Study of Dopamine Mechanism in Striatum When Voluntary Wheel Running Improves Recovery of Cognitive Function After Global Cerebral Ischemia

Yongzhao Fan, Yuhan Wang, Wenliang Ji, Kun Liu*, Xiangjiang Rong*
Capital University of Physical Education and Sports, Beijing, China
*Corresponding author: Kun Liu and Xiangjiang Rong

ABSTRACT: Physical exercise has been demonstrated to be neuroprotective in ischemia models. However, the exact neuroprotective mechanism needs to be further studied. The purpose of this research is to discuss the effect of pre-ischemic voluntary wheel running on levels of dopamine in the striatum and cognition in ischemia rats. 12 Sprague-Dawley rats were divided randomly in following two groups: 0 week pre-ischemia wheel running (0-WR) group and 4 weeks pre-ischemia wheel running (4-WR) group. After training, global cerebral ischemia was induced by two-vessel occlusion (2-VO). Microdialysis and high performance liquid chromatography system (HPLC) were used to collect and determine dopamine in the dialysates of striatum. Passive avoidance was used to test cognitive function 24 h after ischemia. The results showed that striatal dopamine of 0-WR group was rapid increasing after ischemia, but the increase of striatal dopamine level was attenuated in 4 weeks group. Behavioral data indicated that voluntary wheel running promoted cognition recovery after ischemia. Thus, pre-ischemia voluntary wheel running attenuates the increase of dopamine of striatum induced by cerebral ischemia and improves cognitive function in ischemia rats.

1. INTRODUCTION

Cerebral ischemia is a major factor that could cause death and disability in the world. In 2012, there are about 6.7 million people died of cerebral ischemia in the world. Many studies have shown that physical exercise preconditioning ameliorates cerebral ischemia-induced injury. However, the exact mechanisms are poorly understood.

Dopamine is an important monoamine neurotransmitter in the brain, it plays a significant role in memory processes, especially through the interconnection of the striatum and the prefrontal cortex. Brouns R et al. reported that ischemic brain injury displayed relevant interrelations to the change in monoamine neurotransmitters (Brouns et al. 2010). Yoshimoto K et al. found that the 4-VO treatment induced massive increases of DA and 5-HT releases in the nucleus accumbens (Yoshimoto et al. 2009). Lan X et al. found that exercise could improve neurologic function, enhance neuronal plasticity and upregulate the levels of 5-HT, 5-HT1AR and BDNF in cortex tissues of rats with cerebral ischemia (Lan et al. 2014). But whether pre-ischemia exercise protects the brain from ischemia by regulating the dopamine level in the striatum still need to be further explored.

Hence, the purpose of our study is to investigate the effect of pre-ischemic voluntary wheel running on levels of dopamine of striatum and cognition in ischemia rats. It may be of great importance in understanding the neurochemical processes in ischemic stroke and lays fundamental on the prevention of cerebral ischemia.

2. MATERIALS AND METHODS

2.1. Animals and wheel training

Twelve clean grade male Sprague–Dawley rats (8–12 weeks of age, weighing 250–300 g) were provided by Experimental Animal Center of Peking University. The rats were housed under a 12:12-h light/dark cycle with food and water ad libitum. All the rats were divided randomly in following two groups: 0 week pre-ischemia wheel running (0-WR) group and 4 weeks pre-ischemia wheel running (4-WR) group. The rats in 0-WR group were housed in normal cages without any exercise. Rats in 4-WR group were housed in wheel cages with free running for 4 weeks, separately.

2.2. Surgery procedures and In vivo microdialysis

The surgery procedures of stainless steel guide
cannula was lowered into the right striatum and two-vessel occlusion (2-VO) surgery and the In vivo microdialysis as previously described (Liu et al). Each sample was collected in a 250µL tube, which contained 15µL 10mmol HCL and was placed in the ice box. Samples were stored at -80°C before analysis of dopamine concentrations was completed.

### 2.3. High performance liquid chromatography

Dopamine levels in samples were measured using HPLC with electrochemical detection. The flow rate of the mobile phase (50mM NaH2PO3, 2mM decanesulfonic acid, 0.7mM ethylenediaminetetraacetic acid, 11% v/v acetonitrile, and 11% v/v methanol, pH = 6.0 with 6N NaOH) was 1uL/min.

### 2.4. Passive avoidance test

Cognitive function was assessed by applying a step-through passive avoidance task as previously described (Tahamtan et al. 2013). After the learning trial, the retention test was measured 24 h after the learning trial. Each animal was put in the illuminated compartment and the door opened after 2 minutes. Latency to enter the dark compartment was recorded to a maximum of 300s. Animals that did not enter the dark chamber during the retention test were allotted a latency of 300s.

### 2.5. Histological studies

In the end of the experiment, we used 10% chloral hydrate deeply anesthetized animals and used saline and 4% formalin for cardiac perfusion. Coronal sections (100µm) were made with a vibratome. Sections were placed on gelatin-coated slides and were stained with cresyl violet. After that, the brain coronal slices (100µm) were taken for the observation under the microscope and were decided whether microinjection brain regions are striatum in the brain.

### 2.6. Statistical analysis

Microdialysis data were converted to percent of baseline before analysis, and analyzed by GraphPad Prism 5. Dopamine data were analyzed by a two-way analysis of variance (ANOVA) with one repeated measure (time) and an unpaired Student’s t test. Behavior data were analyzed by a paired Student’s t test by GraphPad Prism 5.

### 3. RESULTS

#### 3.1. Dynamic changes of striatal dopamine level of the two groups

Figure 1 shows the level of dopamine in the striatal dialysates from 30 minutes before ischemia to 90 minutes after ischemia. In the process of ischemia, the striatal dopamine level of 0-WR group increased rapidly and reached maximum value at the time point of 30 min after ischemia (P<0.001), then decreased gradually. The striatal dopamine levels of 0-WR group at the time point of 20 min, 30 min, 40 min, 50 min, 60 min after ischemia were significant higher than baseline level before ischemia. The striatal dopamine level of 4-WR group increased slowly and reached maximum value at the time point of 30 min after ischemia, then decreased gradually. There was no significant difference between baseline level before ischemia and those after ischemia in 4-WR group. Compared with 0-WR group, the extracellular dopamine levels of 4-WR group were significant lower at the time point of 50min, 60min, 80min, 90min after ischemia. This result indicated that 4 weeks pre-ischemia wheel running significantly inhibited the rapid increase of striatal dopamine after ischemia.

#### 3.2. Passive avoidance test

Figure 2 shows that the latency time of retention test of the two groups. The latency time of 4-WR group was significant longer than that of 0-WR group in the passive avoidance test. It indicated that cognitive function of 4-WR group rats were better than that of 0-WR group rats (P<0.05).

#### 3.3. Microdialysis probe placements in the striatum

Figure 3 shows microdialysis probe placements in the striatum. Microdialysis probe placements were
located at 0 mm caudal to bregma, 3.0 mm medial-lateral, and 4 mm ventral from the surface of the skull. Animals with incorrect located sites were removed from the study.

Figure 3. Microdialysis probe placements in the right striatum.

4. DISCUSSION

Exercise is an effective way to reduce damage in cerebral ischemia. Researches on the mechanisms of cerebral ischemia are extensive, such as neurotrophic factors, free radical reactions, ionic imbalance and other processes. However, few studies investigated the effect of exercise on monoamine neurotransmitters.

It was reported that ischemia injury of striatum was closely related to the change of extracellular dopamine level. In present study, we found that the extracellular dopamine level of the striatum in 0-WR group showed rapid increasing, reached maximum value at the 30 minutes, and then decreased gradually, this finding was in consistent with other previous studies (Jin, G et al. 2014). Kanthan R et al. reported that the ischemic response of dopamine was correlated with the glutamate levels (Kanthan et al. 1996). The extracellular glutamate level of striatum would be rapid increasing in the process of ischemia and it may induce the release of dopamine in the striatum. In our study, we found that the extracellular dopamine level of 4-WR group increased slowly and reached maximum value at the time point of 30 min after ischemia, then decreased gradually. This finding was in consistent with other previous studies. Mizutani K et al reported that dopamine level was significantly decreased in the exercise group at 10 days after infarction (Mizutani et al. 2013). This could be due to exercise preconditioning upregulated the GLT-1 expression and gamma-aminobutyric level, decreased extracellular glutamate concentration, then reduced the release of dopamine.

In the passive avoidance test, the latency time of the retention test of 4-WR group was longer than that of 0-WR group, this finding was consistent with previous studies (Tahamtan et al. 2013). Park C Y, et al. found that exercise could improve learning ability of ischemia rats by ameliorating degeneration of dopaminergic neurons in the substantia nigra and loss of dopaminergic fibers in the striatum (Park et al. 2013). Our results indicated that as preconditioning intervention, exercise improves behavioral ability following ischemia.

In summary, exercise preconditioning could significantly decrease the overly release of extracellular dopamine of striatum induced by global cerebral ischemia, reduce the cognitive deficits induced by global cerebral ischemia. Thus, scientific and rational exercise preconditioning can be an effective approach to prevent ischemia brain injury.

5. CONCLUSION

According to our results, pre-ischemia voluntary wheel running attenuates the increase of dopamine of striatum induced by cerebral ischemia and improves cognitive function in ischemia rats.

6. ACKNOWLEDGEMENTS

We gratefully acknowledge the financial support by NSF of China (Grant No. 30900709), Innovation Platform of Beijing Municipality (Grant No. PXM2015_014206_000053, PXM2015_014206_000072, PXM2015_014206_000051). The authors declare no conflict of interest.

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