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Highlights

▶ Long term physical exercise reduced the levels of ROS and protein carbonyls in the hippocampus of aging rats. ▶ Levels of antioxidant enzymes /Gpx and SOD-1/ were increased by exercise. ▶ AMPK and PGC-1α are important in mediating the beneficial effects of exercise in the hippocampus.

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LONG-TERM EXERCISE TREATMENT REDUCES OXIDATIVE STRESS IN THE HIPPOCAMPUS OF AGING RATS

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Abstract—Exercise can exert beneficial effects on cognitive functions of older subjects and it can also play an important role in the prevention of neurodegenerative diseases. At the same time it is perceivable that limited information is available on the nature of molecular pathways supporting the antioxidant effects of exercise in the brain. In this study 12-month old, middle-aged female Wistar rats were subjected to daily moderate intensity exercise on a rodent treadmill for a period of 15 weeks which covered the early aging period unmasking already some aging-related molecular disturbances. The levels of reactive oxygen species (ROS), the amount of protein carbonyls, the levels of antioxidant intracellular enzymes superoxide dismutases (SOD-1, SOD-2) and glutathione peroxidase (GPx) were determined in the hippocampus. In addition, to identify the molecular pathways that may be involved in ROS metabolism and mitochondrial biogenesis, the activation of 5′-AMP-activated protein kinase (AMPK), the protein level of peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC-1α), nuclear respiratory factor 1 (NRF-1) and mitochondrial transcription factor A (mtTFA) were measured. Our results revealed a lower level of ROS associated with a reduced amount of protein carbonyls in the hippocampus of physically trained rats compared to sedentary controls. Furthermore, exercise induced an up-regulation of SOD-1 and GPx enzymes, p-AMPK and PGC-1α, that can be related to an improved redox balance in the hippocampus. These results suggest that long-term physical exercise can comprises antioxidant properties and by this way protect neurons against oxidative stress at the early stage of aging.

Key words: exercise, hippocampus, aging, ROS, SOD, GPx, AMPK, PGC-1α.

INTRODUCTION

Reactive oxygen species (ROS) are the products of cellular aerobic metabolism. When ROS are presented in physiological concentrations they play an important role in the modulation of gene expression and signal transduction pathways (Sen and Packer, 1996; Finkel, 2003; Esposito et al., 2004). However, when ROS are produced in excess for a considerable time, they can attack cellular macromolecules like proteins, membrane lipids and DNA. Extensive damage to these biomolecules in the brain can cause neuronal dysfunction and trigger apoptosis (Emerit et al., 2004).

A number of studies have demonstrated that increased levels of ROS are involved in the aging process (Gerschman et al., 1954; Harman, 1956; Droge, 2003) and contribute to pathological changes in neurodegenerative disorders (Floyd and Hensley, 2002). The major targets of ROS are the amino acid residues of proteins. The oxidation of lysine, arginine, proline and threonine residues results in carbonyl derivatives that are used as markers of oxidative stress on proteins (Levine and Stadtman, 2001). ROS-induced carbonylation leads to dysfunctional proteins and enzymes with reduced catalytic activity. Enhanced levels of protein carbonyls have been shown to impair cognitive processes and correlate with the progression of several neuronal pathologies (Stadtman, 2001). The accumulation of carbonyl derivatives have been dramatically raised in vulnerable neurons in Alzheimer disease (Aksenov et al., 2001), and Parkinson disease (Offen et al., 1999).

The hippocampus is highly vulnerable to oxidative damage during aging due to the reduced capacity of neurons to maintain redox homeostasis (Serrano and Klann, 2004). Since the hippocampus is involved in certain forms of learning and memory consolidation (Douglas, 1967; Meissner, 1967; Morris, 2006) oxidative damage to this brain area can cause impairment in cognitive functions (Serrano and Klann, 2004). The maintenance of a normal redox state in hippocampal neurons therefore, is important in the prevention of cognitive decline during aging.

The antioxidant defense at first line is constituted by the antioxidant enzymatic actions. Superoxide radicals are converted to hydrogen peroxide by superoxide dismutase (SOD), and the hydrogen peroxide is eliminated by glutathione peroxidase (GPx) and/or catalase. Catalase activity has been found to be low in the brain (Gaunt and de Duve, 1976), the enzyme GPx,

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Abbreviations: AMPK, 5′-AMP-activated protein kinase; CaMKK, calmodulin-dependent kinase; Gpx, GSH peroxidase; mtTFA, mitochondrial transcription factor A; NRF-1, nuclear respiratory factor-1; PGC-1α, peroxisome proliferator-activated receptor gamma coactivator 1-alpha; ROS, reactive oxygen species; RT, room temperature; SOD, superoxide dismutase; TBS-T, Tris-buffered saline Tween-20.

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therefore, is primarily responsible for destruction of excess hydrogen peroxide formed in the nervous tissue (Sinet et al., 1980). The activity of antioxidant enzymes in the brain is modulated by various factors including aging (Venkateshappa et al., 2012) and physical activity (Radak et al., 2001).

Numerous studies indicate that exercise can reduce the risk of oxidative stress-related diseases and play an important role against age-associated cognitive decline (Bergersen and Storm-Mathisen, 2006; Ferrari, 2007; Hollmann et al., 2007; Shin, 2009). It has been proposed that regular physical training induces an adaptation process in ROS-detoxifying systems, resulting in increased resistance of cells to oxidative challenges (Radak et al., 1999a). Most of the beneficial effects of exercise on aged rodents (Devi and Kiran, 2004) and elderlies (Asha Devi, 2009) were related to long-term exercise of moderate intensity, while the acute training was proved to induce oxidative insults in the nervous system (Aydin et al., 2009). The adaptive response to long-term exercise is rather complex and not fully elucidated; it may involve the modulation of redox-sensitive transcriptional factors (Toldy et al., 2005). Peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC-1α) may play an important role in the control of ROS metabolism, since it regulates the expression of ROS-detoxifying enzymes (Valle et al., 2005). In addition, PGC-1α interacts with a broad range of transcriptional factors involved in the regulation of mitochondrial electron transport activity and mitochondrial biogenesis (Ventura-Clapier et al., 2008). PGC-1α has been reported to induce the transcription of nuclear respiratory factor 1 (NRF-1) leading to the increased expression of mitochondrial transcription factor A (mtTFA), a key regulator of mitochondrial DNA replication (Ventura-Clapier et al., 2008).

In the skeletal muscle, phosphorylation of PGC-1α is mediated by AMPK-activated protein kinase (AMPK) (Jager et al., 2007), a molecule emerging as a central regulator of energy balance principally as fuel sensor. The exercise-induced activation of AMPK in skeletal muscle has been confirmed by many studies (Lee-Young et al., 2009; Palacios et al., 2009; O'Neill et al., 2011). Nonetheless, the effects of physical activity on AMPK, PGC-1α and the related molecular processes in the hippocampus are not yet completely elucidated.

The purpose of the present study was to investigate how long-term moderate intensity physical exercise influences the oxidative status in the hippocampus at the early stage of aging, when the neuronal functions have been acknowledged to decline slowly but gradually. To study the impacts of forced physical activity on the hippocampal redox state 12-month-old female rats were subjected to long-term exercise intervention in our experiment. It is known that the normal cyclic female rats show a gradual decrease in serum estradiol level starting at the age of 12 months (Lapolt et al., 1988; Moorthy et al., 2005b). Thus 12 months can be considered as the starting period of "menopause" and the consequent months as the early stage of "postmenopausal" period by attempting to draw reference to human condition. Estrogens, otherwise, can regulate the expression and activity of antioxidant enzymes itself (Moorthy et al., 2005a) and affect mitochondrial functions (Klinge, 2008). Whatever is the initial deteriorating factor during early aging on the redox balance physical activity could be a significant non-pharmacological tool to attenuate the dysregulation of antioxidant system as it has been proposed in the present study.

EXPERIMENTAL PROCEDURES

Animals and treatments

Middle-aged (12 months old) female Wistar rats were selected for the study. Animals were housed in a room maintained 22 ± 1 °C with a 12:12-h light/dark cycle starting the light period at 7:00. Food and tap water were available *ad libitum*. The animals were divided into two experimental groups: one group served as a sedentary control group (n = 6), while the other group was subjected to exercise treatment (n = 6). The exercise protocol included moderate intensity running on a rodent treadmill. Exercise trained rats were first introduced to treadmill running for 2 days on a 0% incline with 10 and 15 m/min, respectively. Afterwards for the next 4 days the running speed of the daily exercise sessions were gradually increased to 60% of the animals' VO2 max assayed earlier in this particular age group. From the second week of the training program, the animals ran daily at 18 m/min, on a 0% incline, for 30 min.

Twenty-four hours after the last exercise treatment session the animals were sacrificed by decapitation under light CO_2 anesthesia and the brains were quickly removed. The two hemispheres were rapidly separated along the midline on an ice-cooled glass plate. The hippocampus was quickly excised bilaterally and immediately frozen on dry ice. The hippocampal samples were stored at −80 °C until processing. All experimental procedures which were carried out on the animals had been approved by the Animal Examination Ethics Council of the Animal Protection Advisory Board at Semmelweis University, Budapest.

Western blots

The hippocampi of each animal were homogenized in lysis buffer containing 137 mM NaCl, 20 mM Tris–HCl pH 8.0, 2% Nonidet P-40, 10% glycerol and protease inhibitors. The homogenate was sonicated for 30 s in a cold pack. Lysates were centrifuged for 15 min at 15,300g at 4 °C and the supernatants were collected and stored at −20 °C until use. The concentration of protein was determined using the Bradford assay (Bradford and Williams, 1976). Twenty micrograms of protein were electrophoresed on 8–12% (v/v) polyacrylamide SDS–PAGE gels. Proteins were electro transferred onto PVDF membranes (Amersham, Piscataway, NJ, USA). The nonspecific binding of immunoproteins was blocked with 5% non-fat dry powdered milk dissolved in Tris-buffered saline Tween-20 (TBS-T) for 2 h at room temperature (RT). After blocking, the membranes were incubated with primary antibodies overnight at 4 °C. Antibodies were dissolved in TBS-T containing 5% non-fat powdered milk. The primary antibodies were: AMPK: 1:2000, #2532 Cell Signaling; p-AMPK: 1:1000, #2531 Cell Signaling; PGC-1α: 1:1000, #sc13067 Santa Cruz; NRF-1: 1:1000, #sc33771 Santa Cruz; mtTFA: 1:750, #sc30963 Santa Cruz; SOD-1: 1:1000, #AV45752 Sigma–Aldrich; GPx-1 (ISO1): 1:1000, #7283P1 Sigma–Aldrich; SOD-2: 1:1000, #SAB1406465 Sigma–Aldrich). The membranes were rinsed in TBS-T followed by 1-h

incubation with HRP-conjugated secondary antibody at RT. After incubation the membranes were repeatedly washed in TBS-T and incubated with an enhanced chemiluminescence reagent (ECL plus, RPN 2132, Amersham). The protein bands were visualized on X-ray films. The bands were quantified by Image J software, and standardized to β-actin (1:2000, #sc-47778 Santa Cruz). With this software the optical density of the protein bands was measured. Results were expressed in relative density units. The phosphorylation of the AMPK was evaluated by dividing the phospho-specific form by the dephospho form of AMPK.

Detection of reactive oxygen species (ROS)

The overall ROS was determined by using modifications of the dichlorodihydrofluorescein diacetate (H_2DCF-DA) staining method (Kim et al., 2000). This assay approximates levels of reactive species, such as superoxide radical, hydroxyl radical, and hydrogen peroxide. H_2DCF-DA (2′,7′-dichlorodihydrofluorescein diacetate, #D-399 Invitrogen) was dissolved at a concentration of 12.5 mM in ethanol before use. For fluorescence reactions, 96-well black microplates were loaded with phosphate buffer (pH 7.4) to a final concentration of 152 μM/well. Then 8 μl diluted freshly prepared hippocampus homogenate and 40 μl of 125 μM dye were added to achieve a final dye concentration of 25 μM. The changes in the fluorescent signal of the oxidized H_2DCF-DA were recorded at three time points (0, 1 and 30 min), using a micro plate fluorescence reader (excitation/emission wavelengths of 485–538 nm, Fluoroskan Ascent FL). The fluorescence intensity unit was normalized with the protein content and expressed in relative unit production per minute.

Detection of protein carbonyls

The levels of oxidized proteins were determined using an Oxyblot kit (S7150, Chemicon/Millipore, Temecula, CA, USA). Proteins were derivatized with 4-dinitrophenylhydrazine (DNPH) for 15 min followed by incubation at room temperature with a neutralization buffer. Derivatized proteins were electrophoresed on a 10% SDS–PAGE and blotted on PVDF membranes. Blots were blocked with 5% non-fat dry milk in Dulbecco's PBS containing 0.05% Tween 20 (PBS-T) for 3 h at 4 °C followed by incubation with anti-DNP primary antibody (1:150, #S7150 Chemicon/Millipore) overnight at 4 °C. After three washes with PBS-T, membranes were incubated for 1 h at room temperature with HRP-secondary antibodies (1:300, #S7150 Chemicon/Millipore). Immunocomplexes were visualized using ECL plus reagent. The bands were quantified by Image J software, and standardized to β-actin (1:2000, #sc-47778 Santa Cruz). Results on the figures were expressed in density units.

Statistical analyses

The results from experimental groups were compared by paired *t*-test. Statistical significance was set at $p < 0.05$. Means and standard errors of means (SEM) were presented to demonstrate the results. All statistical analyses were done applying the Statistica 8.8 program.

RESULTS

Physical exercise decreased the level of ROS by 28% in the hippocampus measured at the age of 15 months after the 15-week-long training period ($t = 3.472$, $p < 0.01$) as it is shown in Fig. 1. In addition, Fig. 2 shows that at the same time the amount of protein carbonyls in the hippocampus decreased in the trained group compared to sedentary controls ($t = 3.179$, $p < 0.01$). In this figure (Fig. 2) the carbonylated bands are also shown. Exercise decreased the densities in most of the proteins visualized.

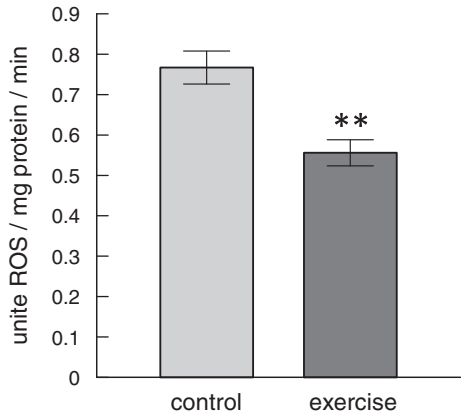

Fig. 1. Reduction of reactive oxygen species (ROS) in the hippocampus by physical exercise as compared to the control (sedentary) group is shown (**$p < 0.01$ vs. control). Columns represent means ± SEMs for six animals per group.

In order to evaluate the protein levels of antioxidant enzymes in the hippocampi of 15–month-old rats, the immunoreactivity of SOD-1, SOD-2 and GPx were measured by Western blot (Figs. 3 and 4). Exercise was effective in up-regulating the expression of SOD-1 in the hippocampus of aging animals ($t = 2.330$, $p < 0.05$; Fig. 3, left side panel; 52% increase). Fig. 3 also presents that there was a trend toward an increased expression of SOD-2 in the trained group ($t = 1.897$, $p = 0.087$; 25% increment). Student *t*-test revealed a significant increment in the GPx protein levels (Fig. 4) in the physically trained group compared to the control group ($t = 2.374$, $p < 0.05$; the increment after exercise was 34%). Upper parts of the figures show the densities of immunoreactive Western blot spots of all animals investigated and the calculated densities were normalized to β-actin and used for statistical processing which are represented by the columns and SEMs.

The phosphorylation of the AMPK was evaluated by dividing its phospho-specific form by its dephospho-form and was normalized to β-actin (Fig. 5). The phosphorylation of AMPK molecules was increased significantly by the exercise treatment ($t = 3.285$, $p < 0.01$; the increment compared to controls was 40%).

PGC-1α, NRF-1 and mtTFA are transcriptional factors involved in the regulation of cell metabolisms and mitochondrial functions. Western blot analysis showed that exercise enhanced the protein level of PGC-1α compared to the control group in the hippocampus ($t = 3.523$, $p < 0.01$; Fig. 6). The increment was 55% which may be considered as a marked enhancement. Fig. 7 shows that the protein levels of NRF-1 is tended to be increased by exercise, however the increment just approached significance level ($t = 2.182$, $p = 0.054$; left side of the figure). Physical activity did not influence the level of mtTFA expression (Fig. 7, right side) shown by Student *t*-test.

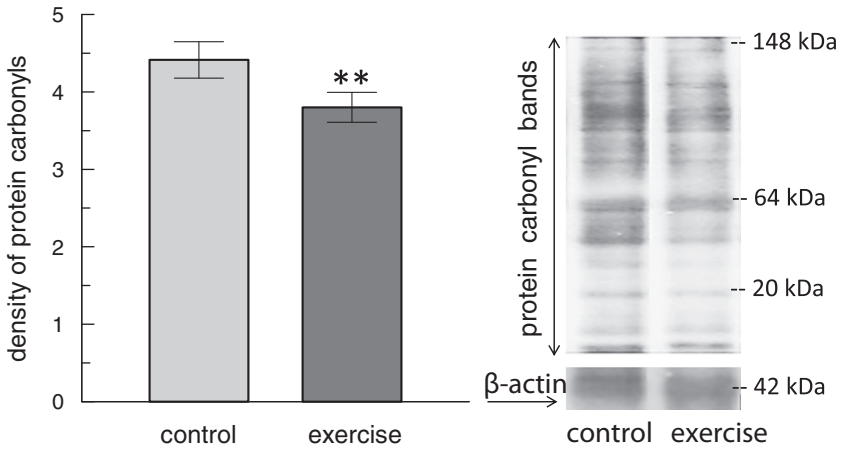

Fig. 2. Protein carbonyl content in the hippocampus decreased by long-term exercise compared to the sedentary control group ($^{*}p < 0.05$ vs. control). Columns represent means ± SEMs for six animals per group. The representative Western blots show the immunoreactivities of protein carbonyls and β-actin (used as a loading control) at the right side of figure.

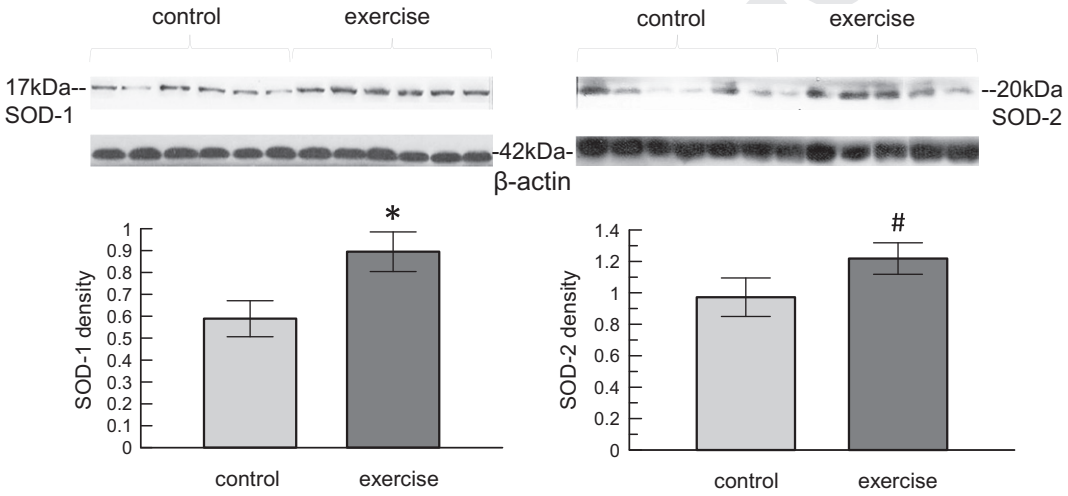

Fig. 3. Intracellular SOD-1 protein levels (left side) and mitochondrial SOD-2 protein content (right side) are shown in the hippocampus of 15-month-old aging female rats. SOD-1 protein levels are increased as a result of exercise ($^{**}p < 0.01$ vs. control). The right side histogram represents that SOD-2 protein levels tended to be increased in exercised rats compared to the sedentary control group ($^{\#}p = 0.087$ vs. control). Columns represent means ± SEMs, $n = 6$. The representative western blots taken from each individual rats show the immunoreactivities of SOD-1 and SOD-2 (upper lines) and below that of β-actin (used as a loading control).

DISCUSSION

The aim of present study was to examine the effects of long-term exercise on the hippocampal oxidative state and the related molecular processes in the course of early brain aging period in female rats. It was found that the long-term physical exercise resulted in reduced levels of reactive oxygen radicals (ROS) in the hippocampus of physically trained group, measured 24 h after the last exercise session under resting state. Due to the lower levels of free radicals the oxidative damage to proteins was significantly reduced in the trained rats. An important finding of this study is that exercise resulted in elevated levels of intracellular antioxidant enzymes in the early phase of aging. The activation of AMPK and the expression of PGC-1α were also promoted by exercise, which can serve for possible molecular pathway regulating the antioxidant gene transcription in the hippocampus.

In this study, the aged animals were subjected to long-term, moderate intensity exercise paradigm, which was effective in reducing the free radical levels in the hippocampus. Previous studies confirmed the role of training intensities in the modulation of redox state in different tissues. A single bout of exercise and exhaustive training have been reported to increase the ROS content and oxidative damage in the muscle and liver (Radak et al., 1999b; Nakamoto et al., 2007) assayed after a 20-h resting state. In contrast, long term training on moderate intensity has been found to reduce oxidative stress markers in the rat brain (Ogonovszky et al., 2005) measured 24 h after the last exercise session, which is in agreement with our observations. As a consequence of reduced ROS levels, lower

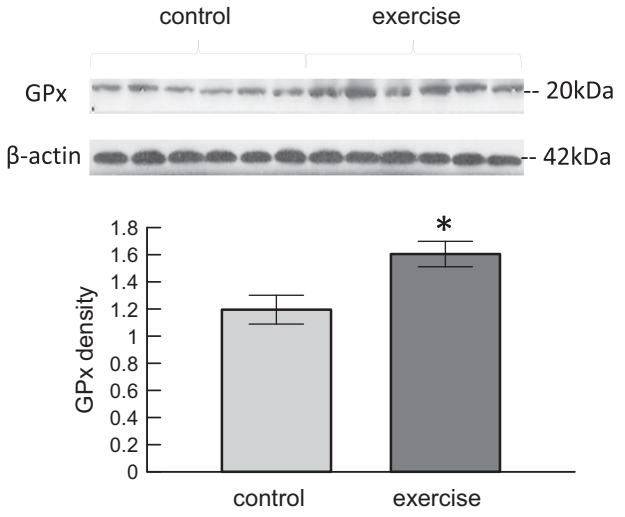

Fig. 4. Physical exercise was effective to increase the levels of GPx protein levels in the hippocampus shown by the columns below (*$p < 0.05$ vs. control, means ± SEMs, $n = 6$). The representative Western blots show the immunoreactivities of GPx and β-actin in all individual rats.

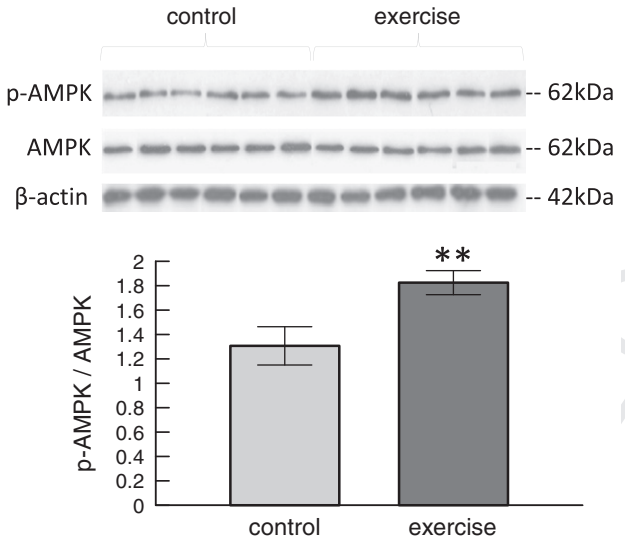

Fig. 5. The activation of AMPK is enhanced by exercise in the hippocampus. The histogram represents that the phosphorylation of AMPK is increased in the physically trained group (exercise) compared to the sedentary control group (**$p < 0.01$ vs. control). The phosphorylation of the AMPK was evaluated by dividing the phospho-specific form by the dephospho-form. Columns represent means ± SEMs for six animals per group. The representative Western blots show the immunoreactivities of p-AMPK, AMPK and β-actin in all animals.

amount of protein carbonyls was detected in the trained animals. Similarly, Radak and co-workers (2001) also found decreased protein carbonyl levels in the brain of 14-month-old male rats exposed to a long-term swimming regime. Together with previous studies our data show that regular exercise can exert beneficial impacts on redox state suggesting that long-term training on moderate intensity could increase the ability of neurons to cope with oxidative stress during the aging process in females as well.

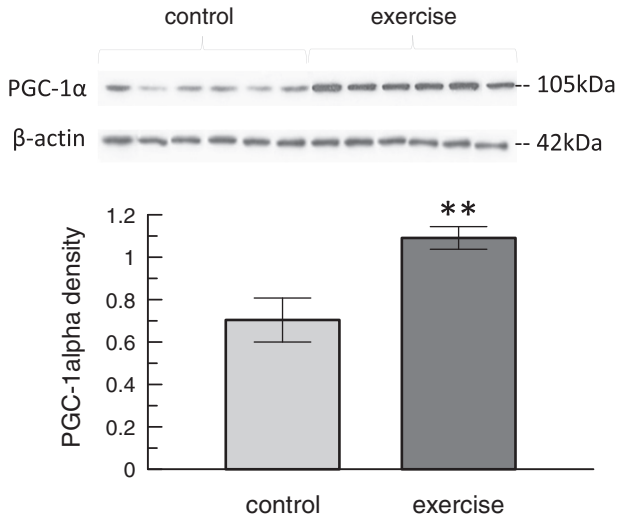

Fig. 6. Exercise up-regulated the PGC-1α protein levels in the hippocampus compared to the control group (**$p < 0.01$). Columns represent means ± SEMs for six animals per group. The representative western blots of PGC-1α and β-actin are shown above.

The beneficial effects of exercise on the oxidative status could be related to the enzymatic adaptation processes. GPx and two types of SOD were assayed, namely copper–zinc SOD (SOD-1) located in the cytosol and mitochondrial manganese SOD (SOD-2). The protein levels of SOD-1 and GPx have been elevated in response to long-term exercise intervention in this study and the level of SOD-2 was only slightly increased. Our results are in agreement with Um and coworkers' data (2011) demonstrating significant increment in SOD-1 Q4 expression after a 3-month-long training period.

In the present experiment, long-term exercise enhanced the protein levels of PGC-1α in the hippocampus. Recently, Steiner et al. (2011) also demonstrated an up-regulation of PGC-1α mRNA expression in specific brain regions of male rodents after 8 weeks of training. PGC-1α could be an important modulator of redox balance in neuronal cells, because it is required for the induction of the gene expression of several antioxidant enzymes including SOD-1, SOD-2 and GPx (St-Pierre et al., 2006). Furthermore, PGC-1α can rescue neurons from oxidative-stress-mediated cell death and plays a role in the prevention of neurodegenerative processes (St-Pierre et al., 2006). The induced expression of PGC-1α thus could serve an explanation for the elevated levels of SOD-1 and GPx found in the hippocampi of trained animals.

Besides its regulatory role on intracellular antioxidant gene transcription, PGC-1α can also influence the mitochondrial functions through increasing the transcriptional activity of NRF-1 resulting in a downstream activation of mtTFA. In the present study no significant increment in the protein levels of NRF-1 and mtTFA were observed, although NRF-1 expression exposed a near significant elevation. Koltai et al. (2012) also showed that exercise increased PGC-1α expression, but the NRF-1 and mtTFA protein remained to be unchanged in the muscle of aged rats. To date, no

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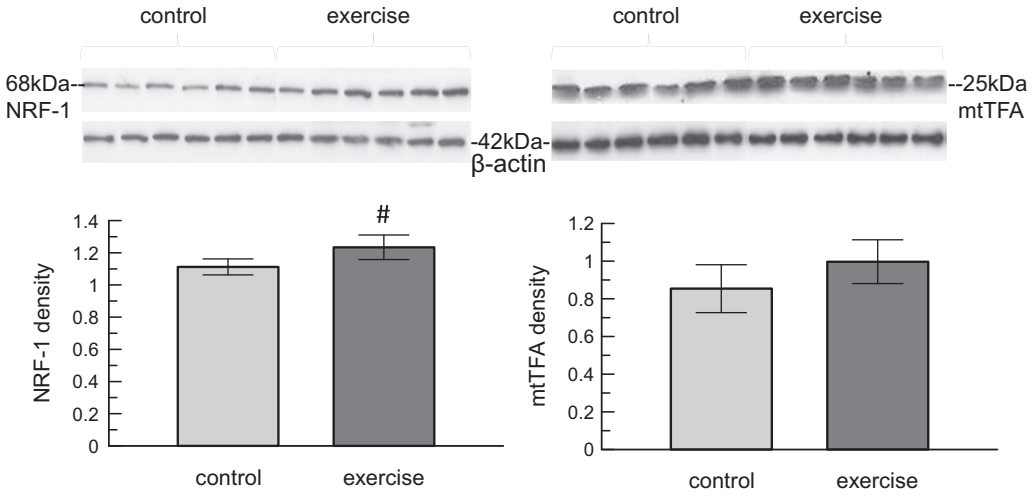

Fig. 7. Elevated (close to significant, $^{\#}p = 0.054$ vs. control) NRF-1 protein level in response to physical exercise is shown in the left panel. The mtTFA protein levels remained unchanged in the hippocampus following exercise (right panel). Means ± SEMs are shown. The representative Western blots show the immunoreactivities of both transcription factors.

studies have examined the effects of physical activity on NRF-1, and mtTFA in the hippocampus. These molecules can share the common components of mitochondrial biogenesis and respiration, however it may be added that the regulation of mitochondrial protein formation might involve multiple, probably cooperative regulatory mechanisms in tissue- and in a cell-specific manner. Therefore, the exact individual contribution of each of the factors supporting mitochondrial functions and mitochondrial biogenesis is rather difficult to dissect (Lopez-Lluch et al., 2008). Moreover deacetylases, such as SIRT-1 exert the posttranslational modification of PGC-1α promoter, which can affect its regulatory role on NRF-1 and mtTFA transcription (Canto and Auwerx, 2009). The complexity of this regulation is further demonstrated by the exercise-intensity-dependent regulation of PGC-1α mRNA induction in the skeletal muscle (Egan et al., 2010). Further research is needed to establish the exercise-mediated molecular pathways involved in mitochondrial functions in cell specific manner.

Our results demonstrated that p-AMPK levels have been increased in the hippocampus following the 15-week-long physical training. The activation of PGC-1α may be linked to the upstream activation of AMPK in the brain. Yu and co-workers (2010) found that the pharmacological stimulation of AMPK promoted the transcriptional activation of PGC-1α in visual cortical neurons. The exercise-induced phosphorylation of AMPK has been well established in skeletal muscle (Canto et al., 2010). The regulation of AMPK activity by physical activity and its effect on hippocampal plasticity and metabolism has been proposed by Gomez-Pinilla et al. (2008). Recently, Bayod et al. (2011) also showed enhanced p-AMPK levels after 36 weeks of physical training in different brain regions including the hippocampus. Based on these observations, it is likely that the activation of AMPK could be a key molecular target of exercise in the hippocampus as well.

The major impact on AMPK signaling is the relationship between neuronal activity and energy demand (Yu and Yang, 2010). Calmodulin-dependent kinases (CaMKKs) have also been found to serve as upstream regulators of AMPK signaling in neurons (Hawley et al., 2005). Physical training elicits neuronal activation and modulates CaMKK (Hescham et al., 2009; Nishijima et al., 2012) which could underlay the stimulation of AMPK by physical activity in the hippocampus. On the other hand, moderate hypoxia and oxidative agents have been also shown to trigger CaMKK and AMPK (Mungai et al., 2011). It is possible that the exercise generates modest amount of ROS, which actually results in the activation of CaMKK/AMPK/PGC-1α pathway and the subsequent activation of antioxidant enzymes. Thus exercise can increase the resistance toward further oxidative insults in long-term. As a consequence of adaptation, the vulnerability of the hippocampus to oxidative stress and diseases could be attenuated by physically active lifestyle.

CONCLUSIONS

In summary, the data from this study demonstrate that long term physical exercise results in lower levels of ROS and protein carbonyls as well as elevated levels of antioxidant enzymes in the hippocampus of aging rats. Physical training, thus, can be an effective option to regulate the oxidative balance and thus delay the onset of oxidative stress-related neurodegenerative processes. This study also provides further evidence that AMPK and PGC-1α could be important molecules in mediating the beneficial effects of exercise in the hippocampus.

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