min at 4 °C. The supernatants were collected and used for quantification of total protein. Equal amounts of protein (20–30 μg) from the tissue extracts under each treatment condition were separated
using SDS polyacrylamide gel electrophoresis and then transferred electrophoretically onto polyvinylidene fluoride (PVDF) membranes. PVDF membranes were blocked by incubation in 5% (w/v) nonfat milk in Tris buffered saline (TBS) with 0.15% Tween 20 (TBS-T) for 1 h. Membranes were then incubated with anti-α-tubulin antibody (1:2000, Sigma–Aldrich, St. Louis, MO, USA) and anti-neurofilament 1 antibody (1:1000, Abcam, Cambridge, UK), followed by anti-mouse IgG horseradish peroxidase-conjugate; anti-BDNF antibody (1:500, Santa Cruz Biotechnology, Santa Cruz, CA, USA), which detects mature (14 kDa) isoform of BDNF, anti-p-TrkA (tyr490) which cross-reacts with tyrosine 490 on TrkB and TrkC as well (1:200, Cell Signaling Technology, Beverly, MA, USA), anti-TrkB (1:200, Santa Cruz Biotechnology), anti-synaptophysin antibody (1:1000, Abcam), anti-PSD-95 antibody (1:1000, Millipore, Billerica, MA, USA), anti-p-GSK-3β antibody (1:1000, Cell Signaling Technology, Beverly, MA, USA), anti-GSK-3β antibody (1:1000, Cell Signaling Technology), anti-p-Akt antibody (1:1000, Cell Signaling Technology), anti Akt antibody (1:1000, Cell Signaling Technology), anti-p-mTOR antibody (1:1000, Cell Signaling Technology), anti-mTOR antibody (1:1000, Cell Signaling Technology), and anti-p-p70S6K (1:1000, Cell Signaling Technology), followed by anti-rabbit IgG horseradish peroxidase-conjugate; and β-neurexin antibody (Santa Cruz Biotechnology), followed by anti-goat IgG horseradish peroxidase-conjugate. After rinsing with 0.15% TBS-T buffer, the immunocomplexes were visualized and quantified using enhanced chemiluminescence (ECL) Western blotting reagents, with chemiluminescence detected using a Las-3000 Image Reader (FujiFilm, Tokyo, Japan) software. Protein levels were normalized to the housekeeping protein α-tubulin to adjust for variability of protein loading and quantified with a densitometer. Data are expressed as the percentage of vehicle control density (deemed to be 100%).

### 2.4. Statistical analysis

A two-way analysis of variance (ANOVA) was performed to determine the individual and interactive effects of treadmill exercise and immobilization stress on the protein levels. Post hoc comparisons were conducted using Scheffe’s test. A p-value <0.05 was deemed statistically significant.

### 3. Results

The results of the two-way ANOVA are summarized in Table 1. Treadmill exercise or stress had a significant individual effect on BDNF, p-Trk receptor, p-Akt, p-GSK-3β, p-mTOR, p-p70S6K, synaptophysin, PSD-95, β-neurexin, and neurofilament 1 levels in the hippocampus (all p <0.01). We observed a significant interaction between exercise and stress for p-p70S6K, β-neurexin, and neurofilament 1 levels (all p <0.01); however, no significant interaction was found for BDNF, p-Trk receptor, p-Akt, p-GSK-3β, p-mTOR, or synaptophysin levels.

### 3.1. Effect of treadmill exercise on the levels of BDNF and Trk receptor phosphorylation

Immobilization stress significantly decreased BDNF expression and Trk receptor phosphorylation in the hippocampus to about 51% and 48%, respectively, compared with the control-sedentary group (each p <0.01; Fig. 1A and B). Running on the treadmill for 5 consecutive days markedly reversed the immobilization-stress-induced decrease in the levels of these proteins (BDNF, 141% of control, p <0.01, Fig. 1A; phosphorylated Trk receptor, 90% of

| Table 1 | Summary of the two-way analysis of variance. |
|-----------------|-----------------|-----------------|-----------------|
|                | Exercise        | Stress          | Exercise × Stress |
|                | F               | p-value         | F               | p-value         | F               | p-value         |
| BDNF p-Trk     | 99.18 ± 21.70   | <0.001          | 31.68 ± 35.36   | <0.001          | 0.60 ± 0.44     | 0.44 ± 0.51     |
| p-Akt          | 43.56           | <0.001          | 65.78           | <0.001          | 0.56            | 0.46            |
| p-GSK-3β       | 27.78           | <0.001          | 39.34           | <0.001          | 2.72            | 0.10            |
| p-mTOR         | 38.78           | <0.001          | 40.53           | <0.001          | 2.24            | 0.14            |
| p-p70S6K       | 58.62           | <0.001          | 101.19          | <0.001          | 10.19           | <0.01           |
| Synaptophysin  | 69.18           | <0.001          | 36.93           | <0.001          | 0.58            | 0.45            |
| PSD-95         | 57.65           | <0.001          | 157.96          | <0.001          | 3.72            | 0.057           |
| β-Neurexin     | 43.85           | <0.001          | 87.48           | <0.001          | 6.94            | <0.01           |
| Neurotrophin 1 | 29.11           | <0.001          | 71.08           | <0.001          | 7.65            | <0.01           |

**Fig. 1.** Effect of treadmill exercise on the levels of brain-derived neurotrophic factor (BDNF) expression and phosphorylated Trk receptor in the rat hippocampus. Rats were subjected to running at a speed of 15 m/min, 30 min/day for 5 days with (+Stress, 2 h daily) or without immobilization stress (−Stress). Bands and values are shown from hippocampal control-sedentary (−Stress, Con), control-exercise (−Stress, Ex), stressed-sedentary (+Stress, Con) or stressed-exercise (+Stress, Ex) groups. Cell lysates were analyzed using SDS-PAGE and Western blotting with anti-BDNF (A) and anti-phospho-tyr490-Trk receptor (B) primary antibodies. A representative image and quantitative analysis normalized to the α-tubulin and TrkB band are shown. Values are expressed as a percentage of the control value (−Stress, Con) and represent means ± SEMs from five animals per group. *p < 0.01 vs. control sedentary, **p < 0.01 vs. stressed sedentary only.
control, p < 0.01, Fig. 1B). Furthermore, exercise increased BDNF expression TrkB receptor phosphorylation under the stress-free condition compared with that in the control-sedentary group (BDNF, 170% of control, p < 0.01; phosphorylated TrkB, 131% of control, p < 0.01).

3.2. Effect of treadmill exercise on phosphorylation levels in Akt, GSK-3β, mTOR, and p70S6K

BDNF activates the PI3K/Akt pathway via the TrkB receptor (Rodgers and Theibert, 2002). Thus, we assessed phosphorylation of GSK-3β and mTOR, the downstream targets of PI3K/Akt. Additionally, we evaluated phosphorylation level of p60S6K, the downstream target of mTOR signaling.

Immobilization stress significantly reduced the level of Akt, GSK-3β, mTOR, and p70S6K phosphorylation (65%, 54%, 39%, and 56%, respectively) compared with levels in the control-sedentary group (each p < 0.01; Fig. 2A–2D). Exercise significantly prevented reduction in phosphorylation of these proteins (phosphorylated Akt, 109% of control, p < 0.01, Fig. 2A; phosphorylated GSK-3β, 74% of control, p < 0.01, Fig. 2B; phosphorylated mTOR, 94% of control, p < 0.01, Fig. 2C; and phosphorylated p70S6K, 132% of control, p < 0.01, Fig. 2D). Moreover, exercise alone significantly increased the levels of phosphorylated Akt, GSK-3β, mTOR, and p70S6K under the stress-free condition (phosphorylated Akt, 153% of control, p < 0.01; phosphorylated GSK-3β, 141% of control, p < 0.05; phosphorylated mTOR, 120% of control, p < 0.01; and phosphorylated p70S6K, 159% of control, p < 0.01).

3.3. Effect of treadmill exercise on synaptic protein levels

Synaptic protein synthesis is dependent on activation of mTOR signaling (Hoeffer and Klann, 2010). To determine whether treadmill exercise increased synaptic proteins levels, we examined the expression of synaptophysin, PSD-95, β-neurexin, and neuroligin 1 in the hippocampus (Fig. 3A–3D).

Immobilization stress produced a significant decrease in pre- and postsynaptic protein expression compared with the control-sedentary group (SYP, 44% of control, p < 0.01, Fig. 3A; PSD-95, 52% of control, p < 0.05, Fig. 3B; β-neurexin, 57% of control, p < 0.01, Fig. 3C; and NLG 1, 51% of control, p < 0.01, Fig. 3D). However, treadmill exercise significantly attenuated the decrease in protein expression caused by immobilization stress (SYP, 98% of control, p < 0.01, Fig. 3A; PSD-95, 124% of control, p < 0.01, Fig. 3B; β-neurexin, 112% of control, p < 0.01, Fig. 3C; and NLG 1, 118% of control, p < 0.01, Fig. 3D). Moreover, exercise significantly increased synaptic proteins levels in the absence of immobilization stress (SYP, 152% of control, p < 0.01, Fig. 3A; PSD-95, 153% of control, p < 0.05, Fig. 3B; β-neurexin, 133% of control, p < 0.01, Fig. 3C; and NLG 1, 134% of control, p < 0.01, Fig. 3D).

Fig. 2. Effect of treadmill exercise on the levels of phospho-Akt, phospho-glycogen synthase kinase-3β (GSK-3β), phospho-mammalian target of rapamycin (mTOR), and phospho-p70 ribosomal protein S6 kinase (p70S6K) in the rat hippocampus. Rats were subjected to running at a speed of 15 m/min, 30 min/day for 5 days with (+Stress, 2h daily) or without immobilization stress (–Stress). Bands and values are shown for hippocampi of control-sedentary (–Stress, Con), control-exercise (–Stress, Ex), stressed-sedentary (+Stress, Con), and stressed-exercise (+Stress, Ex) group. Cell lysates were analyzed using SDS-PAGE and Western blotting with anti-phospho-ser473-Akt (A), anti-phospho-serβ-GSK-3β (B), anti-phospho-ser445-mTOR (C), or anti-phospho-thr389-p70S6K (D) primary antibodies. A representative image and quantitative analysis normalized to the total Akt, GSK-3β, mTOR, or p70S6K band are shown. Values are expressed as a percentage of the control value (–Stress, Con) and represent mean ± SEM from five animals per group. *p < 0.05 vs. control sedentary, **p < 0.01 vs. control sedentary only.
### 4. Discussion

The main findings of the present study were that treadmill exercise for 5 consecutive days activated Akt, GSK-3β, and mTOR the major signaling intermediates of the PI3K pathway via TrkB/BDNF signaling; increased levels of the presynaptic proteins synaptophysin and β-neurexin; and increased expression of the postsynaptic protein PSD-95 and neurelin 1 in the hippocampus of rats subjected to immobilization stress. Moreover, exercise significantly increased the level of these proteins in the absence of immobilization stress.

Stress may cause dysregulation of the hypothalamic–pituitary–adrenal (HPA) axis, leading to excessive glucocorticoid levels, which are associated with depression (Nemeroff and Owens, 2002). The neurotrophic hypothesis of depression is based on the stress-induced down-regulation and antidepressant-induced up-regulation of BDNF expression in the hippocampus (Russo-Neustadt et al., 1999; Adlard and Cotman, 2004). Thus, we chose this model based on previous studies showing that repeated immobilization stress over 1 week caused a reduction in rat hippocampal BDNF expression and an elevation in corticosterone levels (Murakami et al., 2005; Nooshinfar et al., 2011) and that these stress-induced changes were prevented by chronic antidepressants (Nibuya et al., 1995; Ulloa et al., 2010). Our finding that immobilization stress for 1 week markedly decreased BDNF expression in the hippocampus is consistent with previous studies. Corticosterone may be involved in this reduction because exogenous treatment with corticosterone has been shown to reduce BDNF expression (Schaaf et al., 1998). Moreover, our stressed rats showed marked and significant decreases in Akt, GSK-3β, and mTOR phosphorylation and the expression of several synaptic proteins. A previous study by Kozlovsky et al. (2002) reported that GSK-3β phosphorylation did not affect the rat frontal cortex following acute (1 day), subchronic (6 days), or chronic cold immobilization stress (14 days). Our laboratory recently reported that restraint stress for 3 weeks decreased GSK-3β phosphorylation in the rat hippocampus. This discrepancy may be attributed to differences in the experimental paradigms or the brain areas examined.

To our knowledge, the present study is the first to report decreases in Akt and mTOR activity in the rat brain in response to immobilization stress. TrkB receptors activate the PI3K/Akt signaling pathway, leading to GSK-3β inhibition (Li et al., 2007) and mTOR activation (Chiang and Abraham, 2005). Thus, reduced phosphorylation of Akt, GSK-3β, and mTOR under stressful conditions suggests a potential role for BDNF in the regulation of PI3K signaling via trkB receptors in the rat hippocampus. Furthermore, the reduction in mTOR activity may be an important contributing factor to the decrease in the synaptic proteins SYP, PSD-95, β-neurexin, and neurelin 1. In the present study, expression of these synaptic proteins was shown to be down-regulated by immobilization stress. These reductions may be caused by down-regulation of mTOR in response to stress. Our finding of a decrease in the synaptic protein SYP is in agreement with the two previous studies that investigated the effects of immobilization stress on SYP levels in the rat hippocampus (Thome et al., 2001; Xu et al., 2004). Taken together, alterations in the expression of the proteins investigated in the present study may contribute to the molecular...
basis of stress-induced changes in hippocampal synaptic plasticity.

Several studies have suggested that voluntary exercise enhances hippocampal BDNF expression in animals (Adlard and Cotman, 2004; Chen and Russo-Neustadt, 2005; Ding et al., 2011). However, the effects of forced treadmill exercise on stress have not been previously investigated. A previous study reported that low-intensity (15 m/min), but not moderate-intensity (25 m/min), exercise had a beneficial effect in rats (Soya et al., 2007). The investigators reported that increases in mRNA expression of BDNF and c-fos, a marker for neuronal activation, were observed following low-intensity exercise, whereas moderate-intensity exercise elevated corticosterone levels and suppressed the induction of BDNF mRNA. The low-intensity treadmill exercise used in the present study significantly increased hippocampal BDNF levels and suppressed the effect of stress. The exercise-induced increase in BDNF may be a result of increased release of serotonin (5-HT) and/or noradrenaline (NE) (Samorajski et al., 1987; Dey et al., 1992). This explanation is plausible because the effects of exercise may be mediated through increased 5-HT/NE neurotransmission, which activates cAMP/protein kinase A (PKA) and the transcription factor cAMP response element binding protein (CREB) (Nibuya et al., 1995; Jensen et al., 2000). CREB activation increases the expression and secretion of BDNF, which acts via TrkB receptors. Alternatively, the G-protein coupled receptor (GPCR) directly transactivates TrkB receptors through Src tyrosine kinase activity (Lee et al., 2002). Thus, exercise may induce activation of TrkB receptors via Src kinase downstream of GPCR with no BDNF involvement.

Our results showed that treadmill exercise elevated the levels of molecules involved in BDNF/TrkB signaling, such as p-Akt, p-GSK-3β, and p-mTOR, in the hippocampus of rats subjected to immobilization stress. Moreover, we found that exercise elevated levels of these proteins in the absence of immobilization stress. Our study is the first to report a significant change in the level of these proteins in response to immobilization stress and treadmill exercise. The TrkB signaling downstream effectors, p-Akt, p-GSK-3β, and p-extracellular signal-regulated kinase (ERK), are activated in response to voluntary running (Chen and Russo-Neustadt, 2005; Ding et al., 2011; Makena et al., 2012). A recent study reported that voluntary exercise increased p-ERK in the hippocampus of non-maternal-separated rats, and this effect was not observed in maternal-separated rats (Makena et al., 2012). BDNF/TrkB signaling plays principal roles in promoting synaptic plasticity and neuroprotection, which is required for at least some of the known antidepressant actions (Patapoutian and Reichardt, 2001; Segal, 2003). Several studies have shown that in vivo inhibition of GSK-3β in the hippocampus produces antidepressive-like behavior (Gould et al., 2004; Kaidanovich-Beilin et al., 2004; Rosa et al., 2008) and that treatment with some antidepressant drugs increase GSK-3β phosphorylation (Li et al., 2004). Furthermore, inhibition of GSK-3β via Akt activation promotes neuroprotection because activation of GSK-3β facilitates apoptosis (Crowder and Freeman, 2000). Given the antidepressant action of GSK-3β and the neuroprotective action of GSK-3β, it is possible that treadmill exercise exerts antidepressant actions via inhibition of GSK-3β in the hippocampus of stressed rats. Particularly, mTOR activation has been shown to be required for the rapid antidepressant action of ketamine (Maeng et al., 2008; Li et al., 2010). Although 5-HT/NE levels rise rapidly after acute antidepressant treatment, several weeks (3–4 weeks) are required before therapeutic effects are achieved. Recent clinical studies have shown that a low dose of ketamine produces rapid (2 h) and sustained (up to 7 days) antidepressant effects in patients with depression (Berman et al., 2000; Diazgranados et al., 2010). We suggest that activation of mTOR during the treadmill exercise may be associated with the rapid antidepressant actions observed in stressed rats.

To our knowledge, the present study is the first to report a positive effect of treadmill exercise on SYP, PSD-95, NGL 1, and β-neurexin levels in the hippocampus of immobilized rats. Treadmill exercise has been found to have a beneficial effect on the expression of these proteins in the hippocampus of normal rats. Our findings are consistent with those of Ferreira et al. who reported an increase in SYP expression in normal rats after a short period (7 days) of treadmill exercise. Several studies have shown that voluntary exercise elevates SYP and PSD-95 levels in the hippocampus of rats (Lambert et al., 2005; Heschem et al., 2009; Hu et al., 2009). Experimental evidence suggests a positive association between BDNF and SYP (Pozzo-Miller et al., 1999; Vaynman et al., 2006). SYP levels are markedly decreased in the hippocampus of BDNF knockout mice (Pozzo-Miller et al., 1999), and blocking BDNF abrogated exercise-induced increases in SYP levels (Vaynman et al., 2006). Considerable evidence suggests that BDNF and PSD-95 may play a direct role in synaptogenesis by increasing the number and size of dendritic spines in hippocampal neurons (El-Husseini et al., 2000; Tyler and Pozzo-Miller, 2003); these studies suggest that BDNF/TrkB signaling may play an important role in the maturation of excitatory synapses through PSD-95. Recent observations have shown that the application of BDNF to cultured neurons promotes the growth of mature spines by regulating the formation of PSD-95 TrkB complexes (Yoshii and Constantine-Paton, 2010). More specifically, BDNF and PSD-95 have been implicated in N-Methyl-D-aspartate (NMDA) receptor-dependent long-term potentiation (Kovalchuk et al., 2002; Yoshii and Constantine-Paton, 2007). BDNF-null and PSD-95-mutant mice exhibited altered hippocampal long-term memory and impaired learning and memory (Migaud et al., 1998; Pozzo-Miller et al., 1999). No evidence is available concerning the effect of exercise on NGL 1 and β-neurexin levels in response to stress. Particularly, the levels of postsynaptic proteins such as PSD-95 and NGL 1 may regulate the balance between excitatory and inhibitory synapses that is essential for maintaining normal cognitive functioning (Levinson and El-Husseini, 2005). β-neurexin promotes differentiation of excitatory and inhibitory synapses via NGL function (Graf et al., 2004). In this context, treadmill exercise may facilitate synaptogenesis by up-regulating these proteins in the hippocampus. Thus, these effects may be one mechanism underlying the beneficial effect of exercise, which enhances brain function by improving learning, memory, and cognition.

A limitation of the present study was that the kinase activity of Akt, GSK-3β, and mTOR were not examined. Especially, although the phosphorylation of serine2448 of mTOR is often used as a marker of its activation, there are sometimes discrepancies (Mothe-Satney et al., 2004). In the present study, treadmill exercise increased phosphorylation of p70S6K that is induced by activation of mTOR. However, the Additional study including the kinase activity is needed to strengthen the findings of the present work.

The present study is the first to report that treadmill exercise activates PI3K/Akt signaling, which is impaired in the hippocampus of rats subjected to immobilization stress. Moreover, treadmill exercise significantly increased PI3K/Akt signaling in the absence of immobilization stress. Our results provide a clearer understanding of the molecular changes related to exercise-induced antidepressant effects. Furthermore, our findings highlight the importance of physical exercise for the treatment and prevention of depression.

Acknowledgments

This research was supported by Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education, Science and Technology (2011-0002551 to YH Kim and 2012-0003806 to SW Park).


