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Research article

Exercise-induced expression of monocarboxylate transporter 2 in the cerebellum and its contribution to motor performance

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HIGHLIGHTS

- Inhibition of lactate transport via MCT2 in the cerebellum impaired motor performance.
- Acute exercise increased MCT2 expression in the cerebellum at the transcriptional level, but not at the protein level.
- These results suggest that lactate transport via MCT2 in the cerebellum may play an important role in motor performance.

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ABSTRACT

Monocarboxylate transporter 2 (MCT2) is an important component of the lactate transport system in neurons of the adult brain. Purkinje cells in the cerebellum have been shown to have high levels of MCT2, suggesting that this protein has a key function in energy metabolism and neuronal activities in these cells. However, it is not known whether inhibition of lactate transport via MCT2 in the cerebellum affects motor performance. To address this question, we examined motor performance in mice following the inhibition of lactate transport via MCT2 in the cerebellum using α-cyano-4-hydroxycinnamate (4-CIN). 4-CIN or saline was injected into the subarachnoidal space of the cerebellum of mice and motor performance was analyzed by a rotarod test both before and after injection. 4-CIN injection reduced retention time in the rotarod test by approximately 80% at 1 h post-injection compared with pre-injection. No effect was observed at 2 h post-injection or in mice treated with the vehicle control. Because we observed that MCT2 plays an important role in motor performance, we next investigated the effects of acute exercise on MCT2 transcription and protein levels in mice sampled pre-exercise and at 0 and 5 h after 2 h of treadmill running. We found a significant increase in MCT2 mRNA levels, but not of protein levels, in the cerebellum at 5 h after exercise. Our results indicate that lactate transport via MCT2 in the cerebellum may play an important role in motor performance and that exercise can increase MCT2 expression at the transcriptional level.

1. Introduction

Although glucose is considered the primary energy substrate for the adult brain, lactate is also used for various energy consuming activities in neurons; these lactates are produced and released by astrocytes within the brain [1,2]. In order to make use of the lactates, it is necessary to transport them from their site of production to the site of utilization. This transport is carried out by proteins termed monocarboxylate transporters (MCTs). There are 14 subtypes of MCT, and the predominant subtypes found in the brain are MCT1, MCT2 and MCT4 [3]. Interestingly, the distribution and function of MCTs show sub-cellular specificity in the brain [3]. MCT1 is predominantly located in astrocytes [4], and functions to release lactates from the astrocytes. The Km value of MCT1 is ~3.5 mM for lactate [5]. MCT4 is present in astrocytes and has a relatively high Km value for lactate (22–28 mM) [6]. MCT2, the other predominant type in the brain, is present almost exclusively in neurons [7] and has high affinity with lactate (Km: ~0.7 mM) [5]. This suggests that MCT2 facilitates lactate uptake into neurons for utilization as an oxidizable substrate. Lactates are released from glycogen stores in astrocytes, which show enrichment for lactate dehydrogenase (LDH)5 (muscle type) [8,9]. By contrast, there is the enrichment of LDH1 (heart type) in neurons [9].

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Previous studies have demonstrated that lactate transport plays crucial roles for brain function, for example, inhibition of lactate transport suppresses synaptic transmission in patch-clamp recordings [10], and lactate transport is required for memory formation in the hippocampus [11]. In the rat cerebellum, MCT2 has been shown to be expressed strongly and to co-localize with δ2 glutamate receptor (GluD2) in parallel fiber-Purkinje cell synapses [12], suggesting that MCT2 may play an important role in providing lactate as an energy fuel to neurons in the cerebellum. These observations raise the question of whether inhibition of lactate transport in the cerebellum might be associated with a decline in motor performance. At present, it is not known whether inhibition of lactate transport via MCT2 in the cerebellum can affect motor performance.

Recently, Takimoto et al. found that endurance exercise increases the levels of MCT2 in rat brains, although they did not specifically examine the cerebellum [13]. The cerebellum is important for motor control, learning, and carrying out skilled activities. Given that MCT2 in the cerebellum is associated with motor performance, maintenance and enhancement of MCT2 mRNA and protein expression is meaningful. Nutritional metabolic perturbation can change MCT2 protein expression in the brain [14]. Acute exercise also changes metabolism, for example by increasing lactate concentration and consumption in the brain [13,15], but whether acute exercise can change MCT2 mRNA and protein expression in the cerebellum is unknown. It is therefore of interest to determine whether acute exercise increases the level of MCT2 mRNA and protein in this tissue.

In the present study, we injected α-cyano-4-hydroxycinnamate (4-CIN) into the brains of mice to inhibit lactate transport via MCT2 in the cerebellum and subjected the mice to a rotarod test to identify changes in motor performance. We also examined the effects of acute exercise (treadmill running) on MCT1 and MCT2 mRNA and protein levels in the mouse brain (cerebral cortex and cerebellum). We focused on MCT1 and MCT2 because MCT4 has a high Km value for lactate in the brain and blood during endurance exercise [13]. MCT1 and MCT2 isoforms can provide metabolic flexibility across the physiological range of lactate concentrations in the brain. Our results showed that inhibition of lactate transport in the cerebellum dramatically decreased motor performance and that MCT2 mRNA levels increased after acute exercise in the mice.

2. Materials and methods

2.1. Animals

Ten-week-old ICR male mice (CLEA Japan, Tokyo) were used in this study. The mice were housed under 12:12 h light-dark cycle conditions, in an air-conditioned room and provided with standard chow and water *ad libitum*. The present study was approved by the Ethical Committee for Animal Experiments at the University of Tokyo (no. 24-3) and was carried out in accordance with the Guidelines for Research with Experimental Animals of the University of Tokyo and the National Institutes of Health Guide for the Care and Use of Laboratory Animals (revised 1996). All efforts were made to minimize the number of animals used and any discomfort or suffering throughout the course of the experiments.

2.2. In vivo *inhibitor experiment*

Mice were randomly assigned to two groups: 4-CIN injection (n = 4) or saline injection (n = 4) group. The 4-CIN or saline was injected into the subarachnoidal space of the cerebellum of the mice. To clarify effects of 4-CIN and vehicle (saline) injection on motor performance, we measured retention time of staying on a rotating rod before injection (PRE), and 1 and 2 h after injection.

2.3. Microinjection

The mice were anesthetized with isoflurane and were placed on a stereotaxic frame under aseptic conditions. As described previously [16,17], a small hole was made in the occipital bone over the cerebellum using a dental drill, and the dura mater was ablated. A '33′ microsyringe needle was inserted into the subarachnoidal space of the cerebellum. Ten μl 2 mM α-cyano-4-hydroxycinnamate (4-CIN) in 10% DMSO or saline 10% DMSO were injected into the subarachnoidal space at a rate of 1 μl/min. After surgery, mice were kept in a warm cage and allowed to recover completely before the rotarod test.

2.4. Rotarod test

Before the testing sessions, mice were habituated to stay on a stationary rod and then were trained to stay on a rod (5 cm diameter) rotating at a constant rate of 8 rpm for 120 s. The mice were placed on the rotating rod before injection (PRE), and 1 and 2 h after injection. The retention time, that is, latency of falling was recorded automatically. Each trial was terminated once the mouse had stayed on the rod for 120 s.

2.5. Acute exercise experiment

Animals were randomly assigned to three groups: pre-exercise (PRE), and 0 and 5 h after 2 h treadmill running. All mice were familiarized with treadmill running at low speeds (10–20 m/min) for 20 min/day for 2 days. Four days after familiarization, the mice completed 2 h treadmill running (20 m/min) in which the exercise session was divided into two 1 h periods separated by 30 min of rest. The running mice were continuously monitored and gentle touching of the tails was used as an aversive stimulus to encourage the animals to continue running on the treadmill. Five animals from each group were sacrificed by cervical dislocation. The cerebral cortex and cerebellum were rapidly harvested, frozen in liquid nitrogen and stored at −80 °C until further analysis.

2.6. Blood glucose and lactate concentrations

Blood glucose and lactate concentrations were measured in the mice in the acute exercise experiment by collecting blood from the opened chest. Measurements were made using glucose and lactate meters (Arkray, Kyoto, Japan).

2.7. Real time PCR

cDNAs were synthesized using SuperScript VILO (Invitrogen, CA, USA) from total RNAs isolated from the cerebral cortex and cerebellum using TRIZOL (Invitrogen). The cDNAs were analyzed using a 7500 Real-time PCR System (Applied Biosystems, CA, USA) and Power SYBR Green PCR Master Mix (Applied Biosystems). Total RNA isolation and cDNA synthesis were performed according to the manufacturer's protocol. Primers were synthesized by Operon Biotechnologies, Japan: MCT1 *(Slc16a1)* forward primer (5′-TTGTCTGTCTGGTTGCGGCTTGATCG-3′) and reverse primer (5′-GCCCAAGACCTCCAATAACACCAATGC-3′); MCT2 *(Slc16a7)* forward primer (5′-TCTGACAACAGCCAAGAGAAACA-3′) and reverse primer, (5′-TCCGTCGGGAGGAAGTGG-3′). PCR conditions for MCT1 and MCT2 were denaturation at 95 °C for 15 s, followed by annealing and elongation at 60 °C for 1 min. At the end of the PCR, samples were subjected to a dissociation curve analysis. The relative amounts of mRNA were determined by the standard curve method.