Neuroscience Letters 642 (2017) 66–70

Contents lists available at ScienceDirect

Neuroscience Letters

journal homepage: www.elsevier.com/locate/neulet

Regulation of trace elements and redox status in striatum of adult rats by long-term aerobic exercise depends on iron uptakes

Hua-Bo Wu[a], De-Sheng Xiao[b,*]

[a] Department of Food Quality and Safety, School of Food and Biological Engineering, Jiangsu University, Zhenjiang, Jiangsu Province, China
[b] Department of Preventive Medicine, School of Public Health, Guangzhou Medical University, Guangzhou, Guangdong Province, China

HIGHLIGHTS

- Aerobic exercise (AE) decreased striatum Mn, Cu, and Zn contents in rats fed with iron-deficient diet (IDD).
- AE reduced MDA and increased the anti-oxidative variables in striatum of rats fed with iron-overloaded diet (IOD).
- Non-heme iron (NHI) contents were negatively correlated to Cu in striatum of AE rats fed with IDD.
- NHI contents were positively correlated to Cu or Zn in striatum of AE rats with iron-adequate diet.
- NHI contents were positively correlated to Mn or Cu in striatum of AE rats fed with IOD.

ARTICLE INFO

Article history:
Received 22 November 2016
Received in revised form 28 January 2017
Accepted 31 January 2017
Available online 2 February 2017

Keywords:
Aerobic exercise
Striatum
Iron deficiency
Iron overload
Antioxidant

ABSTRACT

We investigated the effects of aerobic exercise (AE) on trace element contents and redox status in the striatum of rats with different diet iron. Weaned female rats were randomly fed with iron-adequate diet (IAD), iron-deficient diet (IDD), and iron-overloaded diet (IOD). After feeding their respective diet for 1 month, the rats fed with same diet were divided into swimming and maintaining sedentary (S) group. After 3 months, the non-heme iron (NHI), Mn, Cu, and Zn in the striatum were measured. Meanwhile, malonaldehyde acid (MDA), total superoxide dismutase activity, hydroxyl radical scavenging activity, and total antioxidant capacity were also analyzed. As compared with respective S rats, Mn, Cu, and Zn contents were significantly decreased in IDDE, but no significantly changes could be seen in IADE or IODE. A negative correlation of NHI with Cu contents in IDDE and positive correlations of NHI with Cu, or Zn contents in IADE, or with Mn or Cu contents in IODE were observed. In addition, striatum MDA was significantly decreased and anti-oxidative variables were increased in IODE compared to IODS. Our results suggest that the modification of trace elements and redox status in the striatum of rats caused by AE depends on dietary iron contents and that AE may also regulate the metabolic relationship of iron storage with other trace elements.

1. Introduction

Fe, Cu, Zn, and Mn are essential trace elements and they play an important role in brain development and functions. However, the overload of these trace elements may damage the tissues or cells since Fe, Cu and Mn are involved in the formation of highly reactive oxygen-derived species (ROS), such as hydroxyl radicals, and induce oxidative stress and lipid peroxidation [10,18]. Although Zn can not directly undergo these redox reactions, it can indirectly displace Fe and Cu from in cell membranes and proteins [4]. The brain is one of the most susceptible organs to peroxidation injury because of high amounts of lipid molecules. The homeostasis of these trace elements is closely interdependent due to their interactions at multiple levels, and the regulations are more complex in the brain than those in other tissues and cells [15].

The striatum is a major nucleus of the basal ganglia and involved in motor activity and cognitive function [22]. It is also one of the most vulnerable to iron deficiency (ID) and iron overload (IO) in several brain regions. ID is quite common in children and adulthood [1,2,12]. Moreover, high iron intake such as high-dosage iron supplement for preventing against ID and dietary excessive iron exposure is not uncommon [17]. ID can lead to dysfunction of

* Corresponding author.
E-mail address: dsxiao@gzhmu.edu.cn (D.-S. Xiao).

http://dx.doi.org/10.1016/j.neulet.2017.01.070
0304-3940/

striatum, especially in the early life and childhood, which remains adulthood even after ID has been corrected [16]. In the aged, striatal iron, jointly with Cu, Zn, or Mn, abnormal accumulation and elevated oxidative stress have been implicated in the development of age-related neurodegenerative diseases [7,24]. Striatal age-dependent iron increase has been considered as a risk factor in neurodegenerative conditions [8]. In animal models, Shukla et al. [23] found that latent iron deficiency produced alterations in various metal levels in different brain regions, and the corpus striatum was the most vulnerable region for such changes. Additionally, previous study has indicated that striatal iron levels may be a biomarker of motor dysfunction in aging and that treatments to reduce accumulation of excess iron in the striatum may have beneficial effects on age-related deterioration of motor performance [5].

Aerobic exercise (AE) is the physical activity of relatively low intensity and long duration that gives the body more oxygen to meet energy requirements during exercise through aerobic metabolism. AE has been proposed to reduce oxidative stress through inducing an adaptation process in antioxidant system in the brain [19]. AE can also improve mental health, including reducing stress and lowering the incidence of depression, as well as increase cognitive capacity. However, it has indicated that AE can also enhance brain ROS formation resulting in cell damages, or cellular protection by the robust adaptation of antioxidant defense system to enhanced ROS formation, depending on exercise protocols and the brain region [6,25]. The present study was designed to investigate whether AE regulates the trace element contents and redox status in the striatum of rats which fed with different dietary iron.

2. Materials and methods

2.1. Animals and treatments

All animal care and experimental procedures conformed to the ethical guidelines of the China Association of Laboratory Animal Care and the ethics committee of Jiangsu University. Female Sprague-Dawley rats post-natal day 21 (PND21) were used in the experiments. The rats were housed in plastic cages on stainless steel grids at 21 ± 2 °C at a relative humidity of 60–65% and a 12 h light/dark cycle with ad labium access to the diets with their respective iron contents and distilled water. Fifty-three rats were randomly assigned to six groups: three sedentary groups respectively with iron-adequate diet (IADS, $n = 8$), iron-deficient diet (IDDS, $n = 8$), and iron-overloaded diet (IODS, $n = 8$), and other three groups respectively with iron-adequate diet (IAD) + AE (IADE, $n = 9$), iron-deficient diet (IDD) + AE (IDDE, $n = 10$), iron-overloaded diet (IOD) + AE (IODE, $n = 10$). After the animals were given the respective diet for 1 month (PND51), AE rats swam for 3 months while other rats were kept sedentary in their cages.

The diets were commercially available semi-purified and pelleted diets from Trophic Animal Feed High-Tech Co., China, on the basis of American Institute of Nutrition-93G standard with or without modifications of iron contents. Iron content was 45 ppm in IAD (code LAD3001G), 12 ppm in IDD (code TP0300), and 1000 ppm in IOD (code TP0450). Diet iron contents were confirmed by flame atomic absorption spectrometry.

The AE rats swam in a glass swimming basin (45 cm width, 80 cm length, 80 cm height) filled with tap water (35 ± 1 °C, 50 cm depth). The swimming was performed between 9:00 and 11:00 a.m., 5 days per week (from Monday to Friday), as described in our previous studies [6]. The total experimental period lasted for 3 months.

2.2. Sample collection

At the end of the experimental, the rats were fasted for about 24 h, and then anesthetized with pentobarbital (at a dosage of 45 mg/kg of body weight). Blood was collected directly by cardiac puncture into tubes treated with ethylene diamine tetraacetic acid. The animals were perfused with 0.9% saline (iron free) via the heart for washing out the blood in the brain, and decapitated. The two hemispheres were rapidly separated along the midline on an ice-cooled glass plate. The striatum was immediately isolated and kept frozen at −70 °C until use.

2.3. Cytosolic sample preparation

The fractions of the striatum were homogenized (1:10, w:v) in ultrapure water on ice and centrifuged at 1500 *g* (4 °C) for 10 min. The supernatant was collected and centrifuged at 35,000*g* (4 °C) for 30 min to further remove cell debris and organelles. The resulting supernatant was designated as the striatum 'cytosolic extract' and was collected. Protein contents were determined using the BCA method [6].

2.4. Hemoglobin (Hb) and plasma iron (PI) concentration assay

Blood Hb concentration was determined by an automated hematology analyzer. Blood was centrifuged at 1500 g (4 °C) for 15 min and PI concentration was measured by the colorimetric method [20].

2.5. Redox status assay

Malonaldehyde acid (MDA) was reacted with thiobarbituric acid (TBA) to form a red adduct with the maximum absorbance at 532 nm. Briefly, 60 μL supernatant, or ultrapure water (served as a reagent blank), or 60 μL of 10 nM Tetraethoxypropane (standard solution), or 60 μL of ethyl alcohol absolute (standard blank) was added with the following reagents: (1) 60 μL of Tris-HCl; (2) 600 μL of 0.1 M HCl, 300 μL of 1%TBA; (3) 300 μL of butyl alcohol. The mixture was incubated in a water bath at 37 °C for 40 min followed by cooling, centrifuging at 1500 *g* for 10 min. The resulting supernatant was assayed at 532 nm.

Total antioxidant capacity (TAOC) was measured according to the colorimetric method by reducing Fe^{3+}–Fe^{2+}, which could react with phenanthroline substance to form stable complex [27]. Hydroxyl radical scavenging activity (RSC) was measured using the hydroxyl radical generated Fenton reagent method [26]. Total superoxide dismutase activity (SOD) was measured using xanthine and xanthine oxidase to generate superoxide radicals that react with 2-(4-Iodophenyl)-3-(4-nitrophenyl)-5-phenyltetrazolium chloride. The reaction gave a red formazan dye product with the maximal absorbance at 450 nm [28].

2.6. Non-heme iron (NHI), Mn, Cu, and Zn contents

NHI, Mn, Cu, and Zn contents were measured based on the trichloroacetic acid precipitation method as previously described [6]. The NHI, Mn, Cu, and Zn concentrations were quantified and normalized by inductively coupled plasma mass spectrometry.

2.7. Statistical analysis

Data were expressed as means ± standard error of mean (SEM). The two-way analysis of variance (ANOVA) was used to analyze the data obtained from experiments. When a significant effect was found, post hoc test was performed with LSD's multiple comparison tests to compare within and between groups. Linear relation