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Forced, moderate-intensity treadmill exercise suppresses apoptosis by increasing the level of NGF and stimulating phosphatidylinositol 3-kinase signaling in the hippocampus of induced aging rats

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ABSTRACT

While nerve growth factor (NGF) activates various signaling cascades, the phosphatidylinositol 3-kinase (PI3-K)/Akt pathway plays a pivotal role in controlling the survival of neurons, although this activity declines during the aging process. We investigated the effect of forced moderate-intensity treadmill exercise on the level of NGF and the PI3-K/Akt signaling pathway in the hippocampus of induced aging rats. Forty-five male Sprague–Dawley rats were divided into the following three groups: (1) control group, in which aging was not induced (CON: $n = 15$), (2) aging-control group, in which aging was induced but the rats were not subjected to exercise (ACON: $n = 15$), and (3) the aging-exercise group, in which aging was induced and the rats were subjected to treadmill exercise (AEX: $n = 15$). D-Galactose (50 mg/kg) was injected into the abdominal cavity for 8 weeks to induce aging. Rats were subjected to treadmill exercise 5 days a week for 8 weeks, and the speed of the treadmill was gradually increased. The protein levels of NGF, P-PI3-K, and P-Akt were significantly high in the AEX group ($p < 0.01$, $p < 0.01$, and $p < 0.001$, respectively). Tyrosine kinase A (Trk A) receptor level was significantly higher in the CON and AEX groups than in the ACON group ($p < 0.01$). TUNEL assay showed a significant reduction in apoptosis in the AEX group ($p < 0.001$). Caspase-3 activation was significantly decreased in the AEX and CON groups ($p < 0.05$). These results show that forced moderate-intensity treadmill exercise increases the level of NGF and activates P-PI3-K to induce P-Akt in order to suppress apoptotic cell death in the hippocampus of induced aging rats.

1. Introduction

Nerve growth factor (NGF) promotes neuronal growth and differentiation (Ahn, 2000). NGF moves by retrograde axonal transport toward the perikaryon after migrating toward the tyrosine kinase A (Trk A) receptor, a high affinity receptor, and P75, a low affinity receptor at the end of the neuronal axon, and this activity is similar to that of brain-derived neurotrophic factor (BDNF). Furthermore, along with BDNF, NGF protects neurons from oxidative damage by activating free radical scavengers (Cheng and Mattson, 1991, 1992; Nistico et al., 1992) as well as reducing levels of reactive oxygen species (ROS) (Mishra et al., 2007) and by playing a role in regenerating or protecting nerve tissues from aging or neuronal damage.

Meanwhile, in aging, several neurobiological changes have been reported in the brain (Porras et al., 1997; Segovia et al., 2001; Segovia and Mora, 2005). For instance, environmental enrichment decrease in this same response of the release of acetylcholine and decrease in the spontaneous motor activity in spite of the release of acetylcholine by a mild stress in the prefrontal cortex does not change in the aging rats (over 24 months) (Segovia et al., 2008). But then, because it is also involved in learning or memory capacity as well as the plasticity of the brain, the level of NGF has been shown to decrease under pathological conditions, such as aging or disease. Silhol et al. (2005) emphasized that the levels of neurotrophic factors and peptide change as people age, and these phenomena are observed outstandingly in the hippocampus. Ang et al. (2003) observed a remarkable deficiency of NGF in MCAO (middle cerebral artery occlusion) rats, a model of focal stroke, as well as in streptozotocin-induced diabetic rats and patients with diabetes (Faradji and Sotelo, 1990). However, external NGF injection was shown to have a positive effect on the microstructures of Schwann cells in rats with diabetic neuropathy (Ahn, 2000). NGF injection

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also influences the proliferation and survival of subcapsular epithelial cells in the rat thymus (Shin, 2003). Based on the findings of Shin, NGF is expected to play an important role in the survival and development of nerve cells and in suppressing apoptotic cell death.

Previous studies have also suggested that NGF is related to the suppression of apoptosis and neuron survival because of the relationship between the Trk A and p75 receptors (Casaccia-Bonnefil et al., 1998), activation of the phosphatidylinositol 3-kinase (PI3-K)/Akt signaling pathway (Shimoke et al., 2005), or activation of phosphorylated proline-rich Akt substrate (*p*PRAS), and MAPK (Gao et al., 2003). Based on the physiological role of NGF (Yamashita et al., 1999) and the relationship between the apoptotic factors (Shimoke et al., 2005), it is estimated that NGF receptors and the PI3-K signaling pathway would strongly influence cell survival, although this relationship was not clear until now. However, it has been clarified that amnesia associated with aging in the hippocampus is closely related to the suppression of neurotrophic factors such as NGF (Gould et al., 1999), and thus, it is likely that an increase in the level of NGF in the hippocampus may contribute to the improvement of memory capacity.

On the other hand, exercise is strongly recommended to prevent diseases and delay the aging process since it is known to promote functional recovery by stimulating the development of brain cells and to improve nervous disorders. It also works advantageously to generate NGF, which protects neuronal cells from cytotoxicity (Neeper, 1995; Gomez-Pinilla et al., 2001; Ang et al., 2003; Albeck et al., 2006; Arida et al., 2007). However, most studies showing the positive effects of exercise on the growth and protection of cells were focused on the results of spontaneous exercise through wheel running, while the results of forced treadmill exercise have not yet been clarified.

Thus, the aim of this study was to verify whether forced moderate-intensity treadmill exercise increases the level of NGF to suppress apoptosis and whether PI3-K signaling is related to the suppression of apoptosis, based on the findings of experiments in induced aging rats. We thus examined the effects of forced moderate-intensity treadmill exercise on the levels of NGF, Trk A receptor, the PI3-K and Akt proteins, and apoptosis by TUNEL assay and caspase-3 activity in the hippocampus of induced aging rats.

2. Materials and methods

2.1. Animals

Male Sprague–Dawley rats ($n = 45$; age, 6 weeks; weight, 200 ± 10 g) were adapted to the laboratory environment (temperature, 22 °C ± 1 °C; relative humidity, 55% ± 3%; 12-h light:12-h dark photoperiod) for 2 weeks. All rats were given free access to water and fed a standard chow diet (protein, 21%; fat, 5%; nitrogen-free extract, 55%; fiber, 4%; and adequate mineral and vitamin contents; Purina Mills Inc., Korea). The rats were housed in pairs. All procedures involving animals were approved by the Ethical Committee of Korea National Sport University and carried out in an ethical manner by following the guidelines provided.

2.2. Experimental procedure

Rats were allocated to the following groups: (1) control group (CON, $n = 15$), (2) aging-control group (ACON, $n = 15$), and (3) aging-exercise group (AEX, $n = 15$). D-Galactose (Sigma, St. Louis, MO, USA) was then injected into the abdominal cavity to induce aging. After being dissolved in distilled water, D-galactose was injected at a fixed time once daily at a dose of 50 mg per kg over the course of 8 weeks to induce aging (Song et al., 1999). Consistent injection of D-galactose is used to develop animal models of aging (Wei et al., 2005), as it accelerates aging by increasing oxidative stress in peripheral nerves and nerve cell death in the hippocampus and weakens recognition and memory functioning in the brain (Cui et al., 2006). Meanwhile, the rats in the AEX group were subjected to moderate-intensity treadmill exercise for 8 weeks on a regular basis of 5 days a week. The speed and duration of the treadmill exercise were gradually increased from about 10–12 m/min for 10 min (grade 0%) in the 1st week to 10–12 m/min for 20 min (grade 0%) in the 2nd week, 18–20 m/min for 20 min (grade 0%) in the 3rd week, 18–20 m/min for 30 min (grade 0%) in the 4th week, and 18–20 m/min for 50 min (grade 0%) in the 5th week.

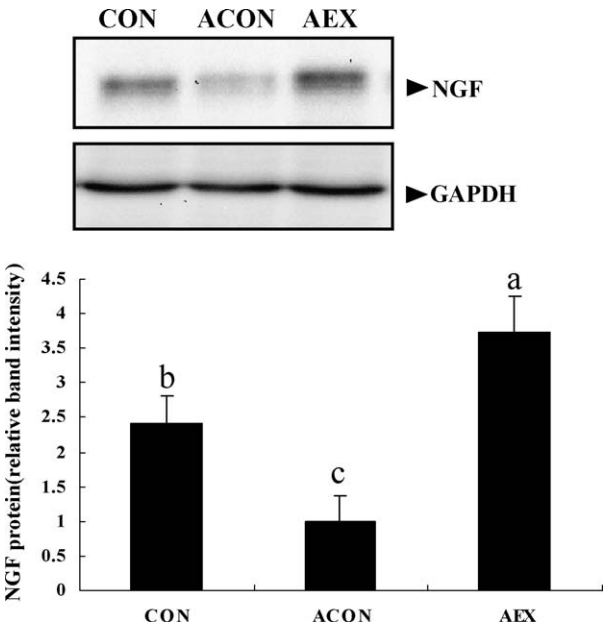

Fig. 1. NGF protein level in the hippocampus of aging rats after moderate-intensity treadmill exercise. CON: control group; $n = 15$, ACON: aging-control group; $n = 15$, AEX: aging-exercise group; $n = 15$. Significantly different among groups at $p < 0.01$. Different letters represent significant variations calculated by one-way analysis of variance (ANOVA) and Tukey's test.

2.3. Tissue preparation

Upon completion of the 8-week exercise program, the rats were anesthetized for 72 h after the final exercise session by intraperitoneal (i.p.) injection of xylazine (8 mg/kg) and ketamine (40 mg/kg). For the TUNEL assay, 5 of 15 rats from each group was selected, transcardially perfused with 50 mM phosphate-buffered saline (PBS), and fixed with a freshly prepared solution consisting of 4% paraformaldehyde in 100 mM phosphate buffer (PB, pH 7.4). The brains were dissected and postfixed in the same fixative overnight and transferred into a 30% sucrose solution for cryoprotection. Coronal sections of 40 μm thickness were made with a freezing microtome (Leica, Nussloch, Germany). For the protein levels and caspase-3 activity analysis, brains of 10 rats from each group were quickly excised, and the hippocampus was dissected and stored at −70 °C.

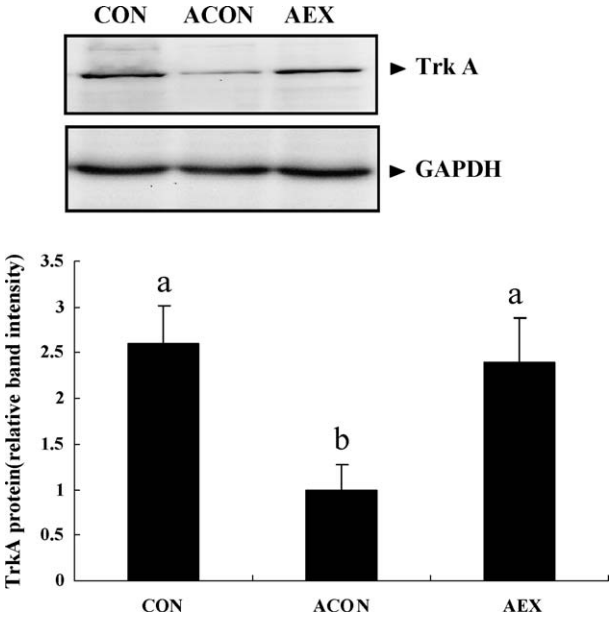

Fig. 2. Trk A receptor protein level in the hippocampus of aging rats after moderate-intensity treadmill exercise. CON: control group; $n = 15$, ACON: aging-control group; $n = 15$, AEX: aging-exercise group; $n = 15$. Significantly different among groups at $p < 0.01$. Different letters represent significant variations calculated by one-way analysis of variance (ANOVA) and Tukey's test.