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Research Report

Exercise increased BDNF and trkB in the contralateral hemisphere of the ischemic rat brain

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Abstract

Previous studies have suggested that brain-derived neurotrophic factor (BDNF) and trkB both have a role in plasticity following brain insults and exercise increases BDNF and trkB mRNA levels in the normal brain. We attempted to determine whether treadmill exercise improves motor function following experimental cerebral ischemia, and whether motor outcome is associated with BDNF and trkB expression. We subjected adult male Sprague–Dawley rats to a permanent ischemia, followed by either 12 days of treadmill exercise or non-exercise. In the exercise group, improvements in the motor behavior index were found and BDNF and trkB proteins in contralateral hemisphere were increased. This study suggests that after permanent brain ischemia, exercise improves motor performance and elevates BDNF and trkB proteins in the contralateral hemisphere.

Theme: Development and regeneration
Topic: Neurotrophic factors: expression and regulation

Keywords: Treadmill exercise; Ischemia; Neurotrophin; Motor behavior index; BDNF; trkB

1. Introduction

Data from several population-based study cohorts indicate that the incidence of stroke in the United States is between 500,000 and 730,000 annually, and that this results in significant morbidity, mortality and disability, particularly among people older than 65 [18]. Exercise has beneficial effects on brain function, including the promotion of plasticity. Exercise is one of the few known interventions associated with recovery enhancement across a variety of neurological disorders. Although behavioral improvements and structural alterations in the brain are well documented, little is known regarding the underlying mechanisms, that are responsible for mediating these enhancements in recovery [7].

Neurotrophic factor might play a role in neuronal survival, proliferation, maturation and outgrowth in the developing brain and neuroprotective functions in mature brain insult. BDNF mRNA was increased bilaterally in dentate granule cells, and in CA1 and CA3 pyramidal neurons in a middle cerebral occlusion rat model [14]. Following ischemia, BDNF mRNA levels were increased at 2, 3 and 12 days after the ischemic event [22].

In animal studies, exercise has been linked to an increase in neuronal activity in the brain. An abrupt interruption of

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prolonged spontaneous running in a rat strain decreased expression of mRNA encoding BDNF and trkB in certain hippocampal areas and this decrease lasted at least 10 days [21]. Voluntary running for 3 weeks increased the mRNA levels of BDNF [19]. Moreover, enriched environmental effects on functional recovery and neurotrophic factor have been actively investigated in ischemic rat models [7,10,17,23], but the exercise effect has not been studied as well. According to a recent review, exercise might act as a gate that primes the brain to respond to environmental stimulation, while simultaneously increasing the ability of neurons to resist insult [3].

We undertook the investigation of the exercise effect on motor function and the expressions of BDNF and trkB in an ischemic rat model. We hypothesized that exercise promotes motor function and changes the expressions of BDNF and trkB. We analyzed ipsilateral and contralateral hemispheres to determine the effects of ischemic injury.

2. Materials and methods

2.1. Experiment I

2.1.1. Experimental design

Thirty-five permanent ischemic middle cerebral artery occlusion (MCAO) rat models and 12 sham operated rats were prepared. The rats were subjected either to exercise (n = 18) or to non-exercise (n = 17) after matching their postoperation 2-day motor behavior scores. Matching was done by the following assortment: mild (12–18 score), moderate (8–11), severe (3–7). Motor behavior scores were measured at 2-, 9- and 16-day postoperatively.

2.1.2. Animal model and treadmill exercise

A MCAO adult male Sprague–Dawley rat (275–325 g) model was used. The protocols for the care and use of animals in this procedure were in compliance with guidelines and were approved by the Catholic University animal care committee. For focal cerebral ischemia, we used Longa's methods as previously described [15]. In brief, after an intraperitoneal injection of 1% ketamine (30 mg/kg) and xylazine hydrochloride (4 mg/kg), the left common carotid artery was exposed at its bifurcation through a midline cervical incision. The branches from the external carotid artery were coagulated and the pterygopalatine artery was ligated with a 5.0 silk suture. A 4.0 nylon monofilament (its tip rounded by heating) was then inserted into the bifurcation site of the common carotid artery. To occlude the origins of the middle cerebral and proximal anterior cerebral artery, monofilament was advanced 16–18 mm into the internal carotid artery from the bifurcation site. The monofilament was secured in place with a ligature and the wound was closed. The animals were allowed to survive for 16 days with food and water ad libitum. Rectal temperature was maintained at 37 ± 1 °C using a thermistor-controlled heating blanket. Exercise groups exercised on a rat treadmill (Columbus instruments, U.S.A) from postoperation 4-day to 15-day, for 12 days at 30 min per day. Treadmill velocity was 10 m/min on the first exercise day, 15 m/min on the second and 20 m/min on the third and subsequent days, the tilting angle was 0°.

2.1.3. Motor behavior index and statistical analysis

Motor behavior index was measured blind to rat groupings by Garcia's score [5] at 10 a.m. on postoperative days 2, 9 and 16 to account for diurnal variation. Six items were measured and the total score ranged from 3 to 18; the higher the score, the better the motor performance. Items 1–4 (spontaneous activity, symmetry of movements, symmetry of forelimbs and climbing the wall of wire cage) measured motor performance, and items 5–6 (reaction to touch on and response to vibrissae touch) measured sensory function (see Appendix A).

The repetitive measured ANOVA in SPSS ver. 12.0 was used to compare the motor behavior indexes of the ischemia-exercise and ischemia-non-exercise groups. The t test and paired t test was used for comparing the densitometry.

2.2. Experiment II

2.2.1. Experimental protocol

On postoperative 16-day, Western blot and immunohistochemistry were performed after sacrifice. The rats were subjected either to Western blot or to immunohistochemistry after matching their postoperation 2-day motor behavior scores. Matching was done by the following assortment: mild (12–18), moderate (8–11), severe (3–7). The exercise and non-exercise groups of the ischemic models and of the sham rats were compared.

In addition, to see temporal change of expression of trkB by the Western blot, 12 rats were added in this experiment. Each four-rat was sacrificed on postoperative days 9, 16 and 23. Exercise was continued by the sacrifice.

2.2.2. Protein extraction from brain

The total protein extract from the brain was prepared as previously described [2,4]. In brief, rats were deeply anesthetized with 1% ketamine (30 mg/kg) and xylazine hydrochloride (4 mg/kg), and rapidly decapitated. Brains were dissected into right and left hemispheres, and placed on ice in 10 volumes of cold homogenization buffer (50 mM Tris, 120 mM NaCl, pH 7.4) to which protease inhibitors (Complete Mini, Gibco, Grand Island, NY) had been freshly added. The tissue was then homogenized and stored at −80 °C. Protein concentrations were determined by using the Bradford method (Bio-Rad, Richmond, CA).

2.2.3. Western blot

Protein extracts from brain tissue (20 μg) were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Protein separation was performed using a