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Caloric restriction increases hippocampal glutamate uptake and glutamine synthetase activity in Wistar rats

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ABSTRACT

Recent studies indicate that caloric restriction (CR) protects the central nervous system from several pathological conditions. The impairment of astroglial cell function, including glutamate uptake, glutamine synthetase (GS) activity and S100B secretion, may contribute to the progression of neurological disorders. The present study aimed to evaluate hippocampal astrocytic changes in response to CR diet, measuring astroglial parameters, such as glutamate uptake, GS activity and the immunocontent of GFAP and S100B. Blood biochemical parameters were also analyzed. Rats (60-day old) were fed *ad libitum* or on CR diets for 12 weeks. CR-fed rats showed approximately 16% less body weight gain than control rats. The CR diet was able to induce a significant increase in glutamate uptake (23%) and in GS activity (26%). There were no statistically significant differences in the immunocontent of either GFAP or S100B. In summary, the present study indicates that CR also modulates astrocyte functions by increasing glutamate uptake and GS activity, suggesting that CR might exert its neuroprotective effects against brain illness by modulation of astrocytic functions.

1. Introduction

Dietary calorie restriction (CR) has been applied for more than 70 years and has wide-ranging health benefits, including extended longevity (Hulbert et al., 2007; Roth et al., 2007). Some recent studies indicate that CR protects the central nervous system (CNS) from age-related diseases, i.e. preventing the symptoms associated with Alzheimer's, Huntington's and Parkinson's diseases (Mattson, 2005). While the neurobiological mechanisms responsible for the role of caloric restriction in protection of the CNS are not fully understood, recent studies suggest that CR regulates adult neuronal stem cells, increase adult neurogenesis in young adults rats, and reduce the age-related decline in neurogenesis in older animals (Levenson and Rich, 2007).

Astrocytes play an active role in brain function by affecting the activity of neurons (Fields and Stevens-Graham, 2002), especially in neuronal development, activity, plasticity, differentiation and maturation (Stevens, 2008; Volterra and Meldolesi, 2005). They may also promote neurogenesis, showing that they are widely involved in neuroprotection (Markiewicz and Lukomska, 2006). Other biochemical parameters have been used to characterize astroglial involvement in neural plasticity and injury, including glutamate uptake, glutamine synthetase (GS) and S100B protein.

Glutamate is the major excitatory neurotransmitter in the central nervous system and its accumulation is implicated in neurodegenerative disorders. Astroglial cells are responsible for major glutamate transport and regulate extracellular levels of glutamate (Hertz, 2006; Magistretti, 2006). The impairment of glutamate transporters causes excitotoxicity and leads to increased ROS production and consequent cell damage (Had-Aissouni et al., 2002). The glutamate homeostasis in the brain is maintained by its well-balanced release, uptake and metabolism. Moreover, astrocytes have a specific enzyme glutamine synthetase (GS) (EC 6.3.1.2) that catalyses the amidation reaction of glutamate to form glutamine. Astroglial glutamate uptake and GS are, respectively, responsible for the removal of glutamate from the synaptic cleft and synthesis/replacement of glutamine to neurons (Bak et al., 2006). Thus, malfunction of astrocytic glutamate transporters will lead to an excessively high extracellular glutamate concentration which may result in neurodegeneration

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caused by the excitotoxic action of glutamate (Schousboe and Waagepetersen, 2005).

In addition to GS, two other proteins are used to characterize astrocytes: glial fibrillary acidic protein (GFAP) and S100B. GFAP is a specific marker of mature astrocytes; CNS injuries are commonly accompanied by astrogliosis, characterized by an increase in GFAP (O'Callaghan et al., 1991). S100B is a Ca^{2+}-binding protein expressed and secreted by astrocytes, having a trophic activity on neuron and glial cells (Tramontina et al., 2006; Van Eldik and Wainwright, 2003). In the developing brain, and following acute glial activation, a glial-derived protein, S100B, acts as a neurotrophic factor and neuronal survival protein. In contrast, overproduction of S100B by activated glia can lead to exacerbation of neuroinflammation and neuronal dysfunction. This duality supports the potential of S100B as a biomarker for brain damage, implicates glial activation as a possible treatment target in acute and chronic CNS disorders, and highlights the dual role of glia in the reparative and pathologic responses to neurologic injury (Van Eldik and Wainwright, 2003).

In this study, we investigated possible specific astrocyte alterations in the hippocampi of Wistar rats in response to CR diet, investigating glutamate uptake, glutamine synthetase activity, GFAP and S100B immunocontent. Blood biochemical parameters were also evaluated.

2. Materials and methods

2.1. Materials

N-methyl-D-glucamine, HEPES and all the other reagents were purchased from Sigma Chemical Co. (St. Louis, MO). L-[^{3}H]-glutamate (specific activity 30 Ci/mmol) was purchased from Amersham International, UK.

2.2. Animal research and diets

Thirty male 60-day-old Wistar rats came from the local breeding colony (ICBS–UFRGS). Animals were maintained in a ventilated room at 21 °C, with free access to water on a 12-h light/dark cycle. The experiments were performed in accordance with the NIH Guide for the Care and Use of Laboratory Animals and were approved by the local authorities. Animals were weight matched and divided into two groups: control (*ad libitum*) and calorie restricted rats (CR) (Table 1) that received regular laboratory chow (Nuvilab-CR1, from Nuvital, Brazil) for 12 weeks. The caloric restriction was progressive, initiated at 10% restriction in the first week, changed to 20% at second week, and to 30% at third week until the end of treatment. The food intake was monitored daily, and the animals were weighed weekly (Chang et al., 2007; Horska et al., 1999).

2.3. Blood sampling and analysis

After treatment, animals were overnight-starved (6th hour) and anesthetized with an intramuscular injection of ketamine and xylazine (75 and 10 mg/kg, respectively). The blood samples were obtained with intracardiac punction, and the animals were killed by decapitation. The blood samples were incubated at room temperature (25 °C) for 5 min and centrifugated at 3200 rpm for 5 min. Serum was stored at −70 °C until the day of the analysis. Biochemical analyses were carried out in a multi-test analyzer (Mega; Merck, Darmstadt, Germany), using specific kits supplied by Merck as follows: total protein (protein-SMT, 1.19703.0001, biuret method); albumin (albumin-SMT, 1.19722.0001, bromocresol method); glucose (GLUC-DH, 1.07116.0001); urea (urea-SMT, 1.19702.0001, UV test); creatinine (creatinine-SMT, 1.19726.0001, UV test); triglycerides (SMT-triglyceride, 1.19706.0001, GPO-PAP method); cholesterol (cholesterol-SMT, 1.19738.0001, CHOD-PAP method). HDL cholesterol was determined using a kit (HDL cholesterol direct FS) from DiaSys (Diagnostic Systems International, Holzheim, Germany).

Table 1
Composition of the laboratory chow.

Composition	(g/kg)
Total fat	110
Sunflower oil	5
Proteins	220
Fibers	30
Ash	60
Vitamins	20
Carbohydrates	520

Commercial non-purified diet, Nuvilab-CR1 (Curitiba, PR, Brazil).

2.4. Hippocampal dissection

The brains were removed and placed in cold saline medium with the following composition (in mM): 120 NaCl; 2 KCl; 1 $CaCl_2$; 1 $MgSO_4$; 25 HEPES; 1 KH_2PO_4; 10 glucose, adjusted to pH 7.4 and previously aerated with O_2. The hippocampi were dissected and transverse slices of 0.3 mm were obtained using a McIlwain Tissue Chopper for subsequent analysis.

2.5. Gutamate uptake assay

Hippocampal slices were then transferred immediately to 24-well culture plates, each well containing 0.3 mL of physiological medium and only one slice. Glutamate uptake was performed, as previously described (Thomazi et al., 2004). Medium were replaced by Hank's balanced salt solution (HBSS) containing (in mM): 137 NaCl; 0.63 Na_2HPO_4; 4.17 $NaHCO_3$; 5.36 KCl; 0.44 KH_2PO_4; 1.26 $CaCl_2$; 0.41 $MgSO_4$; 0.49 $MgCl_2$; 5.55 glucose, in pH 7.4. The assay was started by the addition of 0.1 mM L-glutamate and 0.66 μCi/mL L-[2,3-^{3}H]-glutamate. Incubation was stopped after 5 min by removal of the medium and rinsing the slices twice with ice-cold HBSS. Slices were then lysed in a solution containing 0.5 M NaOH. Sodium-independent uptake was determined using N-methyl-D-glucamine instead of sodium chloride. Sodium-dependent glutamate uptake was obtained by subtracting the non-specific uptake from the specific uptake. Radioactivity was measured with a scintillation counter.

2.6. Glutamine synthetase activity

The enzymatic assay was performed, as previously described (dos Santos et al., 2006). Briefly, homogenized tissue samples (0.1 mL) were added to 0.1 mL of reaction mixture containing (in mM): 10 $MgCl_2$; 50 L-glutamate; 100 imidazole–HCl buffer (pH 7.4); 10 2-mercaptoethanol; 50 hydroxilamine–HCl; 10 ATP and incubated for 15 min at 37 °C. The reaction was stopped by the addition of 0.4 mL of a solution containing (in mM): 370 ferric chloride; 670 HCl; 200 trichloroacetic acid. After centrifugation, the supernatant was measured at 530 nm and compared to the absorbance generated by standard quantities of γ-glutamylhydroxamate treated with ferric chloride reagent.

2.7. ELISA for S100B

ELISA for S100B was carried out as described previously (Leite et al., 2008). Briefly, 50 μL of sample (containing between 5 and 10 ng/μL of total protein) plus 50 μL of Tris buffer were incubated for 2 h on a microtiter plate previously coated with monoclonal anti-S100B. Polyclonal anti-S100B was incubated for 30 min and