Neuroscience Letters 594 (2015) 144–149

Contents lists available at ScienceDirect

Neuroscience Letters

journal homepage: www.elsevier.com/locate/neulet

Research article

A bout of treadmill exercise increases matrix metalloproteinase-9 activity in the rat hippocampus

Takeshi Nishijima*, Masashi Kawakami, Ichiro Kita

Laboratory of Behavioral Physiology, Graduate School of Human Health Sciences, Tokyo Metropolitan University, 1-1 Minami-Osawa, Hachioji, Tokyo 192-0397, Japan

HIGHLIGHTS

- We examined changes in MMP-9 activity in the rat hippocampus following exercise.
- MMP-9 activity in the rat hippocampus was increased 12 h after mild exercise.
- MMP-9 might be a new molecular target regulating exercise-induced hippocampal plasticity.

ARTICLE INFO

Article history:
Received 29 December 2014
Received in revised form 19 March 2015
Accepted 30 March 2015
Available online 1 April 2015

Keywords:
Exercise
Hippocampus
MMP-9
Gel zymography
Extracellular matrix

ABSTRACT

Regular exercise induces a variety of structural changes in the hippocampus of rodents, although the underlying mechanisms remain obscure. Particularly, the possible involvement of molecules regulating the remodeling of the extracellular matrix (ECM) is under-studied. Matrix metalloproteinase-9 (MMP-9), an extracellular protease, plays a critical role in regulating neuronal plasticity by remodeling the ECM in the brain. The current study used gel zymography to examine for changes in the proteolytic activity of MMP-9 in the rat hippocampus following a bout of treadmill exercise at mild (10 m/min) or moderate (25 m/min) intensity. We found that MMP-9 activity was significantly increased at 12 h after mild treadmill exercise. However, the activity of MMP-2 and the expression level of the tissue inhibitor of metalloproteinase-1 (TIMP-1) were unchanged following exercise. These findings suggest that exercise triggers MMP-9 activation in the hippocampus, which might be a new molecular mechanism of exercise-induced hippocampal plasticity.

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1. Introduction

The hippocampus, a highly plastic part of the brain, adaptively responds to physical exercise. Previous studies have demonstrated that regular exercise induces a variety of structural changes in the hippocampus, including neurogenesis [17,22,24], synaptogenesis [3], and angiogenesis [23], which possibly contribute to functional improvement such as better spatial learning [2]. However, the molecular mechanisms underlying these exercise-induced structural changes are still unveiled.

The central nervous system consists of not only cells, *i.e.*, neurons and glia, but also the extracellular environment, which occupies approximately 20% of the brain volume [20]. In order to maintain cellular function, proteolytic disassembly and remodeling of the extracellular matrix (ECM) is critical [26]. In the brain, matrix metalloproteinases (MMPs) are a major family of extracellular proteases that play a pivotal role in activity-dependent remodeling of the ECM [5,12]. Therefore, it is possible that MMPs are involved in regulation of the exercise-induced structural changes in the hippocampus.

MMP-9, forming a subfamily of gelatinase with MMP-2, is a well-characterized extracellular protease in the brain. MMP-9 is mainly released pericellularly by neurons, though it is released to some extent by glial cells as well [11,13,21]. In most studies, MMP-9 is thought of as a key mediator of pathological manifestations. Ischemia- or kainate-induced excitotoxicity is known to induce remarkable MMP-9 activation in the hippocampus, resulting in neuronal damage [7,8,27]. However, MMP-9 also functions in forms of neuronal plasticity such as long-term potentiation [15,25], neurogenesis [1], dendritic remodeling [21], and hippocampus-dependent learning and memory [10,14,15]. Importantly, the proteolytic activity of MMP-9 is controlled by neuronal activity

* Corresponding author at: Graduate School of Human Health Sciences, Tokyo Metropolitan University, 1-1 Minami-Osawa, Hachioji, Tokyo 192-0397, Japan. Tel.: +81 42 677 2963; fax: +81 42 677 2961.
E-mail address: t-nishijima@tmu.ac.jp (T. Nishijima).

http://dx.doi.org/10.1016/j.neulet.2015.03.063
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[4,11,13]. For example, the enhanced neuronal activity triggered by pentylenetetrazole treatment transiently increases the activity of MMP-9, but not of MMP-2, through NMDA receptor activation in the mouse hippocampus [13]. Since treadmill exercise induces glutamatergic NMDA receptor activation in the rat hippocampus [16], we hypothesized that a bout of treadmill exercise would increase MMP-9 proteolytic activity in the rat hippocampus.

To test this hypothesis, we examined temporal changes in the proteolytic activity of MMP-9 in the rat hippocampus following a bout of treadmill exercise. Since hippocampal neuronal activation caused by treadmill exercise is intensity dependent [9,19], rats were subjected to treadmill exercise at either mild (10 m/min) or moderate (25 m/min) speed. The hippocampi were collected at 0, 6, 12, and 24 h after the exercise. The proteolytic activities of MMP-9, as well as of MMP-2, were analyzed by gel zymography. In addition, expression of the tissue inhibitor of matrix metalloproteinase-1 (TIMP-1), an endogenous MMP-9 inhibitor, was also examined.

2. Materials and methods

2.1. Animals

Eight-week-old adult male Wistar rats (SLC, Shizuoka, Japan) were housed in groups 3–4 to a cage under controlled conditions of temperature (22–24 °C) and light (12/12-h light/dark cycle, light on at 5:00), and provided food and water *ad libitum*. All experimental procedures were approved by the Animal Experimental Ethics Committee of the Tokyo Metropolitan University.

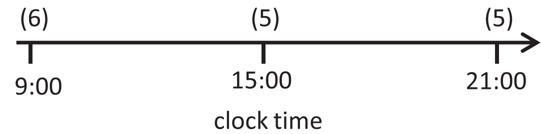
2.2. Experimental designs

This study consisted of three experiments. First, we validated our gel zymography protocol with kainic acid-induced MMP-9 activation in the hippocampus (Experiment 1). Twenty-four hours after an intraperitoneal injection of saline ($n = 2$) or kainic acid (10 mg/kg bw; $n = 2$), rats were deeply anesthetized with pentobarbital sodium and transcardially perfused with cold saline. The hippocampi were quickly dissected out, frozen with liquid nitrogen, and stored at −80 °C. The proteolytic activities of the MMP-9/-2 were analyzed by gel zymography, as described in the following sections.

Next, we examined whether the activities of MMP-9/-2 and the expression of TIMP-1 in the hippocampus show diurnal variation (Experiment 2). Rats were sacrificed and their hippocampi collected at 9:00 ($n = 6$), 15:00 ($n = 5$), and 21:00 ($n = 5$), corresponding to what in Experiment 3 would be at 0, 6, and 12 h after treadmill exercise, respectively (Fig. 1A).

Finally, we examined temporal changes in hippocampal MMP-9/-2 activities and TIMP-1 expression following a bout of treadmill exercise at one of two intensities (Experiment 3). All rats ($n = 100$) were habituated for 7 days to running in the treadmill apparatus (KN-73, Natsume, Japan) for 30 min/day. During the training session, the exercise speed was gradually increased, so that the rats were able to run at a speed of 25 m/min by the last day. After 4 days of rest to allow the short-term effects of the treadmill habituation procedure to decay, the rats were randomly allocated to three groups: control, mild exercise, and moderate exercise. Rats in mild or moderate groups were subjected to treadmill exercise for 30 min at a speed of 10 or 25 m/min, respectively, speeds below and above the lactate threshold (LT; approximately 20 m/min), a validated physiological index of exercise intensity in rats [18]. Rats in the control group were placed on a stationary treadmill for 30 min. Treadmill exercise was started at 8:30 AM, and the hippocampi were collected at 0, 6, 12, or 24 h after the end of exercise (Fig. 1B).

A: Experiment 2



B: Experiment 3

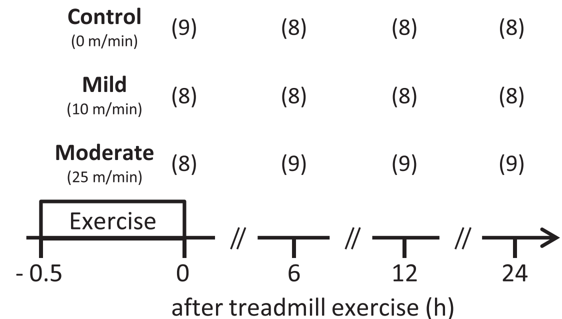


Fig. 1. Timeline of the experiments. In Experiment 2, rats were sacrificed at 9:00, 15:00, and 21:00 (A). In Experiment 3, rats were randomly subjected to a bout of treadmill exercise at either mild (10 m/min) or moderate (25 m/min) intensity for 30 min. Control rats were placed on a stationary treadmill. Rats were sacrificed 0, 6, 12, and 24 h after exercise (B). Figures in parentheses are the number of rats sacrificed at each time points.

2.3. Tissue preparation

The hippocampus from one hemisphere was homogenized in 500 μL of lysis buffer (20 mM Tris–HCl pH 7.6, 150 mM NaCl, 5 mM $CaCl_2$, 1% Triton X-100, 1% glycerol, 500 μM PMSF). The lysates were centrifuged for 10 min at 10,000 rpm at 4 °C and supernatants collected. A portion of the supernatant was mixed with additional lysis buffer containing protease inhibitors (Complete Mini, Roche, Germany), and allocated to protein assay and western blotting, whereas the remainder was used for gel zymography without adding protease inhibitors. Protein concentrations were measured with a BCA protein assay kit (Thermo Scientific, USA).

2.4. Gel zymography

MMP-9/-2 activities in the hippocampus were measured by gel zymography according to a previous report, with minor modifications [13]. Samples containing 1 mg of protein were incubated with 50% Gelatin Sepharose 4B (GE Healthcare, USA) for 24 h at 4 °C with shaking for MMP-9/-2 extraction. After centrifugation (500 × g, 2 min, 4 °C), the pellets were washed three times with working buffer (50 mM Tris–HCl pH 7.6, 150 mM NaCl, 5 mM $CaCl_2$, 0.05% Brij 35), then resuspended in 100 μL of the working buffer containing 10% DMSO. After a 2-h incubation with shaking, 20 μL of the eluates was mixed with 10 μL of Laemmli buffer not containing reducing agent, and subjected to electrophoresis without prior boiling on 10% SDS-polyacrylamide gels containing 0.1% gelatin. After washing twice in 2.5% Triton X-100 to remove excess SDS, gels were incubated in Novex® zymogram developing buffer (Invitrogen, USA) for 48 h at 37 °C to allow for enzymatic digestion of gelatin. Then gels were stained with 0.25% Coomassie brilliant blue G-250 (Sigma–Aldrich) for 30 min and de-stained with 10% methanol containing 7% acetic acid. The total gelatinolytic activities including pro-MMPs were densitometrically quantified using Image-J software (NIH).

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