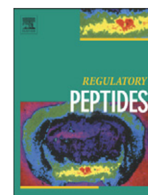
Regulatory Peptides 192–193 (2014) 45–52

Contents lists available at ScienceDirect

Regulatory Peptides

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



Dual effects of carnosine on energy metabolism of cultured cortical astrocytes under normal and ischemic conditions

Yao Shen *, Yueyang Tian, Jianbo Yang, Xiaojie Shi, Li Ouyang, Jieqiong Gao, Jianxin Lu **

Key Laboratory of Laboratory Medicine, Ministry of Education, Zhejiang Provincial Key Laboratory of Medical Genetics, College of Laboratory Medicine and Life Sciences, Wenzhou Medical University, Wenzhou 325035, China

ARTICLE INFO

Article history:
Received 14 February 2014
Received in revised form 9 July 2014
Accepted 11 August 2014
Available online 3 September 2014

Keywords:
Glycolysis
Mitochondrial respiration
Oxidative phosphorylation (OXPHOS)
Oxygen–glucose deprivation (OGD)/recovery

ABSTRACT

Objective: The aim of this study was to investigate the effects of carnosine on the bioenergetic profile of cultured cortical astrocytes under normal and ischemic conditions.
Methods: The Seahorse Bioscience XF96 Extracellular Flux Analyzer was used to measure the oxygen consumption rates (OCRs) and extracellular acidification rates (ECARs) of cultured cortical astrocytes treated with and without carnosine under normal and ischemic conditions.
Results: Under the normal growth condition, the basal OCRs and ECARs of astrocytes were 21.72 ± 1.59 pmol/min/μg protein and 3.95 ± 0.28 mpH/min/μg protein respectively. Mitochondrial respiration accounted for ~80% of the total cellular respiration and 85% of this coupled to ATP synthesis. Carnosine significantly reduced basal OCRs and ECARs and ATP-linked respiration, but it strikingly increased the spare respiratory capacity of astrocytes. The cellular ATP level in carnosine-treated astrocytes was reduced to ~42% of the control. However, under the ischemic condition, carnosine upregulated the mitochondrial respiratory and cellular ATP content of astrocytes exposed to 8 h of oxygen–glucose deprivation (OGD) followed by 24 h of recovery under the normal growth condition.
Conclusions: Carnosine may be an endogenous regulator of astrocyte energy metabolism and a clinically safe therapeutic agent for promoting brain energy metabolism recovery after ischemia/reperfusion injury.

© 2014 Elsevier B.V. All rights reserved.

1. Introduction

Astrocytes are key cells in the mammalian central nervous system (CNS) and play multiple roles in the maintenance of the extracellular environment and in the stabilization of cell–cell communications under physiological and pathological conditions [1,2]. Specifically, astrocytes control the uptake and release of neurotransmitters [3], regulate ionic homeostasis, process and control synaptic information, defend against oxidative stress, influence local blood supply [4], and directly supply neurons with substrates for oxidative phosphorylation (OXPHOS) [5,6]. Recently, a role for astrocyte mitochondrial injury in cerebral ischemia is suggested by the fact that impairment of astrocyte mitochondrial function precedes neuronal death after a brief period of global cerebral ischemia [7]. Thus, we speculated that astrocyte mitochondria may be the primary contributor to the survival of astrocytes themselves and neurons in pathological conditions.

Carnosine, a naturally occurring dipeptide, composed of β-alanine and L-histidine, is widely distributed in tissues including the animal and human brains. The olfactory tract has been demonstrated to contain a very high concentration of carnosine (1–2 mM), whereas the other brain regions contain a much lower carnosine concentration [8–10]. Carnosine has been postulated to have numerous biological roles such as protein glycation inhibitor, anti-inflammatory agent, free radical scavenger, and pH-buffering agent and may serve as a neurotransmitter or a neuromodulator in the olfactory bulb [9,11,12]. However, so far, the physiological functions of carnosine in the brain remain obscure, and a unifying concept has not yet emerged. On the other hand, carnosine has been reported to be synthesized by oligodendrocytes in the CNS, and can be released from oligodendrocytes when the cells receive stimulations. Then the released carnosine is rapidly uptaken by astrocytes by an energy dependent dipeptide transport system [13]. Recently, a line of studies demonstrated that carnosine is neuroprotective in cerebral ischemia in mice and rats [14–16]. A dramatic increase in the number of GFAP-positive astrocytes containing carnosine was observed at the periphery of the infarct area after focal cerebral ischemia [17]. Our previous study also showed that carnosine can prevent the dissipation of mitochondrial membrane potential (ΔΨm) and suppress the increase

* Correspondence to: Y. Shen, Key Laboratory of Laboratory Medicine, Ministry of Education, Zhejiang Provincial Key Laboratory of Medical Genetics, College of Laboratory Medicine and Life Sciences, Wenzhou Medical University, China. Tel./fax: +86 577 86689717.
** Correspondence to: J. Lu, Key Laboratory of Laboratory Medicine, Ministry of Education, Zhejiang Provincial Key Laboratory of Medical Genetics, College of Laboratory Medicine and Life Sciences, Wenzhou Medical University, China. Tel.: +86 577 8668 9805; fax: +86 577 8668 9771.
E-mail addresses: yueshen-2002@163.com (Y. Shen), jxlu313@163.com (J. Lu).

http://dx.doi.org/10.1016/j.regpep.2014.08.005
0167-0115/© 2014 Elsevier B.V. All rights reserved.

of MitoSOX™ Red mitochondrial superoxide indicator intensity induced by oxygen–glucose deprivation (OGD) in cultured cortical astrocytes [18]. Thus, it raises the possibility that carnosine may modify the function of astrocytes in physiological and pathological conditions. However, few reports in previous experiments have demonstrated the effects of carnosine on the function of astrocytes in different conditions, and whether carnosine can regulate astrocytic energy metabolism remains unknown.

Recently, the Seahorse Bioscience XF96 Extracellular Flux Analyzer has been used to continually monitor cell respiration together with extracellular pH as an indicator of anaerobic glycolysis [19]. Therefore, in the present study, we wanted to further characterize the bioenergetic profile of cultured cortical astrocytes and the roles of carnosine in astrocyte energy metabolism under normal and ischemic conditions with the Seahorse Bioscience XF96 Extracellular Flux Analyzer.

2. Materials and methods

2.1. Materials

Carnosine, sodium pyruvate, rotenone, carbonyl cyanide p-trifluoromethoxyphenylhydrazone (FCCP), oligomycin, 2-deoxyglucose (2-DG), β-alanine and L-histidine were obtained from Sigma (St. Louis, MO, USA). Penicillin, streptomycin, L-glutamine, trypsin, poly-D-lysine, Dulbecco's modified Eagle's medium (DMEM), glucose-free DMEM, and fetal bovine serum were from GIBCO-BRL (Grand Island, NY, USA). An Annexin V-FITC/PI apoptosis detection kit, BCA Protein Assay Kit and ATP Assay Kit were bought from Beyotime Institute of Biotechnology (Nanjing, China). XF assay medium and XF calibrant solution were bought from Seahorse Bioscience.

2.2. Primary cortical astrocyte cultures

Primary cultures of cortical astrocytes were prepared from the cortices of newborn Sprague–Dawley rats as previously described [18]. Briefly, the cerebral cortices were trypsinized (0.25%) for 20 min at 37 °C, and then the dissociated cells were seeded onto poly-D-lysine-coated 25 cm^2 flasks. Cells were cultured in high glucose (4.5 g/l) DMEM supplemented with 10% fetal bovine serum, 2 mM glutamine, 100 units/ml penicillin and 100 μg/ml streptomycin. The cultures were maintained at 37 °C under>90% humidity and 5% CO_2. On days 10–11, the confluent cultures were shaken overnight to minimize microglial contamination. More than 95% of the cultured cells were astrocytes as identified by immunofluorescent staining for glial fibrillary acidic protein (GFAP).

2.3. Oxygen–glucose deprivation (OGD) and carnosine treatment

To perform OGD, astrocytes were washed twice and incubated in glucose-free DMEM. Then the cultures were placed in an anaerobic chamber flushed with 5% CO_2/95% N_2 for 30 min. The chamber was then sealed and placed in an incubator at 37 °C. Control groups were cultured in DMEM containing glucose (4.5 g/l) under normoxic condition. For recovery, the cultures were replaced with normal culture medium and returned to the normal culture condition for different recovery times. Carnosine, β-alanine or histidine at a concentration of 5 mM was supplied 30 min before OGD and was present throughout the OGD and recovery processes.

2.4. Extracellular flux technology

The XF96 Extracellular Flux Analyzer (Seahorse Bioscience, Billerica, MA) was used to measure the extracellular flux changes of oxygen and protons in the media immediately surrounding adherent intact cells cultured in a poly-D-lysine-coated XF96-well microplate (Seahorse Bioscience). Thus, the XF technology non-invasively profiles the metabolic activity of cells in minutes, offering a physiologic cell-based assay for the determination of basal oxygen consumption rates (OCRs), glycolysis rates, and respiratory capacity in a single experiment to assess mitochondrial dysfunction. After baseline measurements of OCRs, OCRs and ECARs (extracellular acidification rate) were measured after sequentially adding to each well oligomycin (which blocks the mitochondrial complex V, where the electron chain is coupled to ATP synthesis), FCCP (an uncoupling agent that allows maximum electron transport) and rotenone (which blocks complex I, thereby eliminating mitochondrial respiration). A typical OCR trace from this mitochondrial function assay onhow each parameter is derived is shown in Fig. 1A. All assays were conducted using a seeding density of 10,000 cells/well in 200 μl of DMEM in a XF96 cell culture microplate. The cells were switched to unbuffered DMEM supplemented with 2 mM sodium pyruvate 1 h prior to the beginning of the assay and maintained at 37 °C. Carnosine was not added into the assay medium. Values were normalized to the total protein/well after the completion of the XF assay by the Bradford protein assay.

2.5. ATP quantification

The ATP assay was performed according to the manufacturer's instruction. Briefly, the harvested cells were lysed with a lysis buffer, and then centrifugated at 10,000 $\times g$ at 4 °C for 2 min. The level of ATP was determined by mixing 20 μl of the supernatant with 100 μl of luciferase reagent which catalyzed the light production from ATP and luciferin. Luminance was measured by a monochromator microplate reader. A standard curve was also generated and the protein concentration of each well was determined using the BCA Protein Assay kit. The ATP level in each sample was expressed as nmol/mg protein.

2.6. Flow cytometry analysis of cell death

Cell death was determined using the Annexin V-FITC/PI apoptosis detection kit. Briefly, cells were collected after treatment, washed twice in ice-cold PBS, and then resuspended in binding buffer at a density of 1×10^6 cells/ml. Cells were incubated simultaneously with fluorescein-labeled Annexin V and PI for 20 min and analyzed by flow cytometry. Annexin V-FITC generated signals were detected with an FITC signal detector (FL1, 525 nm). PI signals were monitored using a detector reserved for phycoerythrin emission (FL2, 575 nm). Data were analyzed using Cell Quest software from BD.

2.7. Statistical analyses

All data were expressed as mean ± SEM. Statistical analyses were conducted by SPSS 11.5 for Windows. One-way ANOVA (analysis of variance) followed by LSD (least significant difference) or Dunnett's T3 post-hoc test (where equal variances were not assumed) was applied for multiple comparisons, whereas Student's t-test was used for comparisons between two groups. $P < 0.05$ was considered statistically significant.

3. Results

3.1. Bioenergetic characterization of cultured cortical astrocytes

We first investigated respiration in primary cultured cortical astrocytes, assessed as OCRs, and glycolytic activity, assessed as ECARs. Basal cellular OCRs and ECARs were found to be 21.72 ± 1.59 pmol/min/μg protein and 3.95 ± 0.28 mpH/min/μg protein respectively (Fig. 1B). In the presence of a maximally effective dose of oligomycin (1 μg/ml), OCRs were reduced to 32% of baseline rates, indicating that ~68% of cellular oxygen consumption was related to ATP synthesis. Simultaneously ECARs were increased to 424% of baseline rates, indicating that the cells shifted mitochondrial respiration to

Download English Version:

https://daneshyari.com/en/article/2022400

Download Persian Version:

https://daneshyari.com/article/2022400

Daneshyari.com