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Long-term aerobic exercise increases redox-active iron through nitric oxide in rat hippocampus

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ABSTRACT

Adult hippocampus is highly vulnerable to iron-induced oxidative stress. Aerobic exercise has been proposed to reduce oxidative stress but the findings in the hippocampus are conflicting. This study aimed to observe the changes of redox-active iron and concomitant regulation of cellular iron homeostasis in the hippocampus by aerobic exercise, and possible regulatory effect of nitric oxide (NO). A randomized controlled study was designed in the rats with swimming exercise treatment (for 3 months) and/or an unselective inhibitor of NO synthase (NOS) (L-NAME) treatment. The results from the bleomycin-detectable iron assay showed additional redox-active iron in the hippocampus by exercise treatment. The results from nonheme iron content assay, combined with the redox-active iron content, showed increased storage iron content by exercise treatment. NOx (nitrate plus nitrite) assay showed increased NOx content by exercise treatment. The results from the Western blot assay showed decreased ferroportin expression, no changes of TfR1 and DMT1 expressions, increased IRP1 and IRP2 expression, increased expressions of eNOS and nNOS rather than iNOS. In these effects of exercise treatment, the increased redox-active iron content, storage iron content, IRP1 and IRP2 expressions were competely reversed by L-NAME treatment, and decreased ferroportin expression was in part reversed by L-NAME. L-NAME treatment completely inhibited increased NOx and both eNOS and nNOS expression in the hippocampus. Our findings suggest that aerobic exercise could increase the redox-active iron in the hippocampus, indicating an increase in the capacity to generate hydroxyl radicals through the Fenton reactions, and aerobic exercise-induced iron accumulation in the hippocampus might mainly result from the role of the endogenous NO.

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Introduction

The hippocampus (HC) is a critical brain area for learning, memory and many other behavioral processes [1]. Adult HC relies on iron availability for many essential functions, but is highly vulnerable to iron-induced oxidative stress [2–6]. The hippocampal iron accumulation and oxidative stress have been observed in certain physiological (e.g. normal aging [2], psychological stress [3]) and pathological (e.g. cerebral injury [4], epilepsy [5], Alzheimer's disease [6], surgical trauma [7]) situations. It is believed that the dysregulation of iron homeostasis contributes to an excess of redox-active iron, while the latter generates hydroxyl radicals through the Fenton reaction and Haber–Weiss reaction, thereby triggering the oxidative modification of lipids, proteins and DNA [8].

Aerobic exercise (AE) is the physical activity of relatively low intensity (light-to-moderate intensity) and long duration that gives the body more oxygen to meet energy requirements during exercise through aerobic metabolism. We previously reported that AE could increase nonheme iron (NHI) contents preferentially in the HC among different brain regions [9]. To date, however, there is little information about the hippocampal redox-active iron metabolism and iron homeostasis mechanisms during exercise. A number of studies have demonstrated that exercise exerts diverse effects on the brain, including HC. The acute exercise has transient cognitive impairments and stimulatory effect on

Abbreviations: HC, hippocampus; AE, aerobic exercise; NHI, nonheme iron; ROS, reactive oxygen species; BDI, bleomycin-detectable iron; TfR1, transferrin receptor 1; IRE, iron responsive element; DMT1 + IRE, divalent metal transporter 1 with iron responsive element; DMT1 – IRE, divalent metal transporter 1 without iron responsive element; Fpn, ferroportin 1; IRP1, iron regulatory protein 1; IRP2, iron regulatory protein 2; NO, nitric oxide; NOS, nitric oxide synthase; eNOS, endothelial nitric oxide synthase; nNOS, neuronal nitric oxide synthase; iNOS, inducible nitric oxide synthase; NOx, nitrite plus nitrate; L-NAME, N^{ω}-nitro-L-arginine methyl ester; S1, sedentary group; S2, sedentary+L-NAME group; E1, exercise group; E2, exercise + L-NAME group; TCA, trichloroacetic acid; MDA, malondialdehyde; DTPA, diethylene triamine pentacetate acid; SEM, standard error of mean; ANOVA, two-way analysis of variance.

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the HC [10]. In contrast, regular AE has many cognitive benefits [11] and effects on the HC [12–14], including increases in blood flow, neurotransmitter levels, brain-derived neurotrophic factor, plasticity and neurogenesis, and effective reversal of age-associated volume loss and memory deterioration. AE has been proposed to reduce oxidative stress through inducing an adaptation process in antioxidant systems in the brain [15]. However, its effects on the hippocampal redox state in normal animals have been recently investigated and the findings are quite conflicting, including the increase [16,17], no change [18–20], or the decrease [21] in the oxidative level. It is imperative to investigate the redox-active iron in the HC of non-aged adult during AE because such form of iron is the main source of reactive oxygen species (ROS) and the main determinant of ROS toxicity [8].

Thus, the first purpose in this study was to determine the profiles of hippocampal redox-active iron and cellular iron homeostasis after long-term AE. We applied the bleomycin-detectable iron (BDI) assay because this assay is a reliable and valid measurement for redox-active iron [8,22], and has been proposed for the clinical measurement in the iron-overloaded pathological conditions [22]. It is well known that brain extracellular iron concentration is independent of peripheral iron level and well maintained by brain barriers [23], and neurocytic iron homeostasis is tightly controlled by cellular regulatory mechanisms through altering cellular iron uptake, storage, export, and utilization [23,24]. In the HC, cellular iron uptake is mainly mediated by transferrin receptor 1 (TfR1) for transferrin-bound iron uptake, and by divalent metal transporter 1 (DMT1) for free or loosely bound Fe^{2+} uptake. Ferroportin 1 (Fpn) is well expressed in the HC, which is the sole cellular iron exporter known in mammalian cells [25]. The master regulators are the cytoplasmic iron regulatory protein 1 (IRP1) and 2 (IRP2), both of which reversibly bind to the iron responsive element (IRE) in specific mRNA encoding TfR1, DMT1 + IRE (with IRE), Fpn and iron storage protein ferritin [24]. Unlike DMT1 + IRE, DMT1 − IRE (without IRE) expression is transcriptionally regulated and has been associated with neurotoxicity [26]. In this study, thus, we also investigated the expressions of all these key proteins except the ferritin because cellular storage iron content was also served as an indicator of its expression.

The second purpose in this study was to observe the possible change of nitric oxide (NO) levels by AE and its possible role in the hippocampal redox-active iron metabolism and cellular iron homeostasis. NO is produced from L-arginine by NO synthase (NOS). The HC contains all three isoforms of NOS including endothelial (eNOS), neuronal (nNOS) and inducible (iNOS) [27]. Despite contradictory report [18,28], most of studies have suggested that exercise can increase NO level in the HC, thus extensively involving in exercise-associated functions [27,29]. On the other hand, NO has been proposed as a key regulator of cellular iron metabolism through various mechanisms such as releasing iron from ferritin or IRP1 [30], or inducing the synthesis or degradation of IRP2 [31,32]. NO regulates iron homeostasis in a wide range of physiological and pathological situations of either the peripheral or central nervous system [33]. In this study, we observed the NOx (nitric plus nitrate) levels and the expressions of three NOS isoforms in the HC in an AE rat model, and their changes by an unselective inhibitor of NOS isoforms L-NAME (N^{ω}-nitro-L-arginine methyl ester) treatment for analyzing the relationship of NO with the changes of the hippocampal redox-active iron and iron homeostasis. To the best of our knowledge this is the first report on the effects of exercise on the redox-active iron and cellular iron homeostasis of the HC and their regulation by NO.

Materials and methods

Animals and experimental treatments

All animal procedures were approved by the Ethics Committee of Jiangsu University and the Ethical Guidelines of the China Association of Laboratory Animal Care were strictly followed in this experiment.

It is known that there is a sex-dependent difference in iron metabolism of the brain, including HC [34]. Based on our previous work [9], female rats were used. The female Sprague–Dawley rats (170–190 g) were purchased from the Laboratory Animal Center of Jiangsu University, and maintained in plastic cages at 21 ± 2 °C, with relative humidity of 60–65% and a 12 h cycle of light and dark. After one week of acclimatization, the animals were randomly assigned to one of the following four groups: sedentary (S1, $n = 10$), exercise (E1, $n = 10$), sedentary + L-NAME treatment (S2, $n = 10$), and exercise + L-NAME treatment (E2, $n = 10$). A customized laboratory rodent diet based on AIN93 formula [35] (iron content adjusted to 80 ppm) was supplied by TROPHIC Animal Feed High-Tech Co., China. The rats had ad libitum access to the diet and drinking water (deionized distilled water). During the whole experimental period, L-NAME was freshly dissolved in the drinking water (1 mg/ml) every day and orally supplied to rats in the S2 and E2 groups. Oral administration and the dose of L-NAME treatment were chosen because the efficiency and inhibitory effects on NOS have been investigated [36,37].

According to daily water intake at the beginning of the experiment in this study, an oral dose of L-NAME was approximately 90 mg/kg/d. A similar administration has previously been shown to alter working memory in young rats [37]. L-NAME at a dose of about 100 mg/kg/d by i.p. is also frequently used to inhibit NO synthesis in the brain or brain regions of rats in published literature. This dose of L-NAME by a single i.p. has previously been shown to result in maximal decrease of hippocampal NOx concentrations in microdialysate to 58% of basal in freely moving rats [38]. Obviously, to efficiently inhibit NO synthesis in the brain or brain regions, systemic administration of L-NAME is inevitably concomitant with systemic hypertension because of inhibition of NOS in blood vessel. Despite of this hypertension, during chronic administration of L-NAME, the cerebral blood flow in the brain or its observed different regions, including HC, of rats could maintain at physiological level by a NO-dependent mechanism (cerebrovascular reactivity to the remnant NO production) and a NO-independent mechanism [39,40]. In this study, nonetheless, our analyses of both NOx level and all NOS isoforms expression of the HC could be helpful to indicate whether the effect of L-NAME treatment on iron metabolism in the HC is due to its action on hippocampal NO synthesis.

Exercise protocol

The non-weighted swimming exercise was chosen because rat continuous swimming has been evaluated to be a reliable AE model with a moderate intensity and less emotional stress when several key parameters are appropriate [41], and because this method has been successfully applied in our previous studies [9,42]. In short, the rats in the E1 and E2 groups were subjected to swimming exercise in groups of six in a glass swimming tank of 80 × 80 cm, filled with tap water to the depth of 50–52 cm. This water surface area is sufficient for the animals to continuously swim, and the water depth is enough to prevent against rest by supporting their tails on the bottom of the tank and to eliminate their "bobbing" behavior [41]. The water temperature was kept at 34–36 °C, which can maintain rat core temperature throughout the exercise bout. The swimming was performed between 9:00 and

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