UNIVERSIDADE FEDERAL DO RIO GRANDE DO SUL

FACULDADE DE FARMÁCIA

DISCIPLINA DE TRABALHO DE CONCLUSÃO DE CURSO

Physiologic S100B concentrations reduces glucose uptake in C6 astroglial cells

Krista Minéia Wartchow

Porto Alegre

2013

UNIVERSIDADE FEDERAL DO RIO GRANDE DO SUL

FACULDADE DE FARMÁCIA

DISCIPLINA DE TRABALHO DE CONCLUSÃO DE CURSO

Physiologic S100B concentrations reduces glucose uptake in C6 astroglial cells

Krista Minéia Wartchow

Trabalho de Conclusão do Curso de Graduação em Farmácia

Departamento de Bioquímica

Orientador: Professor Dr. Carlos Alberto Saraiva Gonçalves

Co-orientadora: Dra. Ana Carolina Tramontina

Porto Alegre

2013

Wartchow, Krista Minéia.

Physiologic S100B concentrations reduces glucose uptake in C6 astroglial cells.

Orientador
Professor Dr. Carlos Alberto Gonçalves
Co-orientadora
Dra. Ana Carolina Tramontina

Banca examinadora
Professora Dra. Cristiane Matté
Dra. Daniela Fraga de Souza

Data de apresentação:

Agradecimentos

Gostaria de agradecer ao meu orientador CA por me receber em seu laboratório a mais de 5 anos, e sempre estar disposto a ajudar, sempre com a porta de seu gabinete aberta.

Á minha querida chefinha Ana Carolina, que foi sempre muito mais que uma chefa ou mesmo orientadora, é quem me ensinou tudo o que sei, e é quem eu sei que posso sempre contar. Sem ela perco o meu rumo! Agradeço sempre por tê-la conhecido e tê-la na minha vida. Muito obrigada por tudo sempre Ana! Agradeço também às minhas queridas sub-chefinhas Dani e Lari, elas que sempre estão dispostas a me explicar e me ajudar, independente do assunto. Sinto-me muito sortuda por ter todas vocês ao meu lado.

Andressa querida, um dos meus maiores presentes durante a faculdade, sempre ao meu lado nas risadas e nas angustias, uma pessoa que sempre se pode contar! Obrigada por tudo amiga! E para minhas amigas e colegas de faculdade, que em grupo compartilhamos cada momento, sempre juntas! Obrigada gurias!

E principalmente aos meus pais, Marli e Hans, irmão Christian, e meus avós Opa, Oma e Vóva que sempre estiveram ao meu lado, me apoiando e torcendo. Obrigada pelo amor, paciência, atenção e compreensão constantes. Sem vocês não sou nada! Meu amor é incondicional por vocês! Amo vocês!

William meu amor, obrigada por cada gesto e palavra de carinho durante esta minha caminhada, você foi e é muito importante! Amo-te!

“ Se você quer ser bem sucedido, precisa ter dedicação total, buscar seu último limite e dar o melhor de si mesmo.”

(*Ayrton Senna)*

Sumário

Apresentação

Este trabalho se encontra na forma de artigo, a fim de ser submetido à revista *International Journal of Developmental Neuroscience*. O guia para autores encontra-se anexado, ao final do trabalho.

Physiologic S100B concentrations reduces glucose uptake in C6 astroglial cells

Krista Minéia Wartchow*, Ana Carolina Tramontina, Carlos Alberto Gonçalves

Departamento de Bioquímica, Instituto de Ciências Básicas da Saúde, Universidade Federal do Rio Grande do Sul, Ramiro Barcelos, 2600- Anexo, 90035-003, Porto Alegre, Brazil.

*Corresponding author:

Krista Minéia Wartchow

Fone/ Fax: +55 51 3308 5535.

E-mail address: 00008684@ufrgs.br (C.A. Gonçalves)

Postal address: Ramiro Barcelos, 2600, anexo, laboratório 33. Porto Alegre-RS, Brazil. CEP 99150-003

Abstract

Astrocytes are the most common cells present in the brain and they are responsible for metabolic support, characterized mainly for their close interaction with neurons. Some glial markers are well recognized, as S100B, a calcium binding protein that plays important intra and extracellular roles in central nervous system. This protein is implicated in glial-neuron communication, presenting neurotrophic or neurotoxic properties, depending on its concentration. Among the models available to study astrocytes, C6 glioma cells in later than 100 passages are very useful, because have properties that are characteristic of the glial cells. The objective of the present work is to evaluate the effect of physiological concentration of exogenous S100B on glucose uptake in C6 astroglial cells. We observed a reduction of glucose uptake in the presence of exogenous S100B, and the incubation with anti-S100B antibody reversed this effect. The incubation with anti-RAGE antibody also reversed this effect, demonstrating that this receptor is involved in the S100B effect. However, further studies are necessary to unveil the S100B mechanism of action.

Keywords: Astroglial cells, S100B, Glucose Uptake, Energetic Metabolism

Highlights: We evaluated S100B effect on glucose uptake; Glucose uptake was reduced after addition of S100B; S100B reduces glucose uptake in astroglial C6 cells.

Abbreviations: S100B: calcium binding protein; GFAP: glial fibrillary acidic protein; CNS: central nervous system; RAGE: receptor for advanced glycation end products; FBS: Fetal bovine serum; DMEM: Dulbecco's modified Eagle

medium; EDTA: trypsin/ethylenediaminetetracetic acid; ANOVA: one way analysis of variance; SPSS: Statistical Package for the Social Sciences; GLUTs: glucose transporters proteins; AGEs: advanced glycated end products; MAPKs: mitogen-activated protein kinases.

1. Introduction

Astrocytes are the most common cells present in the brain and they are responsible for many effects of metabolic support, characterized mainly for their close interaction with neurons. Furthermore they are the main non-neural cell type responsible for the maintenance of brain homeostasis (Belanger and Magistretti, 2009). This cells act in the regulation and maintenance of restrictive properties of the blood-brain barrier regulating the accessibility of factors or molecules which are involved in neurogenesis (Sidoryk-Wegrzynowicz and Aschner, 2013).

The astrocytes are also involved in the energy metabolism, and are inserted in the astrocyte-neuron lactate shuttle hypothesis, whit astrocytes contributing to brain glucose uptake (Pellerin and Magistretti, 2004). The uptake depends of glucose coming from the blood circulation, which is taken by the astrocytes and converted to lactate. After, lactate is released on the extracellular space. The glucose processesd by glycolysis is uptakenurs after the activation of Na^+,K^+-ATPase. After that, the lactate is taken up by neurons, and oxidized to produce the energy necessary for cell survival (Pellerin, 2008).

Some glial markers are well recognized, as S100B, a calcium binding protein, and glial fibrillary acidic protein (GFAP), an intermediate filament present in the cytoskeleton (Kimelberg and Nedergaard, 2010). S100B is predominantly expressed and secreted by astrocytes, and plays important intra- and extracellular roles in central nervous system (CNS) (Donato, 2003; Donato et al., 2009, 2013). This protein is implicated in glial-neuron communication, presenting neurotrophic properties at nanomolar levels, like pro-survival effect in neurons and neurite outgrowth (Donato et al., 2009). However at micromolar levels D100B should exert

neurotoxic effects, by release of pro-inflammatory cytokines (Van Eldik and Wainwright, 2003).

The secretion of S100B can be modulated by several compound, as IL-1 β (de Souza et al., 2009), fluoxetine (Tramontina et al., 2008), huperzine-A (Lunardi et al., 2013) and apomorphine (Nardin et al., 2011); high glucose levels (Tramontina et al., 2012) and glutamate (Tramontina et al., 2006), which reduces the secretion.

S100B can act by interaction with the receptor for advanced glycation end products (RAGE), that belongs to the immunoglobulin superfamily (Donato et al., 2009). It was shown that RAGE is the main responsible receptor for S100B intracellular signal transduction (Huttunen et al., 2000), and was found on the cell surface or in the interior of astrocytes (Ponath et al., 2007). Because of these peculiarities, in this work, we denominated C6 glioma cells as C6 astroglial cells.

Among the models available to study astrocytes, C6 astroglial cells in later than 100 passages are very useful, mostly because their similar glial cells properties. Biochemical and metabolic parameters are very similar between astrocytes and glioma C6 cells, including the expression of glutamine synthetase (Bobermin et al., 2012), glutamate uptake activity (Baber and Haghighat, 2010), and the expression of GFAP and S100B (Esposito et al., 2008).

Some animal models have shown reduced levels of extracellular S100B and a decrease in glucose uptake in CNS (Biasibetti et al., 2013; Vizuete et al., 2013), but the link between these two events was not investigated. In order to evaluate the possibility of this link, we decided to evaluate the effect of exogenous S100B on glucose uptake in C6 astroglial cells.

2. Materials and Methods

2.1. Materials

Fetal bovine serum (FBS), Dulbecco's modified Eagle medium (DMEM) and other materials for cell culture were purchased from Gibco BRL (Carlbad, CA, USA). D-[3-^{3}H] glucose (20 Ci/mmol) were purchased from Perkin-Elmer (Boston, MA, USA). S100B protein, Anti-S100B antibody (clone SH-B) and Anti-GFAP (clone G-A-5) were purchased from Sigma-Aldrich (St. Louis, MO- USA). Anti- RAGE (clone N-16) was purchased from Santa Cruz Biotechnology (Santa Cruz CA-USA). Other reagents were purchased from local commercial suppliers (Sulquímica, Labsul or Biogen; Porto Alegre, Brazil).

2.2. C6 Astroglial Cell Culture

The C6 astroglial cell line was obtained from the American Type Culture Collection (Rockville, MA, USA) and cultured according to a previously described procedure (Bobermin et al., 2012; de Souza et al., 2009; Tramontina et al., 2012). Late passage cells (i.e. after at least 100 passages) were seeded in flasks and cultured in DMEM (pH 7.4) containing 5% FBS, 0.1% amphotericin B and 0.032% gentamicin. Cells were maintained at a temperature of 37°C in an atmosphere of 5% CO_2/95% air. At log phase, cells were detached from the culture flasks using 0.05% trypsin/ethylenediaminetetracetic acid (EDTA) and seeded (5×10^3 cells/cm^2) in 24-well plates. The cells were maintened at the same parameters cited before, for three days or until reach confluence.

2.3. Treatments

At confluence, the culture medium was removed, and the cells were treated with different S100B concentrations (0.01, 0.05 and 0.1 ng/ml - pmol) to evaluate the glucose uptake. After determine the high S100B concentration, the cells were incubated with 0.1 ng/ml S100B, in the presence of anti-S100B, anti-RAGE or anti-GFAP (diluted 1:50; 1:100 and 1:500) at the indicated concentrations. After, labeled glucose was added, and the glucose uptake assay was started.

2.4. Glucose uptake assay

Glucose uptake was performed, as previously described (Pellerin and Magistretti, 1994), with some modifications. Briefly, C6 astroglial cells were incubated at 35 °C in a Hank's balanced salt solution (HBSS) containing (in mM): 137 NaCl, 5.36 KCl, 1.26 $CaCl_2$, 0.41$MgSO_4$, 0.49 $MgCl_2$, 0.63 $Na_2HPO_4 \cdot 7H_2O$, 0.44 KH_2PO_4, 4.17 $NaHCO_3$, and 5.55 glucose, adjusted to pH 7.2. The assay was started by the addition of 0.1 µCi/well D-[3-^{3}H] glucose. The incubation was stopped after 15 min by removing the medium and rinsing the cells twice with ice-cold HBSS. The cells were then lysed in a 0.5M NaOH solution. Radioactivity was measured in a scintillation counter. Glucose uptake was calculated by subtracting the non-specific uptake, obtained by the glucose transporter inhibitor, cytochalasin B (25 µM), from the total uptake . This assay demonstrates glucose uptake as an indirect measurement of intracellular tritiated-glucose and derived-metabolites. Results were expressed as nmol/mg protein/min.

2.5. Protein Determination

Protein content was measured by Lowry's method with some modifications using bovine serum albumin as the standard (LOWRY et al., 1951).

2.6. Statistical Analysis

Data are presented as mean ± S.E.M. Each experiment was performed in triplicate from at least three independent cultures. The data were subjected to one way analysis of variance (ANOVA) followed by the Tukey's test. Values of $P<0.05$ were considered significant. All analyses were performed using the Statistical Package for the Social Sciences (SPSS) software.

3. Results

3.1. Glucose uptake is reduced in C6 astroglial cells exposed to different S100B concentrations.

The addition of exogenous S100B reduced the glucose uptake in C6 astroglial cells, when compared to basal conditions (Fig. 1). C6 cells treated with different physiological concentrations (0.01; 0.05; or 0.1 ng/ml) presented the same profile of glucose uptake.

3.2. The effect of S100B on glucose uptake is reversed by the addition of anti-S100B or anti-RAGE.

In order to confirm the specificity of exogenous S100B and to exclude the possible interference of other compounds, we added the antibody anti-S100B (diluted 1:500; 1:100 or 1:50) in the cells previously incubated with 0.1 ng/ml S100B. We observed glucose uptake returning to basal levels (Fig. 2A), which demonstrates that the reduction of glucose uptake was really caused by S100B. In order to evaluate if the effect of S100B occurs via RAGE, we added the antibody anti-RAGE (diluted 1:500; 1:100 or 1:50). We observed the reversal of S100B effects, with glucose uptake returning to basal levels (Fig. 2B).

3.3. Anti-GFAP does not reverse the effect of S100B on glucose uptake.

In order to evaluate if the reversal effect of anti-S100B and anti-RAGE was a simple effect of the antibody conformation or structure, we exposed the cells treated with 0.1 ng/mL S100B to an intracellular protein antibody (anti-GFAP) and no reversal was observed (Fig. 3).

4. Discussion

This study was designed to investigate the effect of S100B upon glucose uptake by astroglial cells, and the possible pathways involved. It is know that S100B, a protein expressed and secreted by glial cells, mainly by astrocytes, can regulate glucose metabolism, cytoskeleton dynamyres and cell proliferation (Donato et al., 2009). S100B, at nanomolar levels stimulates neurite growth and promote neural survival, while micromolar levels results in neurotoxic effects (Van Eldik and Wainwright, 2003). The levels of this protein are elevated in many brain disorders, like, brain ischemia, neurodegenerative diseases and psychiatric disorders (Gonçalves et al., 2008), and because of that, S100B is known as an astrocytic marker of brain injury. In animal models of dementia it was found, reduced levels of S100B in cerebrospinal fluid (CSF) and increased in hippocampus (Biasibetti et al., 2013; Rodrigues et al., 2009, 2010; Vicente et al., 2009). In the same models, it was found a reduction on glucose consumption. Considering that, we hypothesized that might be a link between glucose consumption and S100B.

Our study demonstrates that exogenous S100B in physiological concentrations (pmol) cause a reduction in glucose uptake in astroglial C6 cells. It is unclear if this reduction is due to a decrease in glucose consumption by the cell, or because occurs a blockage of the glucose transporter. In both assumptions, the extracellular concentration of glucose are increased. In order to confirm the specificity of exogenous S100B on the glucose uptake, and to exclude the possible interference of other compounds, we incubated the cells with anti-S100B antibody, to block the S100B effect, and we observed that glucose uptake returned to basal levels.

The energy metabolism homeostasis is essential for the maintenance of the organism, and the specific family of facilitative glucose transporters proteins (GLUTs) is responsible to mediate these processes. In the brain are mainly found the GLUT1 and GLUT3. GLUT1 is present in blood-brain barrier and in glial cells, and GLUT3 mainly in neurons. The remainig transporters have lower levels of expression in CNS (Vannucci et al., 1997). The neuronal glucose transporter GLUT3 is associated with peripheral processes, mostly in cell bodies, which often have a high energy capacity. GLUT1 is abundant in endothelial cells, in astrocytic foot processes associated with blood vessels and in small astrocytic processes located between the neuronal elements (Leino et al., 1997). C6 astroglial cells astrocytic-shaped express mostly GLUT1, in contrast with human astrocytic tumors that express GLUT3 predominantly (Nagamatsu et al., 1996).

S100B can act by binding with RAGE, that was shown to be the responsible receptor for intracellular S100B signal transduction (Huttunen et al., 2000). RAGE is composed of a V-type immunoglobulin domain and two C-type immunoglobulin domains (C1 and C2) (Schmidt et al., 1992). VC1 (complex V) has been show to bind advanced glycated end products (AGEs) and S100B (Dattilo et al., 2007). The structure of VC1 and NMR-based models of the VC1-S100B complex provide detailed insights into the mechanism of ligand recognition and binding, as well as how RAGE can be activated by the diverse range of ligands.

S100B can modulates the receptor RAGE by two pathways, depending on its concentration: nanomolar concentrations activates Ras/Mek/ERK-1/2/NF-kb, and causes an upregulation of the anti-apoptotic factor Bcl-2 in order to protects neurons, while the micromolar concentration activates MEK-ERK1/2 with overproduction of

reatives oxygen species leading to neurotoxicity (Huttunen et al., 2000). To evaluate if the effect of S100B on glucose uptake was mediated by RAGE, we blocked the receptor with an anti-RAGE antibody, which is specific for the C-terminal region of the complex V. We observed that this blockage was capable of prevent S100B effects, with glucose uptake returning to basal levels. This finding demonstrates that the effect of S100B on glucose uptake was really mediated via RAGE.

Many extracellular signals stimulates a specific intracellular signaling cascade that leads to activation of the mitogen-activated protein kinases (MAPKs). The ERK1/2 is the classical mitogen kinase, modulates the cascade of MAPKKKs, and play key roles in cellular proliferation, differentiation, migration, and gene expression (Roux and Blenis, 2004). ERK1/2 has been shown to be critical in neuronal survival, plasticity and regeneration of many types of brain cells (Miloso et al., 2008). Typically, cell surface receptors such as tyrosine kinases and RAGE transmit activating signals to the Raf/MEK/ERK cascade. Treatment with IFNα, especially with IFNα2b in C6 glioma cells, demonstrated by Wang et all decreased glucose consumption and decreased the expression of GLUT1. Moreover, the phosphorylation of MAPK family members (JNK, ERK1/2 and p38MAPK), are involved in the IFN-induced cell proliferation and apoptosis. This study also demonstrates that the activation of JNK and of p-ERK1/2 in astrocytes treated with IFN is related to the reduced consumption of glucose (Wang et al., 2012). Another find that corroborates with the assumption that GLUTs and the cascade of MAPKs are connected is the activation of ERK1/2 by angiotensin, which leads to glucose uptake inhibition in muscle cells (Izawa et al., 2005). The mechanisms which connects RAGE/ERK1/2 and the GLUTs remains inconclusive, however it seems like

that the reduction on glucose uptake find in our study can be modulated by this pathway.

In the last set of results, we decide to evaluate if the effect of S100B and RAGE antibodies on glucose uptake was an unspecific effect of its conformation or structure. Therefore, we treated C6 astroglial cells with an intracellular protein antibody, anti-GFAP, and no effect was observed. These finding confirms the specific effect of the antibodies.

In conclusion, the presented data demonstrates that glucose uptake in C6 astroglial cells was negatively modulated by exogenous S100B in physiological concentrations, and this effect was mediated via RAGE. We hypothesized that activation of ERK1/2 pathway is involved. However, further studies are necessary to clarify this correlation.

5. References

Baber, Z., and Haghighat, N. (2010). Glutamine synthetase gene expression and glutamate transporters in C6-glioma cells. Metab. Brain Dis. *25*, 413–418.

Belanger, M., and Magistretti, P.J. (2009). The role of astroglia in neuroprotection. Dialogues Clin. Neurosci. *11*, 281–295.

Biasibetti, R., Tramontina, A.C., Costa, A.P., Dutra, M.F., Quincozes-Santos, A., Nardin, P., Bernardi, C.L., Wartchow, K.M., Lunardi, P.S., and Gonçalves, C.-A. (2013). Green tea (-)epigallocatechin-3-gallate reverses oxidative stress and reduces acetylcholinesterase activity in a streptozotocin-induced model of dementia. Behav. Brain Res. *236*, 186–193.

Bobermin, L.D., Quincozes-Santos, A., Guerra, M.C., Leite, M.C., Souza, D.O., Gonçalves, C.-A., and Gottfried, C. (2012). Resveratrol prevents ammonia toxicity in astroglial cells. Plos One *7*, e52164.

Dattilo, B.M., Fritz, G., Leclerc, E., Kooi, C.W.V., Heizmann, C.W., and Chazin, W.J. (2007). The extracellular region of the receptor for advanced glycation end products is composed of two independent structural units. Biochemistry (Mosc.) *46*, 6957–6970.

Donato, R. (2003). Intracellular and extracellular roles of S100 proteins. Microsc. Res. Tech. *60*, 540–551.

Donato, R., Sorci, G., Riuzzi, F., Arcuri, C., Bianchi, R., Brozzi, F., Tubaro, C., and Giambanco, I. (2009). S100B's double life: intracellular regulator and extracellular signal. Biochim. Biophys. Acta *1793*, 1008–1022.

Donato, R., Cannon, B.R., Sorci, G., Riuzzi, F., Hsu, K., Weber, D.J., and Geczy, C.L. (2013). Functions of S100 proteins. Curr. Mol. Med. *13*, 24–57.

Van Eldik, L.J., and Wainwright, M.S. (2003). The Janus face of glial-derived S100B: Beneficial and detrimental functions in the brain. Restor. Neurol. Neurosci. *21*, 97–108.

Esposito, E., Iacono, A., Muià, C., Crisafulli, C., Mattace Raso, G., Bramanti, P., Meli, R., and Cuzzocrea, S. (2008). Signal transduction pathways involved in protective effects of melatonin in C6 glioma cells. J. Pineal Res. *44*, 78–87.

Huttunen, H.J., Kuja-Panula, J., Sorci, G., Agneletti, A.L., Donato, R., and Rauvala, H. (2000). Coregulation of neurite outgrowth and cell survival by amphoterin and S100 proteins through receptor for advanced glycation end products (RAGE) activation. J. Biol. Chem. *275*, 40096–40105.

Izawa, Y., Yoshizumi, M., Fujita, Y., Ali, N., Kanematsu, Y., Ishizawa, K., Tsuchiya, K., Obata, T., Ebina, Y., Tomita, S., et al. (2005). ERK1/2 activation by angiotensin II inhibits insulin-induced glucose uptake in vascular smooth muscle cells. Exp. Cell Res. *308*, 291–299.

Kimelberg, H.K., and Nedergaard, M. (2010). Functions of astrocytes and their potential as therapeutic targets. Neurother. J. Am. Soc. Exp. Neurother. *7*, 338–353.

Koch, M., Chitayat, S., Dattilo, B.M., Schiefner, A., Diez, J., Chazin, W.J., and Fritz, G. (2010). Structural basis for ligand recognition and activation of RAGE. Struct. Lond. Engl. 1993 *18*, 1342–1352.

LOWRY, O.H., ROSEBROUGH, N.J., FARR, A.L., and RANDALL, R.J. (1951). Protein measurement with the Folin phenol reagent. J. Biol. Chem. *193*, 265–275.

Lunardi, P., Nardin, P., Guerra, M.C., Abib, R., Leite, M.C., and Gonçalves, C.-A. (2013). Huperzine A, but not tacrine, stimulates S100B secretion in astrocyte cultures. Life Sci. *92*, 701–707.

Miloso, M., Scuteri, A., Foudah, D., and Tredici, G. (2008). MAPKs as mediators of cell fate determination: an approach to neurodegenerative diseases. Curr. Med. Chem. *15*, 538–548.

Nardin, P., Tramontina, A.C., Quincozes-Santos, A., Tortorelli, L.S., Lunardi, P., Klein, P.R., Wartchow, K.M., Bobermin, L.D., Gottfried, C., Elisabetsky, E., et al. (2011). In vitro S100B secretion is reduced by apomorphine: Effects of antipsychotics and antioxidants. Prog. Neuropsychopharmacol. Biol. Psychiatry *35*, 1291–1296.

Pellerin, L. (2008). Brain energetics (thought needs food). Curr. Opin. Clin. Nutr. Metab. Care *11*, 701–705.

Pellerin, L., and Magistretti, P.J. (1994). Glutamate uptake into astrocytes stimulates aerobic glycolysis: a mechanism coupling neuronal activity to glucose utilization. Proc. Natl. Acad. Sci. U. S. A. *91*, 10625–10629.

Pellerin, L., and Magistretti, P.J. (2004). Neuroenergetics: calling upon astrocytes to satisfy hungry neurons. Neurosci. Rev. J. Bringing Neurobiol. Neurol. Psychiatry *10*, 53–62.

Ponath, G., Schettler, C., Kaestner, F., Voigt, B., Wentker, D., Arolt, V., and Rothermundt, M. (2007). Autocrine S100B effects on astrocytes are mediated via RAGE. J. Neuroimmunol. *184*, 214–222.

Rodrigues, L., Biasibetti, R., Swarowsky, A., Leite, M.C., Quincozes-Santos, A., Quilfeldt, J.A., Achaval, M., and Gonçalves, C.-A. (2009). Hippocampal alterations in rats submitted to streptozotocin-induced dementia model are prevented by aminoguanidine. J. Alzheimers Dis. Jad *17*, 193–202.

Rodrigues, L., Dutra, M.F., Ilha, J., Biasibetti, R., Quincozes-Santos, A., Leite, M.C., Marcuzzo, S., Achaval, M., and Gonçalves, C.-A. (2010). Treadmill training restores spatial cognitive deficits and neurochemical alterations in the hippocampus of rats submitted to an intracerebroventricular administration of streptozotocin. J. Neural Transm. Vienna Austria 1996 *117*, 1295–1305.

Roux, P.P., and Blenis, J. (2004). ERK and p38 MAPK-activated protein kinases: a family of protein kinases with diverse biological functions. Microbiol. Mol. Biol. Rev. Mmbr *68*, 320–344.

Schmidt, A.M., Vianna, M., Gerlach, M., Brett, J., Ryan, J., Kao, J., Esposito, C., Hegarty, H., Hurley, W., and Clauss, M. (1992). Isolation and characterization of two binding proteins for advanced glycosylation end products from bovine lung which are present on the endothelial cell surface. J. Biol. Chem. *267*, 14987–14997.

Sidoryk-Wegrzynowicz, M., and Aschner, M. (2013). Role of astrocytes in manganese mediated neurotoxicity. Bmc Pharmacol. Toxicol. *14*, 23.

De Souza, D.F., Leite, M.C., Quincozes-Santos, A., Nardin, P., Tortorelli, L.S., Rigo, M.M., Gottfried, C., Leal, R.B., and Gonçalves, C.-A. (2009). S100B secretion is stimulated by IL-

1beta in glial cultures and hippocampal slices of rats: Likely involvement of MAPK pathway. J. Neuroimmunol. *206*, 52–57.

Tramontina, A.C., Tramontina, F., Bobermin, L.D., Zanotto, C., Souza, D.F., Leite, M.C., Nardin, P., Gottfried, C., and Gonçalves, C.-A. (2008). Secretion of S100B, an astrocyte-derived neurotrophic protein, is stimulated by fluoxetine via a mechanism independent of serotonin. Prog. Neuropsychopharmacol. Biol. Psychiatry *32*, 1580–1583.

Tramontina, A.C., Nardin, P., Quincozes-Santos, A., Tortorelli, L., Wartchow, K.M., Andreazza, A.C., Braganhol, E., de Souza, D.O.G., and Gonçalves, C.-A. (2012). High-glucose and S100B stimulate glutamate uptake in C6 glioma cells. Neurochem. Res. *37*, 1399–1408.

Tramontina, F., Tramontina, A.C., Souza, D.F., Leite, M.C., Gottfried, C., Souza, D.O., Wofchuk, S.T., and Gonçalves, C.-A. (2006). Glutamate uptake is stimulated by extracellular S100B in hippocampal astrocytes. Cell. Mol. Neurobiol. *26*, 81–86.

Vannucci, S.J., Maher, F., and Simpson, I.A. (1997). Glucose transporter proteins in brain: delivery of glucose to neurons and glia. Glia *21*, 2–21.

Vicente, E., Degerone, D., Bohn, L., Scornavaca, F., Pimentel, A., Leite, M.C., Swarowsky, A., Rodrigues, L., Nardin, P., de Almeida, L.M.V., et al. (2009). Astroglial and cognitive effects of chronic cerebral hypoperfusion in the rat. Brain Res. *1251*, 204–212.

Wang, T., Takikawa, Y., Sawara, K., Yoshida, Y., and Suzuki, K. (2012). Negative regulation of human astrocytes by interferon (IFN) α in relation to growth inhibition and impaired glucose utilization. Neurochem. Res. *37*, 1898–1905.

Figure Legends

1) The effect of different concentrations of S100B on glucose uptake by C6 astroglial cells. C6 astroglial cells were cultivated and after confluence, the medium was replaced by HBBS containing different concentrations of S100B (0.01, 0.05 and 0.1 ng/ml), and immediately incubated with [H^3]- glucose for 15 minutes. Data are expressed as mean ± standard error for 5 experiments performed in triplicate. * Significant statistical difference from control, represented by the line on 100%. (One-Way ANOVA, followed by Tukey's test, $p<0.05$).

2) The effect of anti-S100B and anti-RAGE antibodies on glucose uptake by C6 astroglial cells. C6 astroglial cells were cultivated and after confluence, the medium was replaced by HBBS containing A) S100B 0.1 ng/ml and different dilutions of anti-S100B (1:500, 1:100 and 1:50) or B) S100B 0.1 ng/ml and different dilution of anti-RAGE (1:500, 1:100 and 1:50), and immediately incubated with [H^3]- glucose for 15 minutes. Data are expressed as mean ± standard error for 5 experiments performed in triplicate. * Significant statistical difference from control, represented by the line on 100%. (One-Way ANOVA, followed by Tukey's test, $p<0.05$).

3) The effect of anti-GFAP antibody on glucose uptake by C6 astroglial cells. C6 astroglial cells were cultivated and after confluence, the medium was replaced by HBBS containing S100B 0.1 ng/ml and different dilutions of anti-GFAP (1:500, 1:100 and 1:50), and immediately incubated with [H^3]- glucose for 15 minutes. Data are expressed as mean ± standard error for 5 experiments performed in triplicate. * Significant statistical difference from control, represented by the line on 100%. (One-Way ANOVA, followed by Tukey's test, $p<0.05$).

Figures

Fig. 1

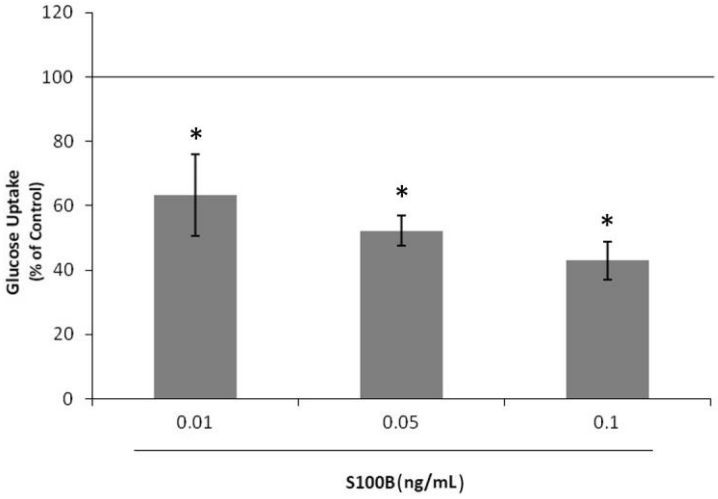


Fig. 2A

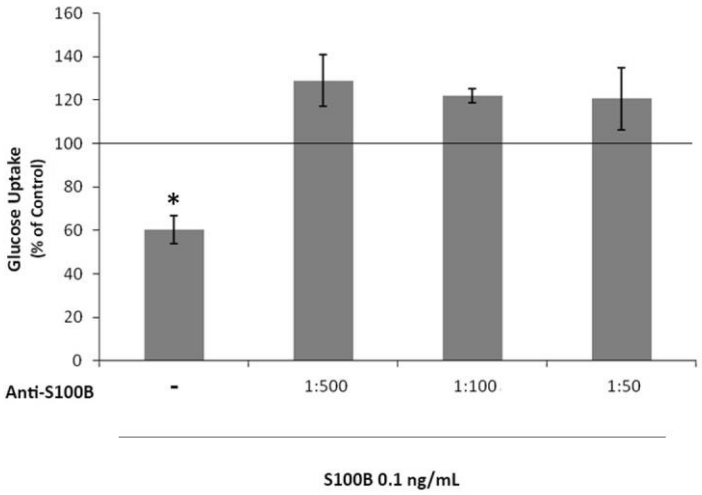


Fig. 2B

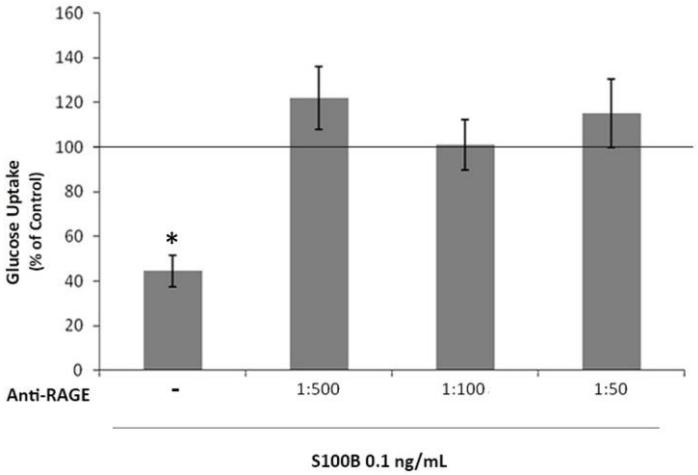
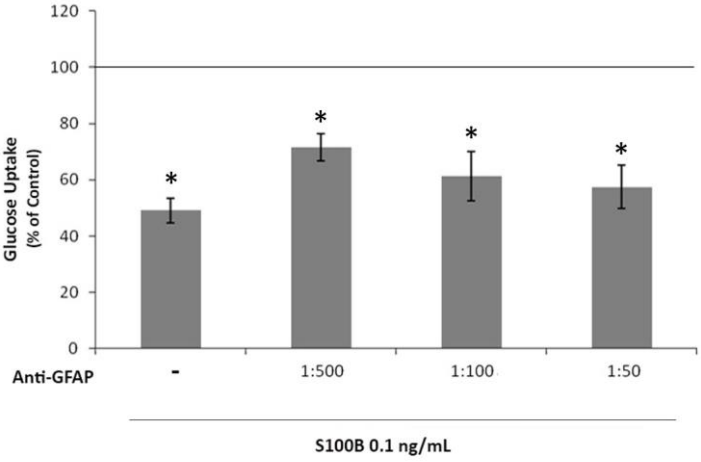

160
140
120
100
80
60
40
20
0
Glucose Uptake (% of Control)
*
Anti-RAGE
-
1:500
1:100
1:50
S100B 0.1 ng/mL

Fig. 3


120
100
80
60
40
20
0
Glucose Uptake (% of Control)
*
*
*
*
Anti-GFAP
-
1:500
1:100
1:50
S100B 0.1 ng/mL

International Journal of Developmental Neuroscience –

Organization of Manuscripts