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Pre-ischemic treadmill training affects glutamate and gamma aminobutyric acid levels in the striatal dialysate of a rat model of cerebral ischemia

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ABSTRACT

Aims: Treadmill training has been shown to improve function in animal models and patients with cerebral ischemia. However, the neurochemical effects of this intervention on the ischemic brain have not been well studied. This study was designed to evaluate the effects of pre-ischemic treadmill training on the release of glutamate and γ-aminobutyric acid (GABA) from the striatum in a rat middle cerebral artery occlusion (MCAO) model.

Main methods: Rats were divided into five groups: sham control without MCAO, and 0, 1, 2 and 4 weeks pre-ischemic treadmill training. After training, cerebral ischemia was induced by MCAO for 120 min, followed by reperfusion. Microdialysis was used to collect dialysates from the striatum immediately before ischemia, and at 40, 80 and 120 min after ischemia, as well as at 40, 80, 120, 160, 200 and 240 min after reperfusion.

Key findings: Pre-ischemic treadmill training decreased glutamate release and increased GABA release during the acute phase of cerebral ischemia/reperfusion. Treadmill training for at least 2 weeks produced statistically significant changes in GABA/glutamate release.

Significance: The present study suggests that treadmill training inhibits the excessive release of glutamate, by stimulating GABA release during the acute phase of cerebral ischemia. This may be one of the important mechanisms to protect the striatal neurons from ischemic damage.

Introduction

Stroke is the third leading cause of death and a major contributor to human disability in many countries (Moseley et al. 2005). Stroke is often fatal and the prognostic impact of treatment is limited, therefore, primary prevention should be employed to reduce the risk of stroke onset (Paganini-Hill et al. 1988).

Treadmill training has been shown to be effective in improving rhythmical walking and to be useful for patients in the acute and chronic phase after stroke (Moseley et al. 2005). Scientists have also found that pre-ischemic locomotor activity effectively enhances survival in gerbils with forebrain ischemia (Stummer et al. 1994). In some laboratories, it has been demonstrated that long-term treadmill aerobic exercise decreases the thrombotic tendency in rat cerebral vessels, and suggested that such exercise has a protective effect on these blood vessels (Sasaki et al. 1995). Another study has shown that pre-ischemic treadmill training reduced cerebral edema and the volume of cerebral infarction in an animal model (Wang et al. 2001). In addition, many studies have found that pre-ischemic treadmill training can retain the integrity of the microvascular system, and reduce the increased permeability of the blood-brain barrier after stroke (Ding et al. 2006). Therefore, it is a fact that pre-ischemic training has a protective effect on subsequent cerebral ischemia. However, the mechanism involved is not well known.

Many studies have shown that, following hypoxic and ischemic brain injury, there is an excessive release of glutamate and over-stimulation of glutamate receptors, which results in neuronal excitotoxicity (Guyot et al. 2001). These increased excitatory neurotransmitters such as glutamate can trigger postsynaptic depolarization and cause influx of positive charges into the cells, which may result in neuronal death (Smith 2004). Humans also possess protective mechanisms, one of which is to release inhibitory neurotransmitters such as γ-aminobutyric acid (GABA) or taurine, which potentially counteracts the effects of excitatory transmitters by hyperpolarizing neuron membrane potential and inhibiting glutamatergic transmission (Costa et al. 2004; Luo and Guo 2005). Some studies have shown that GABA can decrease glutamate and aspartate release from the hippocampus and cortex in global cerebral ischemia, through pre-synaptic inhibition (Ouyang et al. 2004). These findings imply an interaction of GABA and glutamate release. In the present study, we

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focused on the striatum of rats with cerebral ischemia, to examine if pre-ischemic treadmill training changed the extracellular levels of glutamate and GABA. These cerebral regions are vulnerable to ischemia, and the levels of glutamate and GABA, as well as their interaction are closely related to the extent of cell death. The regulatory role of GABA receptors on glutamate release in the cerebral cortex, hippocampus and spinal cord has been reported (Matsumoto et al. 2003; Nelson et al. 2000; Tanaka et al. 2003). The striatum is known to be one of the core regions that is vulnerable to ischemic insult and enriched in both GABA and glutamatergic neurons. Some studies have focused on this region by attempting to block GABA receptors (Ouyang et al. 2007). However, no relevant study has investigated the effect of pre-ischemic training on ischemia of this region.

This study was designed to explore the neurochemical effects of treadmill training on brain ischemic lesions in rats, by evaluating dynamic changes in the key excitatory amino acid glutamate and the principal inhibitory amino acid GABA. Normal rats were subjected to electric treadmill training for different times. A rat ischemia model was established by middle cerebral artery occlusion (MCAO). We used microdialysis to collect the extracellular fluid in the striatum during ischemia/reperfusion in the rat model at different time points, and determine the levels of amino acid neurotransmitters, using high-performance liquid chromatography (HPLC).

Materials and methods

Animal preparation

All experiments were conducted in accordance with the guidelines of the National Institutes of Health Guide for the Care and Use of Laboratory Animals. Thirty clean grade male Sprague–Dawley rats (8–12 weeks of age, weighing 250–300 g) were provided by Shanghai Laboratory Animal Center, Chinese Academy of Sciences. The rats were housed in groups of two, under a 12:12-h light/dark cycle with food and water available ad libitum.

Treadmill training

Rats were assigned randomly to one of the five groups (sham control, 0, 1, 2 and 4 weeks treadmill training) for 4 weeks of pre-ischemic intervention (Zheng et al. 2007). There were 6 rats in each group. The rats in all the training intervention groups (hereinafter referred to as the training group), including the 1-, 2- and 4-week training groups, received 2 days adaptive running exercise of 5–8 m/min for 30 min/day before the formal treadmill training. After adaptive running, the rats started training on an electric treadmill machine (DSPT-202 Type 5-Lane Treadmill; Litai Biotechnology Co., Ltd, China). The rats in the 4-, 2- and 1-week training groups were scheduled to run on the treadmill during all 4 weeks, the last 2 weeks or the last 1 week of the 4-week experiment, respectively. The formal treadmill training was prescribed as 20 m/min, 30 min/day for 5 days per week. The rats in the sham control group and 0-week training group ran freely in their cage for 4 weeks. The rats in all groups were bred under the same conditions. Parameters of treadmill exercise were set at a slope of 0° and belt speed of 20 m/min.

Rat MCAO model

Rats were anesthetized with 12% (v/v) chloral hydrate (0.345 mg/kg, intraperitoneally) at the end of treadmill training. We kept the animals under anesthesia for at least 6 h by gradually supplementing chloral hydrate, based on animal response and their reaction to needle pricks. The rats then were put on a stereotaxic apparatus. The scalp was incised to expose the skull. A hole was made in the skull 0.4 mm behind the bregma, 3.4 mm left to the sagittal suture. A catheter with a stainless steel cannula was inserted into the striatum region (0.4 mm behind the bregma, 3.4 mm left of the sagittal suture, and a depth of 3.0 mm from the skull surface). The cannula was fixed with two screws and dental cement. The left MCA was occluded by the intraluminal suture technique (Longa et al. 1989), with some modifications. In the sham group, the external and internal carotid arteries were isolated but without ligation. The rats were scored after recovery from anesthesia based on a 5-point scale, with reference to the report of Longa et al. 0, no neurological symptoms; 1, unable to completely extend the front jaw on the other side; 2, rotating while crawling, and falling to the other side; 3, unable to walk without help; and 4, unconsciousness. Rats with a score of 1–3 points were considered a successful model and included in the study.

In vivo microdialysis

Artificial cerebrospinal fluid (130 mmol/L NaCl, 2.99 mmol/L KCl, 0.98 mmol/L $CaCl_2$, 0.80 mmol/L $MgC1_2 \cdot 6H_2O$, 25 mmol/L $NaHCO_3$, 0.04 mmol/L $Na_2HPO_4 \cdot 12H_2O$, 0.46 mmol/L $NaH_2PO_4 \cdot H_2O$, pH 7.4) was used as the perfusate for microdialysis and injected with a MD21001 power micro-injector. Rats were anesthetized with chloral hydrate and placed in the prone position in a stereotaxic apparatus. Dialytic samples were taken while rats were unconscious. The balanced microdialysis probe (with 15 kDa's cut-off PES membrane, membrane length 4 mm; MAB6.14.4 probe; Bioanalytical System, Inc., West Lafayette, IN, USA) was inserted into the striatum of the rat at the previously noted location. The inlet tube of the microdialysis probe was connected to a microinfusion pump (MD-0100; Bioanalytical System), and the outlet tube was connected to a 0.5-mL centrifuge tube to collect dialysate for further assay. The system was balanced for 90 min at a flow rate of 1 μL/min. Microdialysis was conducted in the striatum at a flow rate of 2 μL/min for a continuous 10 min, and each sample was collected in a 20-μL vial. Samples were collected at 10 time points, one before ligation, three at 40, 80 and 120 min after ligation, and six samples at 40, 80, 120, 160, 200 and 240 min after reperfusion.

Determination of brain infarction volume

Animals were anesthetized with 2% pentobarbital 24 h after reperfusion. Brains were quickly removed and put in a refrigerator at −20 °C for 10 min. Seven sections of brain tissue were cut at a coronal plane. The thickness of each section was 2 mm. The first cut was at the midpoint between the anterior pole and the optic chiasma. The sections of brain tissue were rapidly put into 2% TTC (2,3,5-triphenyltetrazolium chloride) solution (37 °C) for 30 min, followed by fixation in 4% paraformaldehyde buffer. The sections were photographed 24 h later with a digital camera (DC240; Kodak, USA). The images were processed to calculate the area of infarction with imaging software (Adobe Photoshop 7.0) (pink area was normal brain tissue, and pale areas indicated infarction). The total infarction volume was calculated as the sum of the area of brain infarction multiplied by the thickness of each section (2 mm).

Amino acid measurement by HPLC-FD (fluorescence detector)

The HPLC system used for the separation of amino acid neurotransmitters consisted of an Agilent 1100 LC system (Palo Alto, California, USA) equipped with a column incubator (G1316A), online degasser device (G1322A), four pumps (G1311A), a fluorescence detector (FD, G1321A), hand sampling system and HPLC workstation (Hewlett Packard, Palo Alto, California, USA). HPLC conditions were as follows: precolumn, Security Guard Cartridges C18, 4×3.0 mm (DIKMA, Tokyo, Japan) Inertsil ODS-3 analysis column (250×4.6 mm, 5 μm, DIKMA); mobile phase, 100 mM potassium phosphate buffer (pH 6.0): methanol: acetonitrile (60/30/10 v/v/v); flow rate, 1 ml/min; fluorescent conditions, excitation wavelength of