Neuroscience Letters 662 (2018) 142–146

Contents lists available at ScienceDirect

Neuroscience Letters

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

Research article

High-fat diet reduces the hippocampal content level of lactate which is correlated with the expression of glial glutamate transporters

Sheng-Feng Tsai[a], Yun-Wen Chen[a,b], Yu-Min Kuo[a,c,*]

[a] Institute of Basic Medical Sciences, College of Medicine, National Cheng Kung University, Tainan, Taiwan
[b] Department of Pharmacology, College of Medicine, National Cheng Kung University, Tainan, Taiwan
[c] Department of Cell Biology and Anatomy, College of Medicine, National Cheng Kung University, Tainan, Taiwan

ARTICLE INFO

Keywords:
EAAT1
EAAT2
GLAST
GLT-1
Diet-induced obesity

ABSTRACT

Metabolic disorders hamper the brain metabolism and functions. The astrocytic glucose-derived lactate is known to fill the increased energy needs of neurons during synaptic transmission. However, whether systemic metabolism dysregulation affects the astrocytic lactate metabolism in the brain remain unexamined. To address this question, we adopt a 12-week high-fat diet to induce metabolic disorders in adult mice, and the effects of high-fat diet on the lactate metabolism in the hippocampus were examined. Results showed that a 12-week high-fat diet induced obesity and insulin resistance in mice. High-fat diet also decreased the lactate content levels and the expression of glial glutamate transporters, GLAST and GLT-1, in the hippocampus. Strong correlations between the lactate levels and the levels of GLAST and GLT-1 were evidenced. In conclusion, high-fat feeding induces metabolic disorders and disrupts lactate metabolism in the hippocampus. GLAST and GLT-1 may contribute to the HFD-induced abnormalities of the hippocampal lactate metabolism.

1. Introduction

Lactate, an end product of non-oxidative glycolysis, can be used as an energy source by neurons during neurotransmission in the central nervous system [1]. Astrocytes, a glial cell type, have emerged as energy suppliers necessary for meeting the ample energy demand resulting from synaptic activities in the brain [1]. Compared to neurons, astrocytes have higher rates of glycolysis, but lower rates of oxidative phosphorylation [14,16]. A large portion of glucose, the dominant and essential energy source in the adult brain, entering the glycolytic pathway in astrocytes is released as lactate into the extracellular space [7,26,27]. The astrocytic glucose-derived lactate is known to fill the increased energy needs of neurons during synaptic transmission [13]. It has been reported that the insufficient lactate supplies contribute to the developments of memory deficits and depression [9,34].

Astrocytes are able to closely communicate with surrounding neurons, and provide lactate as energetic substrates to fill neuronal energy needs with a precise spatiotemporal coordination [2,12,22,24,25]. It is known that the call for astrocytes to satisfy the high-energy requirements during neurotransmission is relayed by neurotransmitters released from neighboring activated neurons [35]. In glutamatergic transmission system, the released glutamate is depleted from the synaptic cleft by uptake via the glial excitatory amino acid transporter 1 and 2 (EAAT 1 and 2) or the neuronal EAAT3 [3,29]. In astrocytes, the glutamate transport via EAATs is powered by the sodium gradient across the membrane. EAATs co-transport three Na^+ ions and one H^+ ion, but anti-transport one K^+ ion, while taking up one glutamate molecule in a complete cycle of glutamate transport [40]. In order to counter this glutamate transport-induced perturbation of ion homeostasis, the workload of Na^+/K^+ ATPase increases and significant amounts of ATP are consumed [18]. The subsequent energy insufficiency triggers glycolysis to produce ATP by converting glucose into pyruvate and then to lactate in astrocytes. Lactate is then pumped out from astrocytes into the extracellular space, and is taken up by neurons as energy fuel [10,13,17,30,38]. Taken together, the astrocytic EAAT1 and EAAT2 not only play a critical role in mediating glutamatergic neurotransmission but function as triggers facilitating the lactate production in the astrocytes.

Metabolic disorders are emerging as one of the major medical and public health problems worldwide, and are known to induce adverse effects on the brain functions [28]. However, whether systemic metabolism dysregulation affects the astrocytic lactate metabolism in the central nervous system remain unexamined. To address this question, here, we used high-fat diet (HFD)-induced obesity in mice as an animal model to investigate the effect of metabolic disorders on the lactate metabolism in the hippocampus. Body weight gain and systemic insulin

* Corresponding author at: Department of Cell Biology and Anatomy, College of Medicine, National Cheng Kung University, 1 Ta Hsueh Road, Tainan, 70101, Taiwan.
E-mail address: kuoym@mail.ncku.edu.tw (Y.-M. Kuo).

http://dx.doi.org/10.1016/j.neulet.2017.10.024
Received 9 August 2017; Received in revised form 21 September 2017; Accepted 16 October 2017
Available online 16 October 2017
0304-3940/

sensitivity were used as indicators of metabolic disorders. The hippocampal lactate metabolism was determined by the lactate content level and the expression of lactate metabolism-related proteins abundantly expressed by astrocytes, including GLAST (alternative name of EAAT1 in mice), GLT-1 (alternative name of EAAT2 in mice), phosphofructokinase-1 (PFK1) and 6-phosphofructose-2-kinase/fructose-2,6-bisphosphatase-3 (PFKFB3). PFK1 and PFKFB3 have been known to be critical enzymes involved in glycolysis of astrocytes [14,16].

2. Materials and Methods

2.1. Animals and high-fat diet

All experiments were performed in accordance with the National Institutes of Health Guideline for Animal research (Guide for the Care and Use of Laboratory Animals) and approved by the National Cheng Kung University Institutional Animal Care and Use Committee (IACUC approval number: 104243). Male C57BL/6 mice at 8-week-old were obtained from National Cheng Kung University Laboratory Animal Center (http://www.ncku.edu.tw/animal/eng/nckulac.html) and randomly assigned to Chow and HFD feeding groups. The Chow mice were fed a standard diet (laboratory autoclavable rodent diet 5010, LabDiet, St. Louis, MO, USA) and the HFD mice were fed a diet containing 60 kcal % fat (58Y1, TestDiet, St. Louis, MO, USA) for 12 weeks (from 8 to 20 weeks old). Mice were housed (five per cage) with a stable temperature (24 ± 1 °C), a 12-h light/dark cycle, and unrestricted access to food and water. The housing environment and animal health were monitored by the Laboratory Animal Center. One day after the end of 12-week feeding, the mice were subjected to further behavioral tests or specimen collections.

2.2. Measuring the plasma levels of fasting glucose and insulin

After a 12-h fasting, the mice were anesthetized by pentobarbital (1 g/kg, i.p.) and their blood was collected from the orbital sinus with heparinized capillary tubes. Plasma was obtained after centrifuging the blood at 3,000 × g for 10 min. The glucose levels were measured using a commercial glucose-oxidase kit (code#11538, BioSystems, Barcelona, Spain). The insulin levels were measured using a commercial mouse insulin ELISA kit (10-1247-01, Mercodia, Uppsala, Sweden). The homeostasis model assessment-insulin resistance (HOMA-IR) index was calculated as follows: fasting glucose (mM) × fasting insulin (mU/l)/22.5.

2.3. Lactate level measurements

Both the whole blood level and the hippocampal content level of lactate were quantified using a lactate meter (Lactate Pro2, ARKRAY, Kyoto, Japan) [33]. The whole blood was quickly collected from mice tail vein. We obtained the hippocampal lactate content level by quantifying the lactate concentrations in hippocampus homogenates and then normalized with respective protein concentrations.

2.4. Hippocampal specimen preparation

One day after the end of 12-week HFD feeding, the hippocampus specimens were collected from mice. To suppress the activity of enzymes converting glycogen into lactate in the hippocampus, the anesthetized mice (pentobarbital, 1 g/kg, i.p.) were transcardially perfused with chilled phosphate-buffered saline, and their hippocampi were quickly dissected out and immersed in liquid nitrogen. The frozen hippocampus samples were directly lysed with protein-denaturing reagents for further examination of lactate content levels and immunoblotting.

2.5. Immunoblotting

Immunoblotting of the hippocampal GLAST, GLT-1, PFK1 and PFKFB3 was performed as described previously [36]. However, for preparing multiple transmembrane proteins, e.g., GLAST and GLT-1, we skipped the boiling step to avoid protein aggregations. Relative protein levels (versus β-actin) were estimated by immunoblotting using the following primary antibodies: GLAST (#5684, Cell Signaling Technology, Danvers, MA, USA), GLT-1 (#3838, Cell Signaling Technology), PFK-1 (sc-67028, Santa Cruz Biotechnology, Santa Cruz, CA, USA), and PFKFB3 (#13123, Cell Signaling Technology). The bound antibodies were detected using an enhanced chemiluminescence detection kit (PerkinElmer, Boston, MA, USA). The band densities were measured using an imaging system (BioChemi; UVP, Upland, CA, USA) and analyzed using ImageJ Fiji software (version 1.51K).

2.6. Statistical analysis

Data are expressed as mean ± S.E.M. Significance was set at $p < 0.05$. The results of the body weights, levels of circulating factors, hippocampal lactate content level and hippocampal protein levels were analyzed using unpaired two-tailed Student's t test. The correlations between the levels of lactate and GLAST/GLT-1 in the hippocampus were analyzed by linear regression.

3. Results

3.1. HFD induces metabolic disorders but does not affect the blood lactate concentrations

Our results showed that a 12-week HFD increased the body weight gains in the mice ($t = 9.6$, $d.f. = 8$, $p < 0.0001$, Table 1). Compared to the Chow group, the HFD group had higher levels of fasting plasma glucose ($t = 2.7$, $d.f. = 8$, $p < 0.05$) and insulin ($t = 5.0$, $d.f. = 8$, $p < 0.01$, Table 1). The HOMA-IR index was also increased in the HFD group ($t = 4.5$, $d.f. = 8$, $p < 0.01$). However, HFD did not alter the lactate concentrations in blood ($t = 1.3$, $d.f. = 8$, $p = 0.25$, Table 1). These results indicated that a 12-week HFD effectively induces metabolic disorders in mice without affecting their circulating lactate levels.

3.2. HFD decreases the content level of lactate and downregulates the expression of glutamate transporters in the hippocampus

After establishing the HFD-induced obesity mice model, we further examined the effects of HFD on the lactate metabolism in the hippocampus. The results revealed that HFD decreased the lactate content level in the hippocampus ($t = 2.8$, $d.f. = 8$, $p < 0.05$, Fig. 1). HFD also downregulated the hippocampal expression of GLAST ($t = 4.0$, $d.f. = 8$, $p < 0.01$) and GLT-1 ($t = 2.8$, $d.f. = 8$, $p < 0.05$) (Fig. 2). However, the hippocampal levels of PFK1 ($t = 0.2$, $d.f. = 8$, $p = 0.86$) and PFKFB3 ($t = 0.3$, $d.f. = 8$, $p = 0.77$) were similar between the HFD

Table 1
The effects of a 12-week HFD feeding on body weights, fasting levels of plasma glucose and insulin, HOMA-IR index, and blood level of lactate in mice.

	Chow	HFD	*p* value
Body weight (g)	29.30 ± 1.36	44.70 ± 0.86	< 0.0001
Fasting plasma level of glucose (mg/dL)	83.20 ± 5.63	121.0 ± 12.66	< 0.05
Fasting plasma level of insulin (ng/mL)	0.40 ± 0.06	1.48 ± 0.21	< 0.01
HOMA-IR index	2.26 ± 0.26	12.82 ± 2.33	< 0.01
Whole blood level of lactate (mmol/L)	1.76 ± 0.16	1.44 ± 0.20	=0.25

HOMA-IR index: homeostatic model assessment-insulin resistance index; n = 5.