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Research Report

Different protocols of treadmill exercise induce distinct neuroplastic effects in rat brain motor areas

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ABSTRACT

A variety of exercise protocols have been used to promote experimental neuroplasticity. However, the plastic brain responses generated by several aspects of training (types, frequency, regimens, duration) remain undetermined. The aim of this study was to compare the plastic changes in the glutamatergic system and synaptic proteins in motor cortex, striatum and cerebellum promoted by two different treadmill exercise regimens. The present study analyzed by immunohistochemistry and Western blotting the expression of the subunits of AMPA receptors (GluA1 and GluA2/3) and synaptic proteins (synapsin I and synaptophysin) in adult male Wistar rat brains. The animals were divided into animals subjected to two different frequencies of aerobic exercise groups and sedentary animals. The exercise groups were: intermittent treadmill exercise (ITE) – animals that exercised 3 times a week (every other day) during four weeks, and continuous treadmill exercise (CTE) – animals that exercised every day during four weeks. Our results reveal that different protocols of treadmill exercise were able to promote distinct synaptic reorganization processes among the exercised groups. In general, the intermittent exercise regimen induced a higher expression of presynaptic proteins, whereas the continuous exercise regimen increased postsynaptic GluA1 and GluA2/3 receptors.

1. Introduction

The central nervous system (CNS) can change its structure and function as a result of the interaction of individuals with their environment. Depending on the characteristics of the environment and lifestyle, plastic changes can be enhanced or degraded (Mora et al., 2007). These changes may be responsible for motor learning, memory and plasticity of the CNS (Leasure and Jones, 2008; Arida et al., 2011).

Glutamate is the main CNS excitatory neurotransmitter and its receptors are responsible for activating pathways that promote short and long-term responses (Kind and

Abbreviations: CTE, Continuous frequency treadmill exercise; Cb, Cerebellum; GluA, AMPA receptor; GluA1, AMPA receptor subunit 1; GluA2/3, AMPA receptor subunits 2 and 3; GL, Granular layer of the cerebellar cortex; ITE, Intermittent frequency treadmill exercise; ML, Molecular layer of the cerebellar cortex; PCL, Purkinje cell layer of the cerebellar cortex; SYS, Synapsin I; SYP, Synaptophysin

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Neumann, 2001). The function and dynamic membrane trafficking of ionotropic glutamate receptors, especially α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic (AMPA) receptors, are very important for nearly all aspects of brain function and are involved in synaptic plasticity processes, including long-term potentiation (LTP) and long-term depression (LTD). Indeed, the GluA1 is very important for LTP mechanisms and GluA2 for LTD mechanisms (Huganir and Nicoll, 2013). There are different responses of glutamate concentration and GluA trafficking after running exercise, suggesting that the responses observed in the glutamatergic system may change according to the type, intensity, duration and volume of exercise performed. When exercise causes exhaustion, it is possible to observe a decrease in the concentration of free glutamate in rats (Santos et al., 2007). However, when exercise is performed moderately, there is an increase of glutamate and its receptors. We have demonstrated that continuous treadmill exercise promotes increase of GluA1 and GluA2/3 receptors subunits in the striatum and cerebellum after 7 and 15 days of exercise in the treadmill (Real et al., 2010).

In addition to AMPA receptors, studies also show that the performance of physical exercise is able to increase some proteins involved in plasticity (Derksen et al., 2007; Ferreira et al., 2010; Garcia et al., 2012). Some of these proteins are synaptic proteins, such as synapsin I (SYS) and synaptophysin (SYP), which are in general involved in the regulation of neurotransmitter release from nerve terminals and the turnover of synaptic vesicles in the synaptic terminal (Vaynman et al., 2006). Previous studies showed that a long period of exercise in different modalities can produce alterations of these proteins in the rodent motor cortex, striatum and cerebellum (Lambert et al., 2005; Garcia et al., 2012). On the other hand, when the treadmill exercise is performed for a short period, the expression of these proteins increased only in the cerebellum and striatum (Ferreira et al., 2010).

In summary, various exercise protocols have been used to study its beneficial effects on the CNS, but some neuroplastic responses induced by several aspects of training (types, frequency, volume, and duration) remain undetermined. In fact, depending on the protocol of training used, exercise could be, instead of beneficial, deleterious to the brain by causing increased cytokine production and excitotoxicity (Arida et al., 2011). Thus, the aim of this study was to evaluate if different treadmill exercise regimens could induce different plastic changes in the glutamatergic system and synaptic proteins in brain motor areas. This knowledge could help to understand how different synaptic components contribute to reorganization of motor areas triggered by different exercise regimens.

2. Results

2.1. General aspects

In general, the GluA receptor subunits and the synaptic proteins used in this study are expressed in all brain structures analyzed, but their expression showed different responses for the different frequencies of exercise (3 or 7 times/week) and for the different structures. Overall, the results of immunohistochemistry and Western blotting were similar, with a few exceptions, as described below.

2.2. Corticosterone levels

The evaluation of plasma corticosterone concentration revealed a decrease of the plasma levels for the CTE group (ca. 58%, 2996±266, $P=0.002$). On the other hand, the corticosterone plasma levels of the ITE group (6938±195) were similar to the levels found for the sedentary (SED) group (7172±299)[$F(2,12)=83.331$; $P=0.000001$] (Fig. 1).

2.3. Motor cortex

In the motor cortex, the GluA1 and the GluA2/3 were expressed in the neuropil and cellular bodies of the primary and secondary motor cortices (Fig. 2A). In the immunohistochemistry we analyzed the throughout layer V because it is the output of the motor cortices (Lodato et al., 2015), and in the Western blotting data included all the layers. In the ITE group there was a significant decrease of the number of the labeled cells for both subunits (GluA1 – ca. 35%, 93±2, $P=0.02$; GluA2/3 – ca. 26%, 119±9, $P=0.02$) when compared to the SED group (GluA 1 – 144±13; GluA2/3 – 162±9). The CTE group showed an increase in the number of the cells that express GluA subunits when compared to the SED group (GluA1 – ca. 43%, 205±8, $P=0.008$; GluA2/3 – ca. 30%, 211±5, $P=0.01$) or the ITE group (GluA1 – ca. 120%, $P=0.0005$; GluA2/3 – ca.77%, $P=0.0007$) (Fig. 2B) (GluA1 – [$F(2,6)=37.235$; $P=0.0004$]/GluA2/3 – [$F(2,6)=31.152$; $P=0.0007$]). Protein analysis by Western blotting produced data similar to the immunohistochemistry assays. There was a decrease for GluA1 in the ITE group (0.78±0.03, $P=0.02$) when compared to CTE group (ca.31%, 1.13±0.02), and for GluA2/3 in the ITE group (0.40±0.04) when compared to SED (ca. 60%, 1±0.05; $P=0.0004$) and CTE (ca.66%, 1.18±0.11, $P=0.0002$) (GluA1 – [$F(2,12)=4.7549$; $P=0.03$]/GluA2/3 – [$F(2,12)=30.495$; $P=0.00002$]) (Fig. 2C).

The synaptic proteins (SYS and SYP) exhibited either a cytoplasmic or a diffuse punctate pattern staining throughout the motor cortex (Fig. 3A) and we detected an optical density increase in the ITE group (SYS – ca.47%, 1.47±0.07,

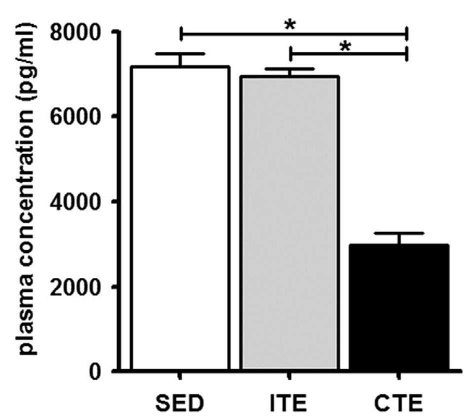

Fig. 1 – Effects of different treadmill exercise regimens on plasma corticosterone concentration. Corticosterone levels was unchanged in the SED and ITE groups and decreased in the CTE group.