Available online at www.sciencedirect.com

SciVerse ScienceDirect

www.elsevier.com/locate/brainres

BRAIN RESEARCH

Research Report

Alteration of protein expression profile following voluntary exercise in the perilesional cortex of rats with focal cerebral infarction

Kenmei Mizutani[a,*], *Shigeru Sonoda*[a], *Keiki Yamada*[b], *Hidehiko Beppu*[a], *Kan Shimpo*[a]

[a]*Division of Biochemistry, Fujita Memorial Nanakuri Institute, Fujita Health University, Mie, Japan*
[b]*Department of Anatomy, Faculty of Medical Technology, School of Health Sciences, Fujita Health University, Aichi, Japan*

ARTICLE INFO

Article history:
Accepted 7 August 2011
Available online 12 August 2010

Keywords:
Neuronal plasticity
Stroke rehabilitation
Functional recovery
Middle cerebral artery occlusion
Voluntary exercise
Antibody microarray

ABSTRACT

Identification of functional molecules in the brain related to improvement of the degree of paralysis or increase of activities will contribute to establishing a new treatment strategy for stroke rehabilitation. Hence, protein expression changes in the cerebral cortex of rat groups with/without voluntary exercise using a running wheel after cerebral infarction were examined in this study.

Motor performance measured by the accelerated rotarod test and alteration of protein expression using antibody microarray analysis comprised 725 different antibodies in the cerebral cortex adjacent to infarction area were examined.

In behavioral evaluation, the mean latency until falling from the rotating rod in the group with voluntary exercise for five days was significantly longer than that in the group without voluntary exercise. In protein expression profile, fifteen proteins showed significant quantitative changes after voluntary exercise for five days compared to rats without exercise. Up-regulated proteins were involved in protein phosphorylation, stress response, cell structure and motility, DNA replication and neurogenesis (11 proteins). In contrast, down-regulated proteins were related to apoptosis, cell adhesion and proteolysis (4 proteins). Additional protein expression analysis showed that both growth-associated protein 43 (GAP43) and phosphorylated serine41 GAP43 (pSer41-GAP43) were significantly increased.

These protein expression changes may be related to the underlying mechanisms of exercise-induced paralysis recovery, that is, neurite formation, and remodeling of synaptic connections may be through the interaction of NGF, calmodulin, PKC and GAP43. In the present study at least some of the participation of modulators associated with the improvement of paralysis might be detected.

1. Introduction

About 20 years ago, it was commonly thought that hemiparesis after stroke hardly recovered; however, it has become widely known that training or rehabilitation brings about some improvement of paralysis by neuronal plasticity in an area adjacent to the lesion and in the contralateral hemisphere (Johansson, 2000; Nudo et al., 1996). In recent research, brain

* *Corresponding author at:* Division of Biochemistry, Fujita Memorial Nanakuri Institute, Fujita Health University, Tsu, Mie 514-1296, Japan. Fax: +81 59 252 0710.
E-mail address: kenmei@fujita-hu.ac.jp (K. Mizutani).

0006-8993/$ – see front matter © 2011 Elsevier B.V. All rights reserved.
doi:10.1016/j.brainres.2011.08.012

imaging using functional MRI (fMRI) has revealed cortical reorganization in these areas in patients with complete or partial upper limb recovery (Feydy et al., 2002). Similarly, morphological studies in brain-damaged rats indicate a structural change of dendritic branches and the spine in the contralesional hemisphere and in the perilesional cortex (Biernaskie and Corbett, 2001; Brown et al., 2008). Another aspect of brain plasticity, growth factors that include nerve growth factor (NGF), glial cell line-derived neurotrophic factor (GDNF), insulin-like growth factor (IGF-I), brain-derived neurotrophic factor (BDNF) and other growth-promoting substances, enhance neuronal and/or glial cell differentiation, neurite outgrowth and remodeling after stroke (Ding et al., 2006; Kleim et al., 2003; Lykissas et al., 2007). Although these structural changes are linked to the outcomes of rehabilitation, little is known about the relationship between functional recovery and molecular mechanisms in the brain. What happens in the brain? Of course, after cerebral infarction the brain shows not only neuronal plasticity, but also a variety of physiological phenomena, such as inflammatory responses, oxidative stress after reperfusion, necrosis, neuronal degeneration and loss through post-ischemic apoptosis, changes energy metabolism, formation of edema, tissue repair, neuroprotection, ischemic tolerance and glial dynamics related to angiogenesis (Buga et al., 2008; Krupinski et al., 1996).

These global changes following cerebral infarction have been mainly observed in natural recovery without intervention (Keyvani et al., 2002; Raghavendra et al., 2002), and little research has paid attention to the molecular changes caused by movement or rehabilitation. Therefore, we focused on molecular alteration with/without exercise, and verified how voluntary exercise after brain infarction influences these phenomena following cerebral infarction.

In this study, to explore the difference in the appearance of functional molecules, we analyzed the protein expression profile in the cerebral cortex adjacent to the infarction area using antibody microarray and Western blotting at the same time as observing exercise-induced functional recovery in rats with cerebral infarction.

2. Results

2.1. *Effect of voluntary exercise on motor performance with the rotarod test*

Motor performance analysis using the accelerated rotarod test in the infarction-CNT group and infarction-EX group is shown in Fig. 1. The mean latency until falling from the rotating rod decreased to approximately 1/5 at 2 days after infarction in the infarction-CNT group (36.7±46.0 s), and in the infarction-EX group (37.1±46.9 s) compared before producing cerebral infarction, the variance of mean latency was not different between the infarction-CNT group and infarction-EX group at the time of pre-infarction and 2 days after infarction. Similarly, the mean latency 5 days after surgery was not significantly different; however, the mean latency in the infarction-EX group (104.1±55.4 s) was significantly longer than in the infarction-CNT group (52.3±48.9 s) 7 days after surgery ($P<0.01$), and this difference was seen until 12 days after cerebral infarction. Voluntary exercise using a running wheel for 12 h a day resulted in extended latency with the rotarod test, that is, improved motor coordination and performance in rats with cerebral infarction.

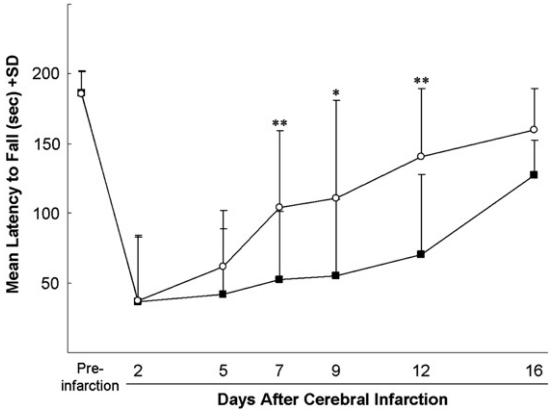

Fig. 1 – Behavioral and motor functional analysis using rotarod test. The time course of improvement in motor coordination and performance, and the time until falling off the rotating rod were measured pre-infarction and 2, 5, 7, 9, 12 and 16 days after surgery. The infarction-EX group (open circle) was compared with the infarction-CNT group (closed square), and significant differences are indicated as $^{*}P<0.05$, $^{**}P<0.01$.

2.2. *Alteration of running distance using a running wheel*

The running distance by voluntary exercise in the infarction-EX group for protein expression analysis is shown in Fig. 2. The mean distance using a running wheel for 12 h/day was 402.2±113.7 meters (m) at the time of pre-infarction. On the first day of exercise (initiated from 2 days after infarction), the distance decreased by approximately half (198.8±37.8 m) of the running distance without cerebral infarction. Subsequently, the distance gradually increased, and became 362.8±194.6 m on the fifth day of exercise.

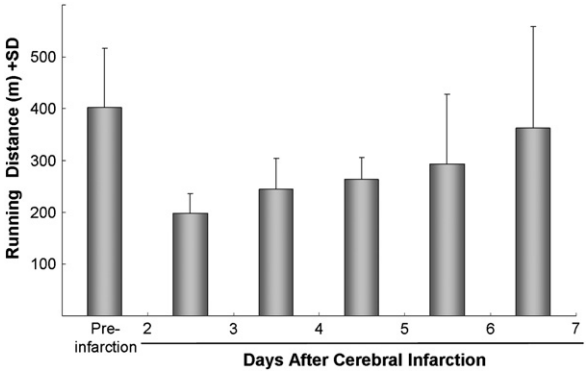

Fig. 2 – Running distance using a running wheel. The running distance (meters) of voluntary exercise by six animals used for protein analysis is shown (mean±SD). Each running distance was measured on a running wheel for 12 h/day in the dark period at pre-infarction and for 5 days from 2 days after cerebral infarction.