The effects of aerobic training on malondialdehyde level of brain premotor cortex of young male rats following an acute bout of exhaustive endurance exercise

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Abstract

Introduction: Physical exercise is a widely accepted behavioral strategy to enhance overall health, including mental function. However, there is controversial evidence showing brain mitochondrial dysfunction, oxidative damage after high-intensity exercise, which presumably worsens cognitive performance.

Purpose: The aim of this study was to investigate whether 8-week treadmill training could modulate exercise-induced oxidative stress in the brain premotor cortex of rats following an acute bout of exhaustive endurance exercise.

Methods: For this reason the study was carried out with 12 week-old male rats (N =32) were randomly divided into two groups (N=16): non-runners control (SED), running exercise (ET). The exercise schedule consisted of progressive treadmill running for 5 days week over 8 weeks. To see the effects of endurance training on acute exhaustive exercise induced oxidative stress, (SED) and (ET) rats were further divided into two groups: animals killed at rest and those killed after an acute bout of exhaustive endurance exercise, in which the rats run at 30 m/min (10% uphill) until exhaustion.

Results: After a single bout of exhaustive treadmill running, increased significantly the lipid peroxidation level of brain premotor cortex in (SED) and (ET) rats (p<0.05).

Conclusion: As a result, it is concluded that the performed 8 weeks exercise could not prevented the increased significantly the lipid peroxidation level of brain premotor cortex response to acute bout of exhaustive exercise. These results indicate that intense exercise can have some deleterious effect on brain premotor cortex.

Keywords: acute exercise, oxidative stress, brain premotor cortex

INTRODUCTION

There is mounting body of evidence, which suggests that regular exercise improves brain function and causes structural, biochemical and physiological adaptation via different pathways (Radak et al., 2013). However, this phenomenon might be also interpreted in different way: exercise attenuates the inactivity-caused deteriorative effects on the CNS. Either interpretation could be correct, as it appears that ROS, and the changes in redox homeostasis could play a role in the very complex mechanism by which exercise training benefits the brain (Radak et al., 2013). However, these exercise-induced outcomes are dependent on the parameters of physical training, such as intensity and duration of physical training (low- to moderate-intensity programs) (Radak et al., 2001). These exercise parameters modulate the formation of mitochondrial reactive oxygen species (ROS) as a consequence of the increased oxygen intake by the tissues (Aguiar et al., 2008) leading to oxidative signaling optimization in several tissues and regulating cell signaling pathways and gene expression. However, depending on concentration, location and context, ROS can be either “friends” or “foes” regarding overall brain functions (Aguiar et al., 2010). Therefore, the complex neurobiolgy of exercise generally demonstrates U-shaped dose−response curves, where low doses are stimulatory and high doses inhibitory (Hu et al., 2009). Regular exercise (low- to moderate-intensity programs) causes an adaptation of the cellular antioxidant system, i.e., some papers demonstrate a significant increase in antioxidant enzymes activities, what increases resistance against oxidative stress and therefore reduces cellular oxidative damage and malondialdehyde level in the brain (Liu et al., 2000; Rybak et al., 1995). On the other hand, high-intensity exercise, direct evidence of increased exercise-induced ROS production is still scarce but it is supported by oxidative stress imbalance in several tissues after exercise (Aguiar et al., 2008; Ji, 1999). The brain has a large potential oxidative capacity and high oxygen consumption (Calabrese
The brain antioxidant capacity is limited by a high content of easily oxidizable fatty acids (Ozakaya et al., 2008) and free iron and low levels of antioxidants enzymes and substrates (Calabrese et al., 2005) Brain oxygen consumption (Ise and Secher, 2000) and ROS production (Aguiar et al., 2010) increase during exercise. Under extreme conditions, such as in high-intensity physical exercise, ROS production may be more strongly and persistently increased, and the antioxidant response may not be sufficient to reset the system to the original level of brain redox homeostasis (Aguiar et al., 2010; Rosa et al., 2007). Free radicals generate a cascade producing lipid peroxidation. Lipid peroxidation is one of the main events induced by oxidative stress. Lipid peroxidation can produce a range of enzymatically damaging consequences (Kovacheva and Ribarov, 1995). Extensive lipid peroxidation is shown to cause membrane disorganization, by peroxidizing mainly the polyunsaturated fatty acids and phospholipids leading to alterations in the ratio of polyunsaturated fatty acids to other fatty acids. Thus, lipid peroxidation is considered as a serious consequence of free radical toxicity leading to profound changes in the membrane structure and function that may even cause cellular death (Ji, 1999). There have been many reports showing that acute and high-intensity exercise causes oxidative stress, free radical generation, increases in oxidative damage biomarkers such as thiobarbituric acid reactive substances, effects on mitochondrial function, and decreases in levels of antioxidants (Aguiar et al., 2008) and enhance its lipid peroxidation in the brain (Rosa et al., 2007; Tsakiris et al., 2006; Turgut et al., 2002). Rosa et al. (2007) demonstrated that the 10 days of intense and exhaustive running program induces an increased significantly Brain oxidative stress was evaluated by lipid peroxidation and protein oxidation in mice. There was a remarkable memory reduction of exercised animals in comparison with the control group (Rosa et al., 2007). Tsakiris et al. (2006), who also reported that either short or prolonged enforced swimming (2 h and 5 h of forced swimming) exercise induces oxidative stress in the rat brain (Tsakiris et al., 2006). Turgut et al. (2002), also found that 30-minute acute swimming exercise is lead to an increase in MDA levels in the brain tissues of rats (Turgut et al., 2002). However, the effects of high-intensity exercise on either oxidative damage or antioxidant status of the brain are still conflicting, and some authors did not observe high-intensity exercise induced alterations in the brain lipid peroxidation or in the antioxidant enzymes activities (Acikgoz et al., 2006; Liu et al., 2002; Somani et al., 1996). Surprisingly, there are few studies on the effects of exercise on oxidative status in brain premotor cortex, one of the most vulnerable brain regions to oxidative. A standing question for planning the design of studies using the therapeutic potential of exercise is whether after 8 weeks of regular aerobic training influences the malondialdehyde level of brain premotor cortex of rats caused by an acute bout of exhaustive endurance exercise. To resolve these questions, in this study, we investigated the malondialdehyde level of brain premotor cortex level was measured in brain of rats at before and immediately the following an acute bout of exhaustive endurance exercise.

Methodology

Animal care

Male Wistar rats weighing 200–232 g (n = 32, 12 weeks old) were purchased from Shahid Beheshti University of Medical Sciences and Health Services and were used in this study. All rats were housed in conventional clear Polycarbonate cages, four rats per cage, in a room with the temperature regulated at 22 ±2°C, humidity 45-65% and in daily light / dark cycle (12h) (0700-1900 h dark; 1900-0700 h light), given standard rat chow and tap water ad libitum. All procedures were approved by the Tehran University Animal Care and Usage Committee and followed the guidelines established by American Physiological Society.

Experimental design

The animals were housed for two weeks prior to any special treatment. In the third-week all the animals were randomly divided mainly into two groups, group1, sedentary (Sed N=16), group2, exercise trained (ET n=16). Two groups were further divided equally into two groups where the rats were studied at rest and immediately after exhaustive exercise. During the training period, the animals in the group2, was run on the treadmill 5 days a week for 8 weeks. Experiments were conducted between 10:00 and 12:00 h.

Training and Acute Exhaustive Exercise

After divided, the animals in the group (ET) were performed aerobic exercise on a treadmill for a period of eight weeks before the training, the group (ET) rats were introduced to treadmill running through the use of one 5-25 minute running session on a rodent treadmill at a speed of 16/7 m/min and a 0-2% uphill grade (1 session a day, 5 times/wk, 1 wk). (Sen, Marin, Kretzschmar, & Hanninen, 1992). The treadmill was equipped with an electric shock grid on the rear barrier to provide exercise motivation to the animals. The exercise protocol was performed in inclined treadmill one session a day during five days a week for 8 weeks. The exercise protocol was arranged as follows: in the first two weeks animals run with a speed of 16/67-18/33 m/min for 35-40 minutes and 3-4% uphill grade, in the following 3 weeks running speed was increased to 20 m/min and 5% grade uphill for 40 minute and in the last 3 weeks, treadmill speed was adjusted to 25 m/min for one hour and 8-10% uphill grade. During the eighth week of the training program, the groups (Sed) were also introduced to 8 weeks of regular aerobic training influences the malondialdehyde level of brain premotor cortex of rats caused by an acute bout of exhaustive endurance exercise.
treadmill running at speed of 16/7-20 m/min, for 15 min day, for 5 days, before sample collection. This regimen was used to ensure that untrained rats could also tolerate the acute exhaustive exercise without having a significant training effect (Sen et al., 1992). At the end of the training period and after 2 days at rest, half of all rats were randomly selected into the acute exhaustive exercise group (each group N=8 , totality N=16). In acute exhaustive exercise, running speed was 25 m/min (10% uphill gradient) for the first 10 min; after that the speed was increased gradually to 30 m/min , and kept constant until the rats were exhausted. The loss of the righting reflex when the rats were turned on their backs was the criterion of exhaustion. To eliminate diurnal effects, the experiments were performed at the same time (08.30–12.30 hours) (Brooks & White, 1978). Immediately after exhaustion exercise, animals were sacrificed with Chloroform and then their brain were quickly removed. From the whole brain, the premotor cortex carefully separated by the Cuello AC,1983 surgical procedure (Cuello, 1983). The specimens were stored at -20° C until assay. The other half of all rats (N=16) underwent anesthesia immediately before the acute exhaustive exercise, the premotor cortex was obtained according to the same program. These samples were used for the measurement levels of total protein concentration and MDA.

Biochemical analysis

Lipid peroxidation

Lipid peroxidation in premotor cortex were estimated by measuring with the thiobarbituric acid reactive substance (TBARS) by the method of Satho, 1978 Quantification of thiobarbituric acid reactive substances was determined at 532 nm by comparing absorption to standard curve of malondialdehyde (MDA) equivalents generated by acid catalyzed hydrolysis of 1, 1, 3, 3-tetramethoxypropane. Values of MDA were expressed as nmol per g of tissue (Kei, 1978).

Protein determination

Quantitative protein determination was achieved by absorbance measurements at 595 nm according to Bradford’s method (1976), with bovine serum albumin as standard (Bradford, 1976).

Data Analysis

The Statistical Package for Social Sciences (SPSS, Ins, Chigaco, IL) version 17 was used for all analyses. Statistical significance was set at a level of P< 0.05, and data were expressed as the mean ± SEM. One-way ANOVAs with Tukey’s post-hoc tests and Independent and dependent t-test, were used to compare group means.

RESULTS

Body weight and time of exhaustion

There were no significant differences in mean weight among the two groups in the beginning of experiments. (Table 1). At the end of the 8 weeks of the experimental period the mean weight of the ET group was significantly higher than ET group (p<0.05) (Table 1). The mean exhaustion time of treadmill running to exhaustion was 12/57±1/74, 39/66±8/28, min for SED and ET groups respectively. Exhaustion time was significantly longer in ET group compared with SED group (p<0.05) (Table 1).

Malondialdehyde level

Malondialdehyde results are presented in (Fig and Table. 1). The results shows that there were no significant differences in malondialdehyde level of brain premotor cortex among Sed and ET groups before and after Exhausted. Malondialdehyde level of brain premotor cortex of rats was significantly increased after exhaustion in the Sed and ET groups (p<0.05).

<table>
<thead>
<tr>
<th>Table 1.</th>
<th>Effects of 8-week training and acute exercise protocols on body weight and TBARS levels in the brain premotor cortex. Values are expressed as mean ± SEM.</th>
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<tbody>
<tr>
<td><strong>Group</strong></td>
<td><strong>body weight (g)</strong></td>
</tr>
<tr>
<td></td>
<td>Beginning of Experiments</td>
</tr>
<tr>
<td>SED</td>
<td>211±10</td>
</tr>
<tr>
<td>ET</td>
<td>218±14</td>
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</tbody>
</table>

TBARS, thiobarbituric acid-reactive substances; MDA, malondialdehyde; SED, sedentary; ET, exercise trained.* Significant difference between two groups (p<0.05).† Significant difference between two time measured in the each group (p<0.05).
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Discussion and Conclusion

The findings derived from present study shown that malondialdehyde level of brain premotor cortex increased significantly after a single bout of exhaustive treadmill running in the Sed and ET groups compared to pre-exhaustive. The results of this research are in line with those of the researchers who argue that intense and/or acute exercise increases the level of oxidative stress in the brain done by Rosa et al., (2007), Tsakiris et al., (2006), Turgut et al., (2002). Rosa et al., (2007) demonstrated that the 10 days of intense and exhaustive running program induces an increased significantly Brain oxidative stress was evaluated by lipid peroxidation and protein oxidation in mice (Rosa et al., 2007). Tsakiris et al., (2006), who also reported that either short or prolonged enforced swimming (2 h and 5 h of forced swimming) exercise induces oxidative stress in the rat brain (Tsakiris et al., 2006). Turgut et al., (2002), also found that 30-minute acute swimming exercise is lead to an increase in MDA levels in the brain tissues of rats are also parallel to the elevated MDA levels in the brain tissues in our study (Turgut et al., 2002).

Brain is an organ very sensitive to oxidative stress and this is partly due to the high metabolic rate and the large amount of iron and copper found in the organ. Brain oxygen consumption (Ide and Secher, 2000) and ROS production (Aguirre et al., 2010) increase during exercise. Under extreme conditions, such as in high-intensity physical exercise, ROS production may be more strongly and persistently increased, and the antioxidant response may not be sufficient to reset the system to the original level of brain redox homeostasis (Rosa et al., 2007). These interact with the diffusible hydrogen peroxide and result in the generation of the extremely reactive hydroxyl radical that yields damage to proteins, lipids and DNA (Halliwell, 2001). Hydrogen peroxide is generated by a number of systems, including reactions catalyzed by monoamine oxidases A and B with a described location of neuronal and glial mitochondrial membranes (Gershon et al., 1990). Besides the possible iron-hydrogen peroxide interactions, Ca^{2+}-associated reactive oxygen species (ROS) generation is also a potent source of ROS in the brain. Both inhibition and activation of neurons activates Ca^{2+}-traffic and the excess of glutamate could result in large increases in ROS production (Allan Butterfield, 2002). Neuronal membranes are packed with phospholipids containing polyunsaturated fatty acid esters, which are very sensitive to attack of ROS, causing a chain reaction which generates lipid radicals and extensive membrane damage. Nicotinamide adenine dinucleotide phosphate NAD(P)H oxidases are potent cellular generators of superoxide including neurons and glia (Lambeth, 2007). NAD(P)H oxidase ROS generation can be influenced by free fatty acids especially mono and polyunsaturated long-chain fatty acids, which could increase ROS production (Schönfeld and Wojtczak, 2008). Despite the fact that brain is well protected by the blood brain barrier, it is important to note that it cannot provide full protection against circulating inflammatory agents that can generate radicals in the brain (Farkas et al., 2006). Free radicals generate a cascade producing lipid peroxidation. Our results are in inconsistent with those obtained with exhaustive training in the treadmill, showing that it did not modify TBARS levels in the rat brain done by Liu et al., (2000), Somani and Husain, (1996), Acikgoz et al., (2006). Accordingly, Liu et al., (2000) demonstrated that acute exhaustive exercise does not alter protein and DNA damage in the whole brain (Liu et al., 2000). Somani & Husain, (1996) also show that acute exercise in treadmill increases lipid peroxidation in striatum, but not in the cortex, cerebellum, medulla and hypothalamus (Somani and Husain, 1996). Acikgoz et al., (2006) demonstrated that the Exhaustive exercise did not change superoxide dismutase and glutathione peroxidase enzyme activities and thiobarbituric acid reactive substances levels neither immediately (0 min) nor at 3, 6, 12, 24 or 48 h after the cessation of exercise in the brain (Acikgoz et al., 2006). However, the effects of exercise on oxidative damage brain status are conflicting.
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Interestingly, there is evidence that such varied outcomes might occur because of biases induced by the distinct kinds and intensities of physical activities protocols used (Ramsden et al., 2003). As well as probably dependent on the duration of exercise training, and the age, sex, and strain of rats (Radak et al., 2013).

**Conclusion:** The performed 8 weeks exercise could not prevented the increased significantly the lipid peroxidation level of brain premotor cortex response to acute bout of exhaustive exercise. These results indicate that intense exercise can have some deleterious effect on brain premotor cortex.

**REFERENCES**


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