Original Article

Glutamate Impairs Mitochondria Aerobic Respiration Capacity and Enhances Glycolysis in Cultured Rat Astrocytes*

YAN Xu[1,2,3,4], SHI Zhong Fang[1,2,3,4], XU Li Xin[1,2,3,4], LI Jia Xin[1,2,3,4], WU Min[1,2,3,4], WANG Xiao Xuan[1,2,3,4], JIA Mei[1,2,3,4], DONG Li Ping[1,2,3,4], YANG Shao Hua[5], and YUAN Fang[1,2,3,4,#]

1. Department of Pathophysiology, Beijing Neurosurgical Institute, Beijing Tiantan Hospital, Capital Medical University, Beijing 100050, China; 2. Beijing Key Laboratory of Central Nervous System Injury, Beijing Neurosurgical Institute, Beijing Tiantan Hospital, Capital Medical University, Beijing 100050, China; 3. Beijing Key Laboratory of Translational Medicine for Cerebrovascular Disease, Beijing Tiantan Hospital, Capital Medical University, Beijing 100050, China; 4. China National Clinical Research Center for Neurological Diseases, Beijing Tiantan Hospital, Capital Medical University, Beijing 100050, China; 5. Department of Pharmacology and Neuroscience, University of North Texas Health Science Center, Fort Worth, Texas 76107-2699, USA

Abstract

Objective To study the effect of glutamate on metabolism, shifts in glycolysis and lactate release in rat astrocytes.

Methods After 10 days, secondary cultured astrocytes were treated with 1 mmol/L glutamate for 1 h, and the oxygen consumption rates (OCR) and extra cellular acidification rate (ECAR) was analyzed using a Seahorse XF 24 Extracellular Flux Analyzer. Cell viability was then evaluated by MTT assay. Moreover, changes in extracellular lactate concentration induced by glutamate were tested with a lactate detection kit.

Results Compared with the control group, treatment with 1 mmol/L glutamate decreased the astrocytes' maximal respiration and spare respiratory capacity but increased their glycolytic capacity and glycolytic reserve. Further analysis found that 1-h treatment with different concentrations of glutamate (0.1-1 mmol/L) increased lactate release from astrocytes, however the cell viability was not affected by the glutamate treatment.

Conclusion The current study provided direct evidence that exogenous glutamate treatment impaired the mitochondrial respiration capacity of astrocytes and enhanced aerobic glycolysis, which could be involved in glutamate injury or protection mechanisms in response to neurological disorders.

Key words: Astrocytes; Glutamate; Mitochondrial metabolism; Glycolysis; Lactate

Biomed Environ Sci, 2017; 30(1): 44-51 *doi: 10.3967/bes2017.005* *ISSN: 0895-3988*

www.besjournal.com (full text) *CN: 11-2816/Q*

*This work was supported by the National Natural Science Foundation of China, No. 81271286; Beijing Natural Science Foundation, No. 7152027 to YUAN Fang; and Innovation Foundation of Beijing Neurosurgical Institute, No. 2014-11 to YAN Xu.

#Correspondence should be addressed to YUAN Fang, Professor, PhD, Tel: 86-10-67096750, E-mail: florayuan@vip.sina.com

Biographical note of the first author: YAN Xu, male, born in 1984, PhD, Research Assistant, majoring in brain injury.

INTRODUCTION

The mammalian brain has high energy requirements. Approximately 20% of the oxygen and 25% of the glucose consumed by the human body are consumed by cerebral metabolism, yet the brain constitutes only -2% of the total body weight[1]. In addition to this extremely high energy consumption, the brain appears to have very limited energy storage. Energy supply and expenditure are tightly coupled with neurovascular and neurometabolic mechanisms. Astrocytes, which are the most abundant cells in the mammalian brain and vastly outnumber neurons, are important in the neurovascular coupling of neuronal activity and cerebral blood flow (CBF), which provides an uninterrupted supply of oxygen and glucose to meet the high metabolic demand of the neurons[2-3].

Glutamate, an important excitatory neurotransmitter in the central nervous system, has several functions in the nervous system. It acts both pre- and postsynaptically by activating glutamate receptors, which are responsible for excitatory neurotransmission and are pivotal elements of complex systems underlying synaptic plasticity, learning, memory and other fundamental events/functions in neurophysiology[4]. In addition to being an excitatory transmitter, much of the glutamate taken up by glutamate transporters in astrocytes is destined for oxidative phosphorylation to generate energy, which requires conversion to the tricarboxylic acid (TCA) cycle intermediate a-ketoglutarate[5].

Astrocytes are important in glutamate uptake, metabolism, and release in the central nervous system. Recent studies indicated that disturbance of astrocyte metabolism is a significant cause of neuronal dysfunction and neurodegenerative processes[6-7]. Glutamate is also involved in pathological conditions such as cerebral ischemia and other neurodegenerative diseases. In these instances, extracellular glutamate levels are increased significantly, resulting in neurotoxic effects. Numerous studies have shown that nervous system diseases could be regulated by the dysfunctions of glutamate receptors and/or glutamate uptake[8-9]. Although previous studies have shown that glutamate administration could increase glycolysis and acidification in astrocytes[10-11], the effect of glutamate on cellular metabolism and changes in glycolysis in rat astrocytes remains to be fully characterized. In the current study, astrocytes were cultured, identified and exposed to glutamate. The respiration and glycolysis changes in cultured astrocytes were analyzed using a Seahorse XF-24 Metabolic Flux Analyzer. Lactate release was also detected to confirm the shift to glycolysis in astrocytes after glutamate exposure.

MATERIALS AND METHODS

Materials

All of the rats were provided by the Institute of Laboratory Animal Science affiliated with the Chinese Academy of Medical Sciences [Certificate No, SCSK (Beijing) 2005-0013]. The animal protocol was approved by the Animal Care and Use Committee of Beijing Neurosurgical Institute and was consistent with the National Institutes of Health Guide for the Care and Use of Laboratory Animals. Dimethyl Sulphoxide (DMSO) and 3-(4,5-dimethyl-thiazol-2-yl)-2,5-diphenyltetrazoliumbromide (MTT) were obtained from Sigma (St.Louis, MO, USA). Trypsin, Minimum Essential Medium (MEM) and fetal bovine serum (FBS) were obtained from GIBCO-BRL (Grand Island, NY, USA). Rabbit anti-glial fibrillary acidic protein (GFAP) was purchased from Dako (Glostrup, Denmark). Mounting medium containing 4',6-Diamidino-2-phenylindole dihydrochloride DAPI (ZLI-9557) and goat anti-rabbit secondary antibodies conjugated to fluorescein isothiocyanate (FITC) (ZF-0311) were obtained from ZSGB-BIO (Beijing, China). Cell Lysis Buffer and Enhanced BCA Protein Assay Kit were obtained from Beyotime (Haimen, China). The XF Glycolysis Stress Test Kit, XF Cell Mito Stress Test Kit, and XF Base Medium were acquired from Seahorse Bioscience (North Billerica, MA, USA). Sodium pyruvate, glucose and L-glutamine were purchased from Sigma-Aldrich (Saint Louis, MO, USA). The Lactate Assay Kit was obtained from Biovision (California, USA).

Astrocyte Culture and Identification

Astrocyte cultures were prepared according to a previously described method[12] with slight modifications[13]. One-day-old Sprague Dawley rats were anesthetized with ether, and then dipped into 75% alcohol for sterilization. The cerebral cortex was removed from the skulls, and the meninges were carefully stripped away. The cerebral tissues were cut into small pieces and dissociated into single cells by gentle pipetting. After filtering, the isolated cells