BRAIN RESEARCH 1388 (2011) 48–55

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BRAIN RESEARCH

Research Report

Cerebral metabolism after forced or voluntary physical exercise

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ARTICLE INFO

Article history:
Accepted 24 February 2011
Available online 10 March 2011

Keywords:
Cerebral metabolism
Exercise
HIF-1α
PFK-1
GLUT
Glycolysis

ABSTRACT

The pathophysiology of stroke, a leading cause of morbidity and mortality, is still in the process of being understood. Pre-ischemic exercise has been known to be beneficial in reducing the severity of stroke-induced brain injury in animal models. Forced exercise with a stressful component, rather than voluntary exercise, was better able to induce neuroprotection. This study further determined the changes in cerebral metabolism resulting from the two methods of exercise (forced versus voluntary).

Adult male Sprague–Dawley rats were randomly assigned to 3 groups: the control group (no exercise), the forced treadmill exercise group, and the voluntary running wheel exercise group. In order to measure the extent of cerebral metabolism in animals with different exercise regimens, mRNA levels and protein expression of glucose transporter 1 and glucose transporter 3 (GLUT-1 and GLUT-3), phosphofructokinase (PFK), lactate dehydrogenase (LDH), and adenosine monophosphate kinase (AMPK) were measured utilizing real-time reverse transcription polymerase chain reaction (PCR) analysis as well as Western blot analysis. Phosphorylated AMPK activity was also measured using an ELISA activity kit, and hypoxic inducible factor (HIF)-1α was measured at transcription and translation levels.

The data show that the forced exercise group had a significant ($p < 0.05$) increase in cerebral glycolysis, including expressions of GLUT-1, GLUT-3, PFK, LDH, phosphorylated AMPK activity and HIF-1α, when compared to the voluntary exercise and the control groups. Our results suggest that the effects of different exercise on HIF-1α expression and cerebral glycolysis may provide a possible reason for the discrepancy in neuroprotection, with forced exercise faring better than voluntary exercise through increased cerebral metabolism.

1. Introduction

Stroke, as one of the current leading causes of death and injury, commands a great deal of attention from the medical research world. An expanding literature has developed to substantiate the beneficial effects of exercise on stroke-induced brain injury in animal models (Stummer et al., 1994; Endres et al., 2003; Ding et al., 2005; Guo et al., 2008a; Guo et al.,

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doi:10.1016/j.brainres.2011.02.076

2008b); however, the underlying mechanisms of exercise-induced neuroprotection against stroke remain unclear. Many studies have solely employed a forced treadmill exercise regimen in which the stress associated with the regimen may act as a confounding variable. In our previous study, we used different exercise regimens, forced exercise on the treadmill and voluntary exercise on a running wheel (Hayes et al., 2008), and found that forced treadmill exercise induced stronger neuroprotection as compared to the other groups in the experiment over the same period. It is clear that uncovering the underlying mechanisms could help elucidate observations of exercise-induced neuroprotection, and this could lead to the development of better strategies for both the treatment and prevention of stroke.

Multiple studies have indicated that physical exercise increases metabolism and glycolysis in skeletal muscles (Zorzano et al., 2005; McGee and Hargreaves, 2006; Burgomaster et al., 2007) which leads to increased production of metabolite transport proteins and increased efficiency during this period of relative hypoxia. Whether exercise increases metabolism and glycolysis in the brain tissue remains a question as the lack of energy is seen as one of the main culprits in cell death; an increased ability to produce energy, as a result of exercise, could be considered a strong survival mechanism. Thus, as it is in muscle tissue, exercise could increase the brain's ability to produce energy under hypoxic conditions via an up-regulation in the compounds necessary for glycolysis and other metabolic processes. A recent human study has suggested that endurance training attenuates the cerebral metabolic response to submaximal exercise, as reflected in a lower carbohydrate uptake and maintained cerebral oxygenation (Seifert et al., 2009). It is currently thought that the continued ability to produce energy in a hypoxic state is due to hypoxic inducible factor (HIF). HIF-1α, a subunit of HIF-1, plays a key role in maintaining oxygen homeostasis in the body (Iyer et al., 1998; Semenza, 2000). In normoxic conditions, HIF levels are maintained at low levels by oxygen dependent hydroxylase enzymes. When a hypoxic situation arises, the two hydroxylase enzymes lose their ability to function and the degradation of HIF-1α does not occur properly, leading to an increase in HIF-1α concentrations (Bracken et al., 2006). Thus, increased levels of HIF-1α may indicate a hypoxic situation and a change in metabolism.

In order to follow changes in metabolism, markers such as glucose transporter 1 (GLUT-1), glucose transporter 3 (GLUT-3), phosphofructokinase (PFK), lactate dehydrogenase (LDH), and 5′-AMP-activated protein kinase (AMPK) were measured as these glycolytic proteins play a significant role in cerebral metabolism. The GLUT-1 transporter serves to maintain a basal level of glucose transport in endothelial cells of the blood brain barrier (BBB). The GLUT-3 is found in high concentrations in neurons of the brain and can be up-regulated in states of hypoglycemia (Maurer et al., 2006). PFK-1 is a key regulatory enzyme for glycolysis and has been shown to be upregulated in times of metabolic demand (Minchenko et al., 2003). LDH is involved in the inter-conversion of lactate and pyruvate which can then be used as an alternative energy source when oxygen is lacking for oxidative phosphorylation (Schurr, 2002; Rossignol et al., 2003). Finally, AMPK (5′-AMP-activated protein kinase), a metabolite and stress-sensing kinase, regulates homeostasis and serves as an energy sensor in all eukaryotic cells (Kahn et al., 2005). AMPK is activated when cellular ATP is depleted or when the AMP/ATP ratio within the cell is increasing, as seen in conditions of ischemia, hypoxia, heat shock, or glucose deprivation. By comparing expressions of these proteins associated with cerebral glycolysis, we were able to gain a better understanding of how different exercise regimens affect cerebral metabolism which. This in turn helped to shed light on both the nature of neuroprotection as well as its underlying mechanisms following an ischemic event (Hayes et al., 2008).

2. Results

2.1. *Running distances*

We monitored the running distances of rats in both the voluntary and forced exercise groups in order to estimate the amount of exercise in the two groups. The daily average running distances of the individual voluntary exercise group members (4432.4 m) were significantly longer than those of the forced exercise group members (900 m), which is consistent with the findings in our previous study (Hayes et al., 2008). At this level of exercise, voluntary exercise did not induce neuroprotection when compared with that of the forced exercise group. The weight gain was comparable in the two exercise group.

2.2. *HIF-1α up-regulation*

In the course of our PCR analysis, where we measured the levels of HIF-1α mRNA that were synthesized, we found a significant difference ($F_{(2,18)} = 69.8$, $p < 0.01$) between the two groups (Fig. 1A). The voluntary exercise group resulted in a value of 1.63 ± 0.09, while the forced exercise group yielded a value of 2.38 ± 0.11 (Fig. 1A). Post-hoc comparison further indicates that expressions of HIF-1α in the forced exercise group were significantly ($p < 0.01$) higher than those found in the voluntary exercise group. HIF-1α protein levels were also measured and compared with the control group, designated as 1 in Fig. 1B, and we found that the voluntary exercise group measured at 2.24 ± 0.08, while the forced treadmill group was comparatively higher at 2.89 ± 0.23. Statistical analysis demonstrates that there were significant difference among the three groups ($F_{(2,18)} = 46.1$, $p < 0.01$), and a post-hoc analysis further highlights that HIF-1α protein levels in the forced exercise group were significantly ($p < 0.01$) higher than those found in the voluntary exercise group.

2.3. *Cerebral glycolysis after two different exercise regimens*

As a marker of increased glucose transports, GLUT-1 (Fig. 2A) and GLUT-3 (Fig. 3A) mRNAs were measured using real-time PCR. GLUT-1 ($F_{(2,18)} = 3362.4$, $p < 0.01$) and GLUT-3 ($F_{(2,18)} = 1560.2$, $p < 0.01$) levels in the voluntary exercise group were significantly lower than those found in the forced exercise group. Both groups contained significantly elevated mRNA levels when compared to the control group ($p < 0.01$). With respect to the levels of LDH mRNA (Fig. 4A), a significant