

Insulin Stimulates S100B Secretion and These Proteins Antagonistically Modulate Brain Glucose Metabolism

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Abstract Brain metabolism is highly dependent on glucose, which is derived from the blood circulation and metabolized by the astrocytes and other neural cells via several pathways. Glucose uptake in the brain does not involve insulin-dependent glucose transporters; however, this hormone affects the glucose influx to the brain. Changes in cerebrospinal fluid levels of S100B (an astrocyte-derived protein) have been associated with alterations in glucose metabolism; however, there is no evidence whether insulin modulates glucose metabolism and S100B secretion. Herein, we investigated the effect of S100B on glucose metabolism, measuring D-³H-glucose incorporation in two preparations, C6 glioma cells and acute hippocampal slices, and we also investigated the effect of insulin on S100B secretion. Our results showed that: (a) S100B at physiological levels decreases glucose uptake, through the multiligand receptor RAGE and mitogen-activated protein kinase/ERK signaling, and (b) insulin stimulated S100B secretion via PI3K signaling. Our findings indicate the existence of insulin-S100B modulation of glucose utilization in the brain tissue, and may improve our understanding of glucose metabolism in several conditions such as ketosis, streptozotocin-induced dementia and pharmacological exposure to antipsychotics, situations that

lead to changes in insulin signaling and extracellular levels of S100B.

Keywords Astrocyte · Glucose metabolism · Insulin · MAPK · RAGE · S100B

Introduction

Astrocytes, the most abundant cells in the brain tissue, are involved in a number of functions, including maintenance of the brain blood barrier and provision of metabolic support to neurons [1, 2]. The astrocytes are a very heterogeneous cell population, but can be identified by a common marker: the glial fibrillary acidic protein (GFAP). Brain metabolism is highly dependent on glucose derived from the blood circulation, which is taken up by the astrocytes and involved in several metabolic energy pathways, such as synthesis of neurotransmitter, glutathione, lipids. Two other energetic substrates, glycogen and lactate, which are transferred to the neurons upon energetic demand [3].

Although the main entry of glucose into the brain tissue occurs via glucose transporters 1 (in endothelial cells and astrocytes) and 3 (in neurons), in an insulin-independent manner, this hormone affects brain glucose metabolism via the activation of insulin receptors (IR). These receptors are distributed throughout the brain in neurons and glial cells [4–6], acting by PI3K pathway activation [7]. Insulin signaling impairment in the brain tissue has been suggested to be involved in the cognitive alterations that occur in diabetes mellitus and Alzheimer's disease [8, 9].

Another marker used to identify astrocytes is the S100B protein, although it has been also identified in oligodendrocytes and a few neurons [10]. S100B is a calcium-binding protein of 21 kDa that binds and regulates many

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intracellular proteins [11–13], including GFAP [14] and two glycolytic enzymes, fructose-1,6-bisphosphate aldolase and phosphoglucomutase [15, 16]. Moreover, this protein is secreted by astrocytes and its extracellular neurotrophic activity has been described *in vitro* and *in vivo* [17, 18]. The extracellular effect of S100B is triggered by its binding to the multiligand receptor for advanced glycation end products (RAGE) (see Ref. [19]), which, in turn, triggers a biochemical cascade involving the mitogen-activated protein kinases (MAPK); such as ERK1/2, p38 and JNK [12].

Changes in cerebrospinal fluid S100B levels have been associated with alterations in glucose metabolism [20, 21]. S100B is also expressed and secreted by adipocytes [22] and a pioneer work showed that insulin reduces S100B secretion in adipose tissue [23]. More recently, in accordance with these findings, peripheral increments in glucose/insulin levels in healthy human subjects were reported to be accompanied by reductions in serum S100B levels. However, there is no evidence that insulin directly modulates S100B or that extracellular S100B modulates glucose metabolism in the brain tissue [24].

Therefore, we investigated the effect of S100B on glucose metabolism, measuring ^3H -glucose incorporation in two preparations, C6 astroglial cells and acute hippocampal slices. In addition, we investigated the effect of insulin on the S100B secretion.

Materials and Methods

Animals

For the preparation of hippocampal slices, male 30-day-old Wistar rats were obtained from our breeding colony (Department of Biochemistry, UFRGS, Porto Alegre, Brazil). The animals were maintained under controlled light and environmental conditions (12 h light/12 h dark cycle at a constant temperature of 22 ± 1 °C) with free access to commercial chow and water. All animal experiments were carried out in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and were approved by the Federal University of Rio Grande do Sul Animal Care and Use Committee (process number 28035).

Material

Fetal bovine serum (FBS), Dulbecco's modified Eagle medium (DMEM) and other materials for cell culture were purchased from Gibco BRL (Carlsbad, CA, USA). D-[3- ^3H] glucose (20 Ci/mmol) was purchased from Perkin-Elmer (Boston, MA, USA). 4-(2-hydroxyethyl)-

piperazine-1-ethanesulfonic acid (HEPES), S100B protein, anti-S100B antibody (SH-B1), *o*-phenylenediamine (OPD), 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).

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 [25–27]. 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/ethylenediaminetetraacetic acid (EDTA) and seeded (5×10^3 cells/cm 2) on 24-well plates. The cells were maintained under the same conditions, for 3 days or until reaching confluence.

Preparation and Incubation of Brain Hippocampal Slices

Thirty-day-old rats were decapitated and their hippocampi were quickly dissected out and sliced, based on our previous works [28, 29]. Transverse sections (300 μm) of tissue were rapidly obtained using a McIlwain tissue chopper. One slice was placed into each well of a 24-well culture plate. Slices were incubated in oxygenated physiological medium containing, in mM, 120 NaCl, 2.0 KCl, 1.0 CaCl_2 , 1.0 MgSO_4 , 25.0 HEPES, 1.0 KH_2PO_4 and 10.0 glucose, pH 7.4, at room temperature. The medium was changed every 15 min with fresh medium. Following a 120-min equilibration period, slices were incubated in medium in the presence/absence of treatment conditions for 1 h at 30 °C.

Treatments

To evaluate glucose uptake, C6 astroglial cells and hippocampal slices were treated with different concentrations of S100B (0.01, 0.05 and 0.1 ng/mL) for 15 min in DMEM without FBS or 1 h in Hank's balanced salt solution (HBSS), respectively. HBSS containing (in mM): 137 NaCl, 5.36 KCl, 1.26 CaCl_2 , 0.41 MgSO_4 , 0.49 MgCl_2 , 0.63 $\text{Na}_2\text{HPO}_4 \cdot 7\text{H}_2\text{O}$, 0.44 KH_2PO_4 , 4.17 NaHCO_3 , and 5.55 glucose, adjusted to pH 7.2. In order to evaluate

glucose uptake and S100B secretion, cells and slices were treated with regular insulin (0.1–10 μM) (Humalin R, Lilly France).

The cells and slices were incubated with S100B, in the presence of anti-S100B, anti-RAGE or anti-GFAP antibodies in 1:50; 1:100 and 1:500 dilutions. The inhibitors, PD98059 (10 μM , MEK inhibitor), SB203580 (10 μM , p38 inhibitor), SP600125 (10 μM , JNK inhibitor), or LY294002 (10 μM , PI3K inhibitor, used just with regular insulin) were added 15 min before S100B or insulin exposure.

ELISA for S100B

The S100B concentration was determined in the culture medium and in the incubation medium of slices at 1 h. S100B levels were determined by ELISA, as described previously (Leite et al. 2008). Briefly, 50 μL of sample plus 50 μL of Tris buffer were incubated for 2 h on a microtiter plate, previously coated with anti-S100B monoclonal antibody (SH-B1, from Sigma). Anti-S100 polyclonal antibody (from DAKO) was incubated for 30 min and then peroxidase-conjugated anti-rabbit antibody was added for a further 30 min. The color reaction with *o*-phenylenediamine was measured at 492 nm. The standard S100B curve ranged from 0.002 to 1 ng/mL.

Glucose Uptake Assay

After S100B or insulin incubation, in DMEM (for C6 glioma cells) or HBSS (for hippocampal slices), as described in item 2.5, the glucose uptake assay was performed as previously described [30]. Briefly, C6 astroglial cells or hippocampal slices were incubated at 35 $^{\circ}\text{C}$ in HBSS. The assay was started by the addition of 0.1 μCi /well D-[3- ^3H] 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 0.5 M NaOH solution. Radioactivity was measured using a scintillation counter. Glucose uptake was calculated by subtracting the non-specific uptake, obtained by the glucose transport 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.

Protein Determination

Protein content was measured by Lowry's method with some modifications using bovine serum albumin as the standard [31].

Statistical Analysis

Data are presented as mean \pm SEM. Each experiment was performed in triplicate from at least three independent experiments. 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 Graphpad Prism software version 6 (La Jolla, CA, USA).

Results

Glucose Uptake is Reduced in C6 Astroglial Cells Exposed to S100B

The addition of exogenous S100B (at 0.05 or 0.1 ng/mL) reduced the glucose uptake in C6 astroglial cells when compared to basal conditions (Fig. 1a) ($p = 0.0034$ and $f_{(3, 40)} = 5.346$). In order to confirm the specificity of exogenous S100B and to exclude the possible interference of other compounds, we added 0.1 ng/mL S100B to cells, which were previously incubated with the anti-S100B antibody (diluted 1:500; 1:100 or 1:50). Glucose uptake returned to basal levels (Fig. 1b) ($p = 0.0004$ and $f_{(4, 19)} = 9.691$), indicating that the reduction in glucose uptake was caused by S100B. Aiming to evaluate whether the effect of S100B occurs via RAGE, we added the anti-RAGE antibody (diluted at 1:500, 1:100 or 1:50), which abolished the effect of S100B (Fig. 1c) ($p = 0.009$ and $f_{(4, 22)} = 4.421$). In order to evaluate whether the blocking effect of anti-S100B and anti-RAGE was a non-specific effect due to any antibody addition, we exposed 0.1 ng/mL S100B-treated C6 cells to anti-GFAP at the same dilutions. Anti-GFAP, at all dilutions, was unable to affect the S100B-induced decrease in glucose uptake (Fig. 1d) ($p = 0.0001$ and $f_{(4, 20)} = 9.851$).

MAPK Signaling Mediates the S100B-Induced Reduction in Glucose Uptake in C6 Astroglial

MAPK is a well-characterized pathway in RAGE signaling. Therefore, we evaluated whether this pathway is involved in the effect of S100B on glucose uptake, by adding MAPK inhibitors 15 min before S100B treatment. The PD98059 inhibitor, which blocks the upstream kinase of ERK1/2, returned glucose uptake to basal levels (Fig. 2a) ($p < 0.0001$ and $f_{(3, 39)} = 9.741$). PD98059 *per se* was not able to alter glucose uptake. SP600125, a MAPK/JNK inhibitor (Fig. 2b) ($p = 0.0004$ and $f_{(3, 16)} = 10.74$), or SB203580, a MAPK/p38 inhibitor (Fig. 2c) ($p < 0.0001$ and $f_{(3, 16)} = 16.14$), did not alter the effect of S100B on

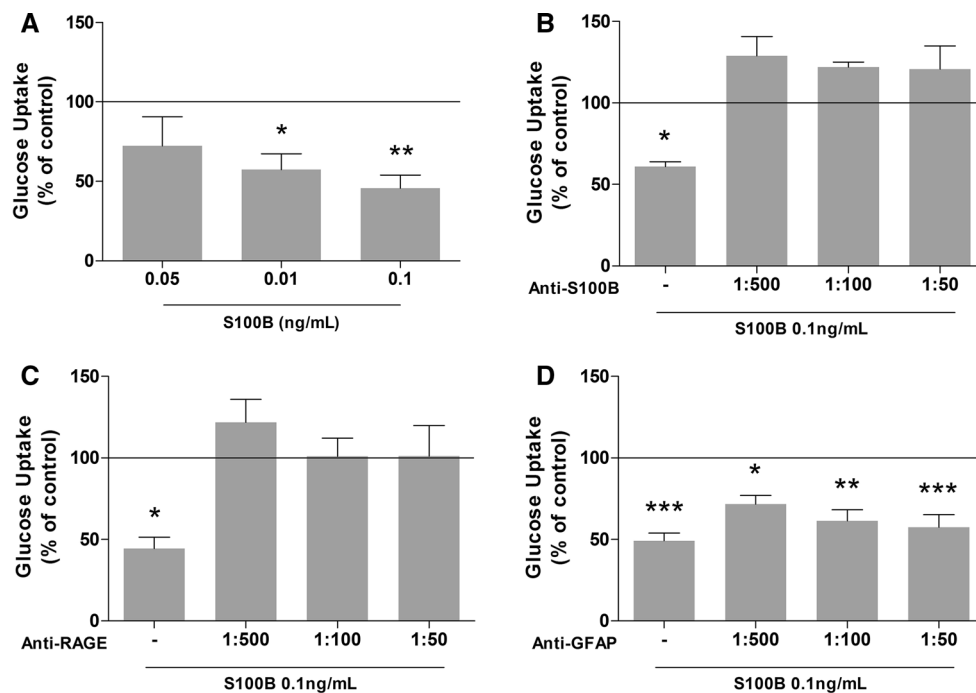


Fig. 1 Evaluation of glucose uptake in C6 astroglial cells exposed to S100B. After confluence, the medium of C6 glial cells were replaced by HBSS containing 0.1 μ Ci/well D-[3-³H] glucose and incubated for 15 min in the presence of S100B at different concentrations (a). C6 astroglial cells were incubated for 15 min in the presence of 0.1 ng/mL S100B and in the presence of different anti-S100B antibody

dilutions (b) or anti-RAGE (c) or anti-GFAP (d). Data are shown as perceptual means \pm SE of six independent experiments performed in triplicate. The line represents control value (100 %). * Significantly different from basal by one-way ANOVA followed by Tukey's test for $p < 0.05$, ** for $p < 0.01$, *** for $p < 0.001$

glucose uptake. Unexpectedly, SP600125 (but not SB203580), alone, caused a decrease in glucose uptake.

Glucose Uptake is Also Reduced in Hippocampal Slices Exposed to S100B

When acute hippocampal slices were exposed to exogenous S100B protein (Fig. 3a) ($p = 0.0152$ and $f_{(3, 26)} = 4.189$), a reduction in glucose uptake was also observed, similarly to effects obtained in C6 astroglial cells. As in C6 glial cells, the addition of anti-RAGE antibody (diluted at 1:500 and 1:100) blocked the effect of S100B on glucose uptake (Fig. 3b) ($p = 0.0055$ and $f_{(3, 28)} = 5.207$).

ERK Signaling is Also Involved in the S100B-Induced Reduction in Glucose Uptake in Hippocampal Slices

In hippocampal slices, we preformed the same treatments with MAPK inhibitors in order to evaluate the pathways involved in the effect of S100B on glucose uptake. We observed that the reduction in glucose uptake induced by S100B was prevented by PD98059, which blocks ERK1/2 activation (Fig. 4a) ($p < 0.0001$ and $f_{(3, 23)} = 19.81$).

Moreover, in contrast to results obtained in C6 cells, SP600125 and SB203580 (inhibitors of Jnk and p38, respectively)—also prevented the reduction in glucose uptake induced by S100B (Fig. 4b) ($p = 0.0004$ and $f_{(3, 20)} = 10.74$) and (4c) ($p < 0.0001$ and $f_{(3, 18)} = 16.14$). In contrast to results in C6 cells, SP600125 alone increased the glucose uptake in hippocampal slices.

Glucose Uptake is Increased in C6 Astroglial Cells and Hippocampal Slices Exposed to Insulin

Aiming to confirm the effect of insulin on glucose metabolism in brain tissue and to evaluate its effect in our preparations, we exposed C6 astroglial cells and acute hippocampal slices to different concentrations of insulin (0.05–10 nM). C6 cells significantly increased glucose uptake at 10 nM (Fig. 5a) ($p = 0.0017$ and $f_{(3, 12)} = 9.526$) and this effect was abolished by incubation with LY294002, an inhibitor of the PI3K pathway (Fig. 5b) ($p = 0.0363$ and $f_{(3, 12)} = 3.931$). The same results were obtained in hippocampal slices. Glucose uptake was stimulated by insulin even at lower concentrations (at 1 nM) (Fig. 5c) ($p = 0.0027$ and $f_{(3, 17)} = 7.099$) and this effect was blocked by incubation with LY294002 (Fig. 5d) ($p = 0.0094$ and $f_{(3, 19)} = 5.082$).

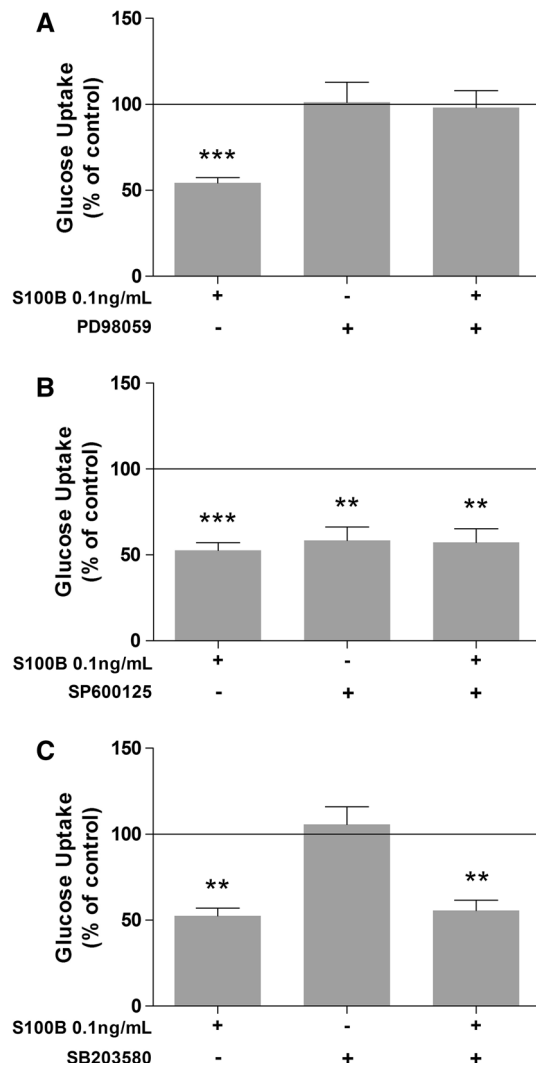


Fig. 2 Effect of MAPK inhibitors on glucose uptake in C6 astroglial cells exposed to S100B. After confluence, the mediums of the C6 astroglial cells were replaced with HBSS containing 0.1 μ Ci/well D-[3- 3 H] glucose and incubated for 15 min in the presence of 0.1 ng/mL S100B and in the absence or presence of 10 μ M PD98059 (a) or 10 μ M SP600125 (b) or 10 μ M SB203580 (c). Data are shown as perceptual means \pm SE of six independent experiments performed in triplicate. The *line* represents control value (100 %). ** Significantly different from basal by one-way ANOVA followed by Tukey's test for $p < 0.01$, *** for $p < 0.001$

Insulin Positively Modulates S100B Secretion in Hippocampal Slices

Insulin (from 0.05 to 10 nM) did not affect S100B secretion in C6 astroglial cells (Fig. 6a) ($p = 0.8735$ and $f_{(3, 41)} = 0.2321$); however, PI3K pathway inhibition with LY294002 increased the secretion of S100B (Fig. 6b) ($p < 0.0001$ and $f_{(3, 35)} = 18.80$). In contrast, in hippocampal slices, insulin directly (at 10 nM) induced S100B secretion (Fig. 6c) ($p = 0.0027$ and $f_{(3,16)} = 7.252$), but

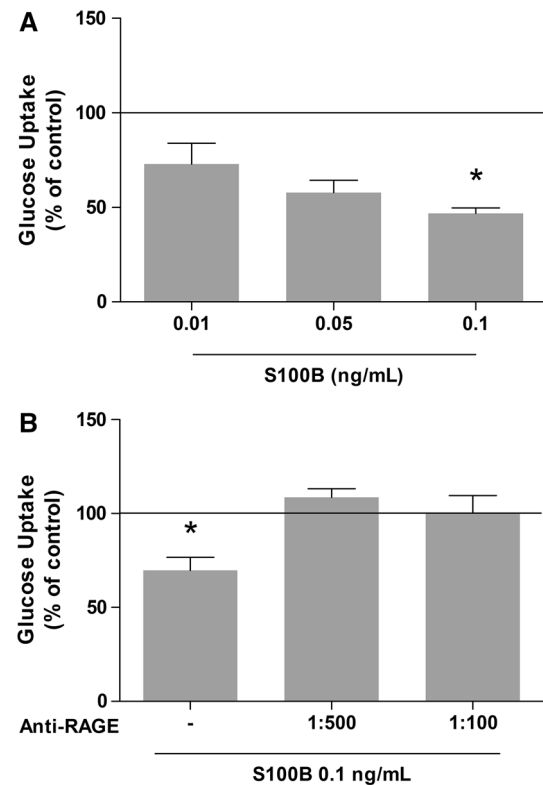


Fig. 3 Evaluation of glucose uptake in hippocampal slices exposed to S100B. **a** Hippocampal slices were treated for 1 h with different S100B concentrations. After the treatment, the slices were incubated with 0.1 μ Ci/well D-[3- 3 H] glucose for 30 min. **b** Hippocampal slices were treated for 1 h in the presence of S100B and in the presence of different concentrations of anti-RAGE antibody and then incubated with 0.1 μ Ci/well D-[3- 3 H] glucose for 30 min. Data are shown as perceptual means \pm SE of six independent experiments performed in triplicate. The *line* represents control value (100 %). * Significantly different from basal by one-way ANOVA followed by Tukey's test for $p < 0.05$

the PI3K inhibitor was not able to prevent this effect (Fig. 6d) ($p = 0.0147$ and $f_{(3, 28)} = 4.168$). Together these results suggest a positive modulation of insulin on S100B secretion.

Discussion

The brain's sensitivity to insulin, particularly with regard to glucose metabolism, is well characterized. Some of our previous studies have provided findings to suggest a connection between extracellular levels of S100B and cell glucose metabolism in brain tissue [21, 32, 33], but this association has not been characterized to date. Moreover, no association between insulin and extracellular S100B, i.e. S100B secretion, has been described. The findings of the present study indicate that extracellular S100B negatively modulates glucose metabolism in the brain tissue, at least

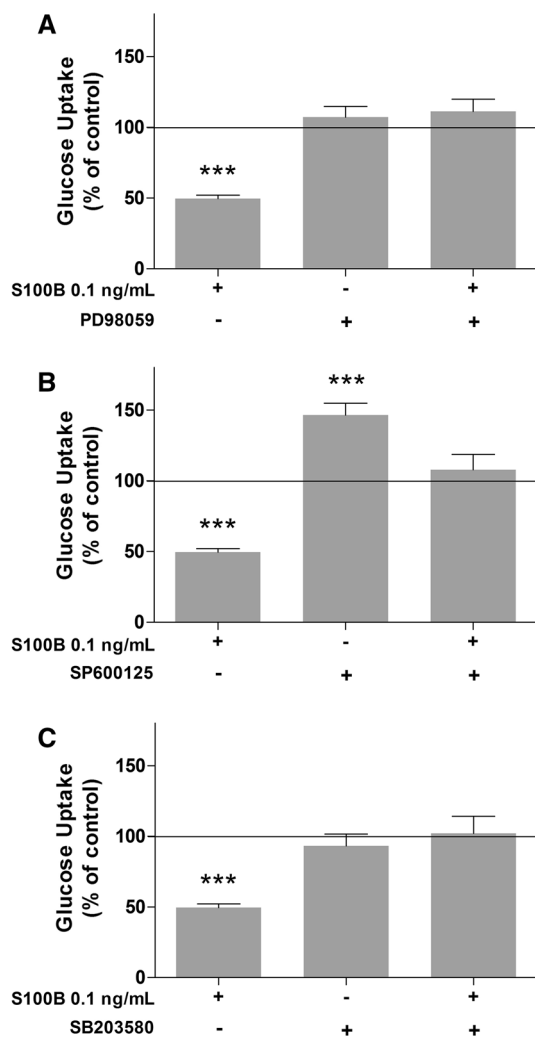


Fig. 4 Effect of MAPK inhibitors on glucose uptake in hippocampal slices exposed to S100B. Hippocampal slices were treated for 1 h with different S100B concentrations. After the treatment, the slices were incubated for 30 min with 0.1 $\mu\text{Ci}/\text{well}$ D-[3- ^3H] glucose in the presence of 10 μM PD98059 (a) or 10 μM SP600125 (b) or 10 μM SB203580 (c). Data are shown as perceptual means \pm SE of six independent experiments performed in triplicate. The line represents control value (100 %). *** Significantly different from basal by one-way ANOVA followed by Tukey's test for $p < 0.001$

in glial cells, supporting an association between S100B and glucose metabolism. Moreover, our data indicate that insulin upregulates S100B secretion. Furthermore, results suggest a possible physiological link between insulin and S100B in glucose metabolism in brain tissue.

We used two preparations in this study, C6 astroglial cells and acute hippocampal slices. C6 glioma cells were employed as they are astroglial-like after 100 passages, expressing glutamine synthetase [25], and demonstrating glutamate uptake activity [34, 35], and the expression of GFAP and S100B [36]. C6 glioma cells and primary astrocytes have different regulatory mechanisms for S100B secretion under different conditions, such as high-glucose

exposure [37]. However, C6 glioma cells secrete lower quantities of S100B, when compared to primary astrocytes, thus facilitating studies using the exogenous addition of S100B [27]. On the other hand, we also used hippocampal slices, which are more complex cell preparations that resemble in vivo conditions. Hippocampal tissue contains functional and interactive neurons and astrocytes that secrete S100B, but also in lower quantities than in primary astrocytes in culture [38]. Although isolated glial cells and hippocampal slices may exhibit different responses to pharmacological stimulation (e.g. [38]), results can help us to understand biochemical changes in vivo. Moreover, we used low concentrations of S100B exogenous in these experiments, which were much lower than μM toxic levels reported in vitro, allowing us to speculate regarding an extracellular physiological role of this protein in glucose metabolism.

S100B induced a decrease in glucose uptake, in C6 astroglial cells and hippocampal slices, and this effect was mediated by RAGE. Moreover, in both preparations, this effect involved ERK, a classical pathway involved in RAGE activation by S100B [12, 39]. Another MAPK, p38, seems to also be involved in this S100B-induced effect, as demonstrated by results obtained with a p38 inhibitor in acute hippocampal slices. Moreover, we observed that SP600125 (an inhibitor of JNK) *per se* inhibited glucose uptake in C6 glial cells and stimulated glucose uptake in hippocampal slices. It is unclear, at this moment, whether these effects are mediated by JNK or involve unspecific targets of this compound.

We should draw attention to an aspect of our experimental conditions, which does not represent a limitation, but may limit an exact conclusion; C6 cells and hippocampal slices were incubated with D-[3- ^3H] glucose, which is uptaken and metabolized. However, herein, we use glucose metabolism as synonymous of glucose uptake. The S100B effect involves glucose uptake, but at this moment, we cannot say whether it directly involves glucose transport and/or glucose consumption in its various metabolic fates in glial cells. Nevertheless the final effect of S100B on glucose metabolism is clear.

Insulin, at 1 and/or 10 nM, was able to increase glucose metabolism in C6 astroglial cells and acute hippocampal slices, and this effect involved PI3K signaling. Insulin (at 10 nM) was able to induce an increase in S100B secretion in hippocampal slices, but not in C6 astroglial cells. However, inhibition of PI3K increased S100B secretion in C6 glial cells, suggesting that insulin signaling maybe increase S100B secretion. This link could help to explain decreases in the cerebrospinal fluid S100B levels under conditions of impaired insulin signaling, such as STZ-induced dementia [32, 40] or reduced insulin signaling such as in the ketogenic diet [41].

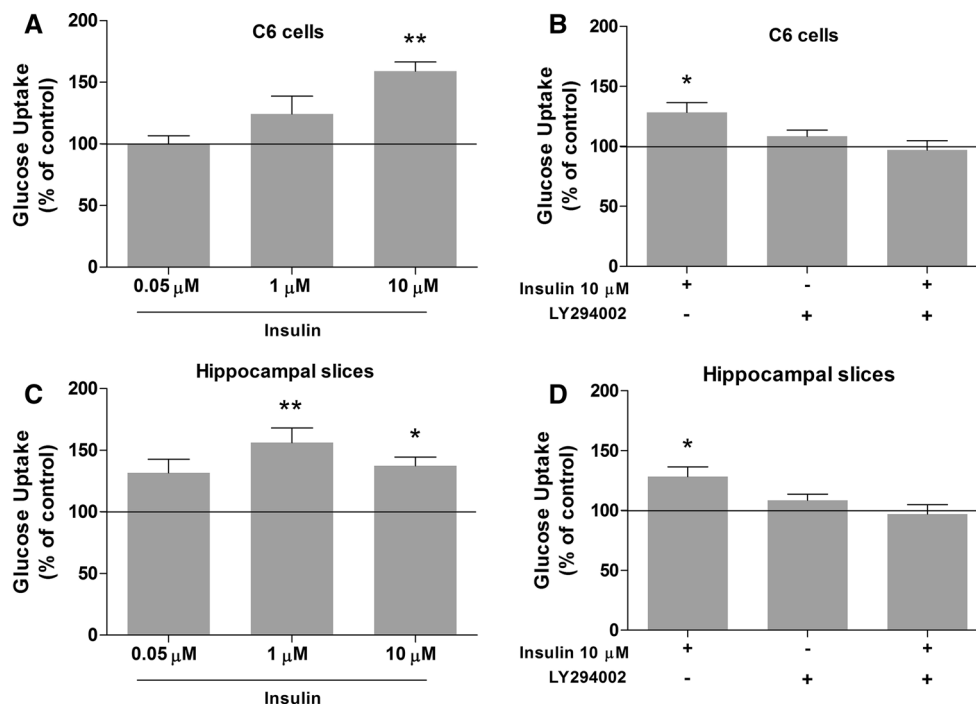


Fig. 5 Evaluation of glucose uptake in the presence of exogenous insulin and PI3K inhibitor. **a** After confluence, the C6 glial cells were treated with different insulin concentrations for 1 h. **b** After confluence, the C6 cells were treated in the presence of 10 μM insulin and 10 μM LY294002, a PI3K inhibitor for 1 h. **c** Hippocampal slices were treated with different insulin concentrations for 1 h. **d** Hippocampal slices were treated in the absence and presence of 10 μM

insulin and in the presence of 10 μM LY294002. Slices were then incubated with 0.1 $\mu\text{Ci/well}$ D-[3- ^3H] glucose for 30 min in the presence of treatments. Data are shown as perceptual means \pm SE of six independent experiments performed in triplicate. The *line* represents control value (100 %). * Significantly different from basal by one-way ANOVA followed by Tukey's test for $p < 0.05$, ** for $p < 0.01$

Other observations could also be related to this link. For example, antipsychotics appear to decrease insulin sensitivity [42], as well as S100B secretion [43, 44]. Moreover, antidepressants, such as fluoxetine, which increase S100B secretion [45] seem to increase insulin sensitivity [46]. These observations do not clarify the dilemma of which event occurs first, but indicate the possibility of glial S100B secretion modulation by insulin.

In addition, recent data from adipocytes suggest that the super expression of RAGE (the receptor for S100B) reduces insulin sensitivity [47], indicating a possible cross-talk between insulin and S100B. Ongoing experiments in our lab using RAGE silencing will help to clarify this possibility in brain tissue. However, independently of this other possibility, we conceive a link between insulin and S100B in the modulation of brain glucose metabolism (see Fig. 7). Insulin stimulates brain glucose metabolism, while S100B does the opposite. However, insulin also stimulates S100B, which would work as a brake or negative feedback for the action of insulin. This brake could have different activity in neuron and glial cells, due to the different amounts of RAGE, in physiological and pathological conditions. For example, increased RAGE has been reported in diabetes

mellitus and Alzheimer's disease [48]. Such an this increase could result in a decrease in glucose metabolism via the activation of S100B. It is possible that S100B secretion, in this case, is induced by cytokines for example [26], because insulin signaling is impaired. Moreover, the beta-amyloid peptide (rather than S100B) may activate RAGE in Alzheimer's disease [49].

This insulin-S100B axis of regulation in the brain is different from that of the adipose tissue, where insulin decreases S100B secretion [23]. Accordingly, increases in glucose/insulin levels in healthy human subjects were recently reported to be accompanied by a reduction in serum S100B levels [24]. It is not known whether extracellular S100B in adipose tissue also decreases glucose uptake, but RAGE overexpression in adipocytes is associated with the suppression of glucose transporter type 4 (dependent on insulin) and attenuates insulin-stimulated glucose uptake [47]. At this moment, it can only be speculated that, in a glucose-rich environment, the insulin-S100B axis drives glucose to adipocytes and saves astrocytes from massive flux. On the other hand, under fasting conditions, this brake would be turned off in the brain tissue [21].

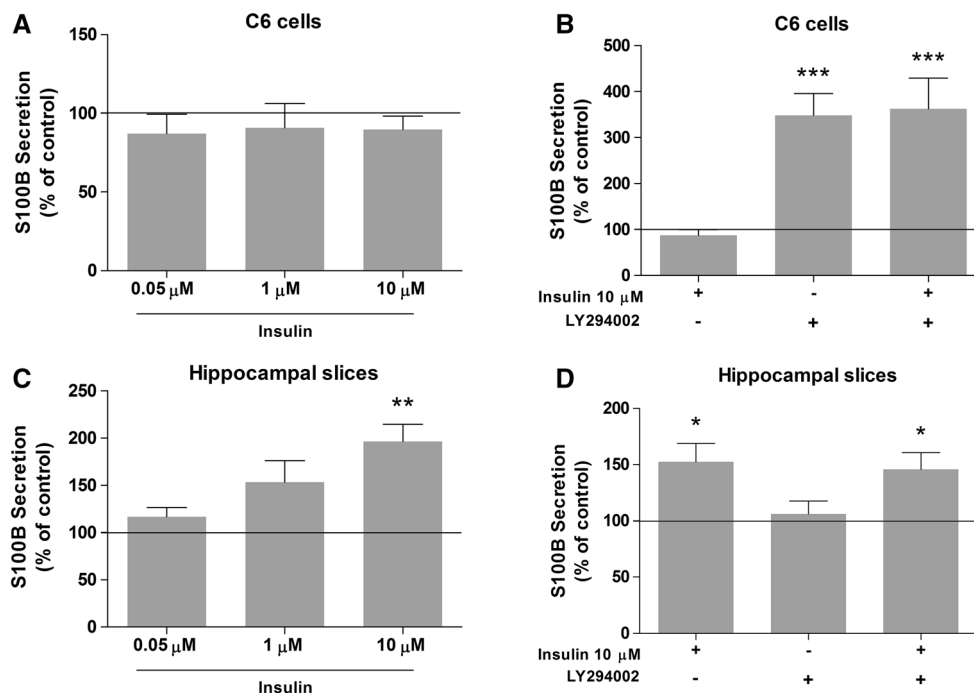


Fig. 6 Evaluation of S100B secretion in the presence of exogenous insulin and PI3K inhibitor. **a** After confluence, the medium of the C6 astroglial cells was replaced by FBS-free medium with different insulin concentrations for 1 h. **b** After confluence, cell medium was replaced by FBS-free medium in the absence and presence of 10 μM insulin and in the absence and presence of 10 μM LY294002, a PI3K inhibitor for 1 h. **c** Hippocampal slices were treated with different insulin concentrations for 1 h. **d** Hippocampal slices were treated in

the absence and presence of 10 μM insulin and in the absence and presence of 10 μM LY294002, a PI3K inhibitor for 1 h. The S100B-secreted content was measured by ELISA. Data are shown as perceptual means ± SE of six independent experiments performed in triplicate. The *line* represents control value (100 %). * Significantly different from basal by one-way ANOVA, followed by Tukey’s test for $p < 0.05$, ** for $p < 0.01$, *** for $p < 0.001$

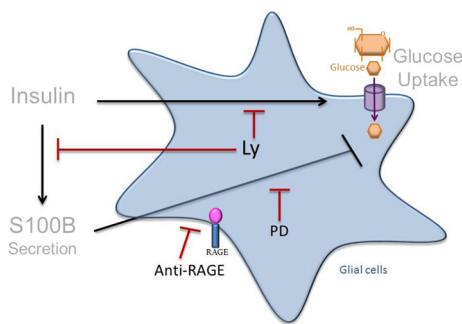


Fig. 7 Mechanism of action of insulin and S100B on glial glucose metabolism. Insulin stimulates S100B secretion from glial cells, as well as glucose metabolism. Both actions are inhibited by the addition of LY294002 (LY), a PI3K inhibitor. Extracellular S100B, in turn, inhibits glucose uptake and/or metabolism in glial cells and this effect is blocked by the PD98059 inhibitor (PD), which blocks the upstream kinase of ERK1/2, and anti-RAGE antibody

Conclusions

In summary, data from C6 astroglial cells and acute hippocampal slices indicate that: (i) S100B, at physiological levels, decreases glucose metabolism via RAGE/ERK signaling, and (ii) insulin stimulates S100B secretion via

PI3K signaling. Such associations allow us to conceive an insulin-S100B mediated modulation of glucose utilization, which furthers our understanding of glucose metabolism in several conditions of physiological (e.g. ketosis), pathological (e.g. Alzheimer’s disease) and pharmacological exposure (e.g. antipsychotics).

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