Physiology & Behavior 147 (2015) 78–83

Contents lists available at ScienceDirect

Physiology & Behavior

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

Comparing interval and continuous exercise training regimens on neurotrophic factors in rat brain

Mohammad Esmaiel Afzalpour [a,*], Hossein Taheri Chadorneshin [b], Mohsen Foadoddini [c], Hossein Abtahi Eivari [d]

[a] Department of Physical Education and Sport Sciences, University of Birjand, Birjand, Iran
[b] Exercise Physiology, University of Birjand, Birjand, Iran
[c] Atherosclerosis and Coronary Research Center, Birjand University of Medical Sciences, Birjand, Iran
[d] Department of Clinical Biochemistry, Gonabad University of Medical Sciences, Gonabad, Iran

HIGHLIGHTS

- High intensity exercise training increases BDNF and GDNF in the brain.
- Interval training increases BDNF and GDNF in the brain more than continuous training.
- There is positive correlation between H_2O_2 and TNFα with BDNF and GDNF in the brain.

ARTICLE INFO

Article history:
Received 25 December 2014
Received in revised form 27 February 2015
Accepted 6 April 2015
Available online 11 April 2015

Keywords:
Brain-derived neurotrophic factor
Glial cell line-derived neurotrophic factor
Hydrogen peroxide
Tumor necrosis factor alpha
Interval training
Continuous training

ABSTRACT

The research literature suggests that oxidative stress and pro-inflammatory factors influence neurotrophins in vitro. However, there is insufficient information about their effects on exercise training conditions, especially during high intensity trainings. This study aimed to compare the effects of 6 weeks of high intensity interval and continuous training regimens on brain-derived neurotrophic factor (BDNF), glial cell line-derived neurotrophic factor (GDNF), hydrogen peroxide (H_2O_2), and tumor necrosis factor alpha (TNF-α) in the rat brain. For this purpose, twenty-four Albino Wistar rats were divided into sedentary control (SC), high intensity interval training (HIIT), and continuous training (CT) groups. Both HIIT and CT regimens increased H_2O_2 level and TNF-α concentration in the brain, and the alterations made were greater following HIIT than CT. In addition, both HIIT and CT regimens increased BDNF and GDNF concentrations significantly, with a higher elevation following HIIT than CT. Furthermore, H_2O_2 level and TNF-α concentration correlated positively with both BDNF and GDNF concentrations. Generally, high intensity interval training regimen, rather than continuous training regimen, is highly potential to improve BDNF and GDNF through a greater increase in H_2O_2 and TNF-α as oxidative stress and pro-inflammatory factors.

1. Introduction

Brain-derived neurotrophic factor (BDNF) and Glial cell line-derived neurotrophic factor (GDNF) are small peptides which belong to the family of neurotrophic factors and are distributed in different regions of central nerve system (CNS) [1]. BDNF modulates brain development and neuroplasticity and neurite outgrowth, thereby improving memory and preventing Alzheimer and depression [2–4]. Further, GDNF improves motor function by protecting dopaminergic, cortical, and motor neurons which in turn prevents the occurrence of Parkinson and Amyotrophic Lateral Sclerosis diseases [5–7]. However, the available evidence suggests that concentrations of BDNF and GDNF can be modulated by altered redox homeostasis [1,8,9], pro-inflammatory conditions [10–13], and exercise training [1,6,14,15].

In vitro, accumulating evidence has shown that the expression of BDNF and GDNF may be influenced by hydrogen peroxide (H_2O_2) [1,8,9] and tumor necrosis factor alpha (TNF-α) [10–13]. Nuclear factor-kappa B (NF-κB) and cAMP response element bind protein (CREB), as two main transcription factors, play important roles in the expression of BDNF and GDNF [9,11,12]. TNFα increases in the brain through microglia activation and peripheral TNF-α crossing from blood–brain barrier [11,16,17]. By interaction with its receptors, TNF-α induces neurotrophin expression not only through the activation of NF-κB but also via the activation of CREB [11,12]. In this context, it has been

Abbreviations: BDNF, Brain-derived neurotrophic factor; CNS, Central nerve system; CREB, cAMP response element bind protein; CT, Continuous training; GDNF, Glial cell line-derived neurotrophic factor; H_2O_2, Hydrogen peroxide; HIIT, High intensity interval training; IGF-1, Insulin-like growth factor-1; NF-κB, Nuclear factor-kappa B; SC, Sedentary control; TNF-α, Tumor necrosis factor alpha; VO_2max, Maximal oxygen uptake.

* Corresponding author at: Faculty of Physical Education and Sport Sciences, University of Birjand, Shokat Abad, Birjand, South Khorasan, Iran.
E-mail address: mazalpour@birjand.ac.ir (M.E. Afzalpour).

http://dx.doi.org/10.1016/j.physbeh.2015.04.012
0031-9384/

shown that TNF-α induces BDNF and GDNF expression in astrocytes [12,13], trigeminal ganglion neurons [10], and dorsal root ganglia [18]. Besides, exogenous and astrocytes-released TNF-α induces nerve growth factor and GDNF production in astrocytes [13]. However, anti-TNF-α treatment inhibits BDNF release from astrocytes [12]. Similarly, H_2O_2 upregulates BDNF and GDNF expression by increasing translocation of p65:p50 of NF-κB complex from cytoplasm to nucleus [1,19–21]. Also, the activation of NADPH oxidase (as a stress oxidative resource) increases BDNF expression by enhancing CREB phosphorylation [9]. In this context, it has been indicated that H_2O_2 injection to rats increases GDNF protein concentration in cervical spinal cord region [1]. Another study has pointed out that H_2O_2 increases GDNF protein in neuron-glia mixed cultures and protects dopaminergic neurons [21]. However, it has been shown that antioxidant N-acetyl-L-cysteine inhibits BDNF release from the microvascular endothelial cell line of the brain [8], and N-tert-butyl-α-phenylnitrone reduces BDNF protein of the cervical spinal cord in rats [1].

Identifying the factors which regulate the brain BDNF and GDNF availability is an important goal for brain health and function [22]. Exercise training accounts for a non-pharmacological approach to modulating neurotrophins [4]. It is maintained that chronic swimming exercise [3], treadmill running [1,5,6], and wheel running [22–24] elevated neurotrophins in different regions of the nerve system of young [15], adolescent, and old animals [7,25]. In addition, it has been shown that low to moderate exercise training improves memory function and balance coordination through increasing BDNF and GDNF concentrations in hippocampus [3] and striatum [5], respectively. Furthermore, daily and alternating days of wheel running increase BDNF concentration in hippocampus [22]. BDNF protein elevates 2 weeks after the exercise ends, while it progressively declines following 4 weeks of detraining [24]. Also, it has been shown that long-term running at moderate intensity [5,23], contrary to short-term running [26], increased GDNF concentration in the striatum and sciatic nerve.

Although low to moderate exercise training regimens increase BDNF and GDNF concentrations in different regions of the brain [3,5,22,23], effects of intensive exercise trainings on BDNF and GDNF have not yet been well established. The importance of this issue becomes even more evident since we know that intensive exercise trainings increase H_2O_2 [27] and TNF-α production [28]. In reality, interactive effects of H_2O_2 and TNF-α on neurotrophin adaptations induced by intensive exercise training have not yet been sufficiently investigated. Furthermore, it seems that intensive interval and continuous training regimens may activate stress oxidative resources [29] and antioxidant system differently [30], and thereby produce different levels of H_2O_2. Therefore, it is proposed that neurotrophin adaptations may be influenced differently by intensive interval and continuous exercise training regimens. Many people do not have enough time for exercise, and it is necessary to examine the effects of exercise training intensity on health improvement, especially neurotrophin adaptations. Hence, the aim of the present study was to compare the effects of high intensity interval and continuous training regimens on BDNF and GDNF concentrations in the rat brain and determine their relations with H_2O_2 and TNF-α as two possible modulating mechanisms.

2. Materials and methods

2.1. Animals

All animal experiments conformed to the guidelines for the use and care of laboratory animals ("Principles of laboratory animal care", NIH publication No. 86-23. Revised 1996), and the study was approved by the ethics committee of Birjand University of Medical Sciences in Iran. Twenty-four mature male Albino Wistar rats (3 months old) with a weight equal to 282 ± 14 g were prepared from the laboratory of bearing and multiplying at the Mashhad University of Medical Sciences in Iran. The rats were housed in standard cages of polycarbonate (20 × 59 × 38 cm) in a room at a temperature of 22 ± 2 °C with a 12:12-h reverse light–dark cycle starting the light period at 7:00 am. The rats had free access to tap water and standard rat food (Javaneh Khorasan Company, Iran). All the animals were checked daily for clinical signs of diseases. The animals were randomly divided into three equal groups (n = 8) of sedentary control (SC), high intensity interval training (HIIT), and continuous training (CT).

2.2. Exercise training protocols

Exercise training was performed on a 12-lane treadmill because the intensity and duration of exercise could be controlled easily [15]. The animals were familiarized with running on a motor-driven treadmill (5 days, 10 min/day at a speed of 10 m/min) [31]. Continuous and interval exercise trainings were performed on the basis of overload principle for 6 weeks, 6 sessions per week (Table 1) [32]. Overload was exerted by increasing time and intervals in CT and HIIT groups, respectively. At the beginning and end of continuous and interval exercise training regimens, warm-up and cool-down were performed at 16 m/min. This intensity corresponds to 68% maximal oxygen uptake (VO_2max). Besides, intensities of continuous and interval exercise training regimens correspond to 80 and 95–100% VO_2max, respectively. Active rest was performed between intervals in HIIT group for 60 s at 16 m/min [32]. The rats were motivated to run via the electrical shocks at the rear of the treadmill and by gentle prodding using a sponge [1]. Each animal was assigned to a fixed lane and all the activities were performed in the respective lane during the entire training program to minimize novelty confound [26]. The rats of the SC group were transported daily to the training room, exposed to the same environment as the exercising groups, and placed on the treadmill without running for as long as the exercising groups were on the treadmill [5].

2.3. Tissue preparation

To avoid data misinterpretation due to the remaining effects of the last exercise session, the rats were sacrificed by decapitation under deep anesthesia (Ketamine, 60–80 mg/kg and Xylazine, 8 mg/kg; IP) 48 h after the last exercise session [5] between 10:00 and 11:00 am. The whole brain of each rat was removed and dissected in less than 5 min and washed by normal saline to remove excess surface blood. The brain was rapidly submerged in liquid nitrogen for 2 min and finally stored at −80 °C for further analysis.

2.4. Biochemical assays

In order to measure all the parameters from the same region and because of different distributions of GDNF [20] and BDNF [33] in the brain, we selected the whole brain for biochemical assays [2]. Each brain was dipped in liquid nitrogen and smashed into a fine powder [7,34]. For total proteins of GDNF, BDNF and TNF-α assays, we added 1 ml 1× Phosphate Buffered Saline and Protease Inhibitor Cocktail (#GB-326-1, ProBlock™-50, Goldbio technology CO, USA) to the microtube containing 52–87 mg powdered brain tissue and stored them overnight at −20 °C. After two freeze–thaw cycles which were performed to break the cell membranes, the homogenate was vortexed (Stuart Mixers Vortex, SA8™, England) and centrifuged (Eppendorf Centrifuge, Mini Spin^R, Germany) for 5 min at 5000 ×g, at 2–8 °C. For H_2O_2 assay, after the addition of 400 μl of 1× Phosphate Buffered Saline to 54–97 mg of powdered brain tissue and performing vortex, the sample was centrifuged for 15 min at 7000 rpm. The supernatant was removed and the assays were carried out immediately according to the manufacturer's instructions. We used the commercially 96-well ELISA kits to measure the protein contents of total GDNF (#CSB-E04566r, Cusabio Biotech CO., LTD. Sino-American), total BDNF (#CSB-E04504r, Cusabio Biotech CO., LTD. Sino-American) and total TNF-α (#865.000.096, Diaclone SAS., France) in the brain. The sensitivities of the kits were less than 7.81,