Biochemical and Biophysical Research Communications 405 (2011) 691–696

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

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

Insulin increases glutamate transporter GLT1 in cultured astrocytes

Yi-Fei Ji, Si-Min Xu, Jia Zhu, Xin-Xin Wang, Ying Shen *

Department of Neurobiology, Key Laboratory of Medical Neurobiology of Ministry of Health of China, Zhejiang Province Key Laboratory of Neurobiology, Zhejiang University School of Medicine, Hangzhou, Zhejiang 310058, PR China

ARTICLE INFO

Article history:
Received 24 January 2011
Available online 1 February 2011

Keywords:
Glutamate transporter
GLT1
Astrocyte
Insulin
Excitotoxicity
Growth factor
Akt

ABSTRACT

The astroglial cell-specific glutamate transporter subtype 2 (excitatory amino acid transporter 2, GLT1) plays an important role in excitotoxicity that develops after damage to the central nervous system (CNS) is incurred. Both the protein kinase C signaling pathway and the epidermal growth factor (EGF) pathway have been suggested to participate in the modulation of GLT1, but the modulatory mechanisms of GLT1 expression are not fully understood. In the present study, we aimed to evaluate the effects of insulin on GLT1 expression. We found that short-term stimulation of insulin led to the upregulation of both total and surface expressions of GLT1. Akt phosphorylation increased after insulin treatment, and triciribine, the inhibitor of Akt phosphorylation, significantly inhibited the effects of insulin. We also found that the upregulation of GLT1 expression correlated with increased kappa B motif-binding phosphoprotein (KBBP) and GLT1 mRNA levels. Our results suggest that insulin may modulate the expression of astrocytic GLT1, which might play a role in reactive astrocytes after CNS injuries.

1. Introduction

Glutamate is the most abundant excitatory neurotransmitter in the brain, but excessive levels of extracellular glutamate are excitotoxic and lead to severe neuronal death, which has been implicated in neurodegenerative diseases and traumatic brain injury [1,2]. The astroglial cell-specific glutamate transporter subtype 2 (excitatory amino acid transporter 2, GLT1) accounts for more than 90% of synaptic and extrasynaptic glutamate clearance in the central nervous system (CNS) [3–5]. Thus, GLT1 plays an important role in preventing excessive excitotoxicity that occurs in nerve damages.

Previous work revealed that Akt induces the expression of GLT1 through increased transcription [6]. Protein kinase C (PKC) can regulate endocytosis and degradation of this transporter in a ubiquitin-dependent manner [7–10]. Protein interacting with C kinase 1 (PICK1) was also shown to interact with GLT1b, thereby regulating the modulation of GLT1 function by PKC [11]. Conversely, some evidence suggests that unknown neuronal secreted factors can regulate the expressions and cytoplasmic clusters of GLT1 and glutamate aspartate transporter (GLAST) [12–18]. Limited evidence has shown that GLT1 expression is increased by epidermal growth factor (EGF)/Akt signals [6], particularly in astrocytes incubated in an astrocyte-defined medium (ADM) [19] that may contain a number of growth factors [20,21]. These data suggest that growth factors might be implicated in the modulation of GLT1 expression.

Although these experiments promote comprehension of the modulation of GLT1, how GLT1 activity is regulated by growth factors remains to be elucidated. In the present work, we aimed to explore whether insulin regulates GLT1 expression in astrocytes. Our results indicate that short-term stimulation of insulin can actively regulate astrocytic GLT1 expression and function, which require Akt activation and correlate with increases in both kappa B motif-binding phosphoprotein (KBBP) expression and GLT1 mRNA levels.

2. Materials and methods

2.1. Astrocyte cultures

All animal experiments were specifically designed to minimize the number of animals used and were approved by the Animal Experimentation Ethics Committee of Zhejiang University. Cortical astrocytes were derived from brain cortices of E18 Sprague–Dawley rats [15,22]. Cortices were dissected and incubated with 0.25% trypsin–EDTA for 20 min at 37 °C. Tissue was triturated and suspended in Dulbecco's modified Eagle's medium (DMEM, GIBCO, Carlsbad, CA). Cells were plated at a uniform density of 2×10^5 cells/ml and maintained as described previously [10]. In these cultures, approximately 95% of the cells were astrocytes based on immunocytochemical criteria, as shown by positive staining for glial fibrillary acidic protein (GFAP) (Supplemental Fig. 1).

* Corresponding author. Address: Department of Neurobiology, Key Laboratory of Medical Neurobiology of Ministry of Health of China, Zhejiang Province Key Laboratory of Neurobiology, Zhejiang University School of Medicine, 388 YuHangTang Rd., Hangzhou, Zhejiang 310058, PR China. Fax: +86 571 88208240.
E-mail addresses: yshen@zju.edu.cn, jiyifei2003@gmail.com (Y. Shen).

0006-291X/$ - see front matter © 2011 Elsevier Inc. All rights reserved.
doi:10.1016/j.bbrc.2011.01.105

Oligodendrocytes and microglial cells growing on top of the confluent astrocyte layer were removed by shaking at 200 rpm for 2 h at 37 °C, and the culture medium was then replaced. Administration of 200 nM insulin overnight did not visibly change astrocyte growth (Supplemental Fig. 1).

2.2. Antibodies and reagents

Antibodies against S6, phosphorylated S6 (Ser240/Ser244) (pS6), Akt, and phosphorylated Akt (Ser473) (pAkt) were purchased from Cell Signaling (Danvers, MA). GFAP and GAPDH antibodies were from Abcam (Cambridge, UK). Triciribine (TCBN) and proteinase inhibitor cocktails were from Merck Chemicals (Darmstadt, Germany). Horseradish peroxidase-conjugated secondary antibodies for immunoblotting were from GE Healthcare (Waukesha, WI). 4′,6-Diamidino-2-phenylindole (DAPI), Alexa Fluor-conjugated secondary antibodies for immunofluorescence, neurobasal medium, and B27 supplements were purchased from Invitrogen (Carlsbad, CA). The antibodies against GLT1 and glutamate transporter subtype 1 (GLAST) were gifts from Dr. Michael Robinson (University of Pennsylvania, Philadelphia, PA). All other reagents were purchased from Sigma (St. Louis, MO), unless stated otherwise.

2.3. Western blotting and cell surface biotinylation

Astrocyte cultures were lysed and harvested in RIPA buffer containing proteinase inhibitor cocktails (Merck Chemicals, Whitehouse Station, NJ). The concentration of proteins in each sample was measured by BCA protein assay. Equal quantities of proteins were loaded and run on SDS–PAGE gels. The products were then transferred to PVDF membranes (Immobilon-P, Millipore, Billerica, MA), immunoblotted with antibodies, and visualized with enhanced chemiluminescence (ECL, Thermo Scientific, Rockford, IL). Film signals were digitally scanned and quantitated using ImageJ 1.42q (National Institutes of Health, Bethesda, MD). GAPDH immunoreactivity was set as the loading control in each experiment, and all data were normalized to the corresponding control.

After the biotinylation of surface proteins with a membrane-impermeant reagent, sulfo-NHS-SS-biotin (Thermo Scientific), and quenching of the excess biotin, biotinylated proteins were batch extracted with avidin-conjugated beads as described previously [10]. Equivalent proteins of lysate and biotinylated (cell surface) fractions were loaded onto SDS–PAGE gels. After separation and transfer, the PVDF membranes were probed with antibodies.

2.4. RNA preparation and real-time polymerase chain reaction (PCR)

Changes in mRNA expression were examined by real-time PCR using an ABI Prism 7500 Sequence Detection System (Applied Biosystems, Carlsbad, CA). cDNA was amplified using SYBR Premix Ex Taq (TaKaRa, Dalian, China) in the presence of primer oligonucleotides specific for GLT1 and β-actin. Quantification was performed using the comparative cycle threshold method, with the β-actin expression level as the internal control. The following forward (F) and reverse (R) primer sequences were used to amplify GLT1 and β-actin: GLT1-F, 5′-GTTCAAGGACGGGATGAATGTCTTA-3′; GLT1-R, 5′-CATCAGCTTGGCCTGCTCAC-3′; actin-F, 5′-TCACCCACACTGTGCCCATCTATGA-3′; and actin-R, 5′-CATCGGAACCGCTCATTGCCGATAG-3′.

2.5. Immunostaining

Cultured astrocytes were fixed with precooled (−20 °C) methanol for 5 min. For GFAP staining, astrocytes were permeabilized and exposed to anti-GFAP antibody (1:600) overnight at 4 °C. For membrane GLT1 staining, the astrocytes were incubated with GLT1 antibody (1:600) in PBS with 0.1% BSA overnight at 4 °C, and then incubated with Alexa Fluor-conjugated secondary antibodies (1:1000) for 1 h at room temperature in the dark. Immunocytochemistry images were obtained using a fluorescent microscope (BX61, Olympus, Tokyo, Japan) at a resolution of 1024 × 1024 pixels. Fluorescent photomicrographs were converted to pseudocolor images using Olympus Image-Pro 6.3 (Olympus). MetaMorph 5.0 software (Universal Imaging, West Chester, PA) was used to analyze cell images for quantitation.

2.6. Electrophysiology

Whole-cell patch-clamp recordings were performed in the cultured astrocytes at room temperature, according to previous work [23]. Patch pipettes were pulled from borosilicate glasses with a perpendicular pipette puller (Narishige Instruments, Tokyo, Japan). The series resistance of the pipette was between 3 and 7 MΩ when it was filled with an intracellular solution containing (in mM) 135 Cs-methanesulfonate, 10 CsCl, 0.2 ethylene glycol bis(β-aminoethyl ether) *N*,*N*′-tetraacetic acid, 10 Hepes, 4 Na-ATP, and 0.4 Na-GTP (310 Osm; pH 7.3, adjusted with KOH). Cells were voltage-clamped at −80 mV using a MultiClamp 700A amplifier (Molecular Devices, Foster City, CA). The analog signals were sampled at 10 kHz and low-pass-filtered at 2 kHz using pClamp 9.0 (Molecular Devices). To measure transporter currents, we used a rapid solution changer (RSC-160, Biological Science Instruments, Claix, France) to apply drug solutions to recorded cells. Solutions were made with Ringer's solution containing (in mM) 145 NaCl, 3 KCl, 2 $CaCl_2$, 10 Hepes, and 10 glucose (310 Osm; pH 7.4, adjusted with NaOH). The solutions in the tubes were fed into the perfusion bath by gravity at a flow rate of 0.5 ml/min.

2.7. Statistical analysis

Statistical differences were determined using either Student's *t*-test for two-group comparisons or one-way ANOVA with Tukey's test for multiple comparisons among more than two groups. Statistical significance was accepted at $P < 0.05$. Data in the text and figures are presented as mean ± SEM.

3. Results

3.1. Insulin treatment increases both total and surface expressions of GLT1

To detect the expression of GLT1, we starved the astrocytes for 16 h in DMEM containing 0.1% FBS before performing experiments throughout the present work [18]. To determine the effects of insulin on GLT1 expression, we added 200 nM insulin to the cultures for 1 h. Similar doses and durations have been utilized for observation of the effects of insulin in previous work [24,25]. We found that insulin exposure increased total GLT1 expression (Fig. 1A). By contrast, GLAST was unaffected. Biotinylation was then used to examine the cell surface expression of GLT1. As shown in Fig. 1D, insulin increased GLT1 levels at the cell surface but left GLAST levels unaltered. To further confirm these findings, we conducted two more lines of experiments: first, whole-cell patch-clamp recordings in the cultured astrocytes were performed to record the transporter-mediated currents. Rapid application of 100 μM glutamate induced inward currents with an average peak current of 145 ± 22 pA ($n = 18$), as shown in Fig. 1G ("insulin −"). Insulin treatment produced a large increase in peak currents ("insulin +") with an average of 250 ± 29 pA ($n = 16$; $P < 0.05$ compared with "insulin −"). Unfortunately, because there is no GLAST-specific antagonist, we were unable to block GLAST-mediated

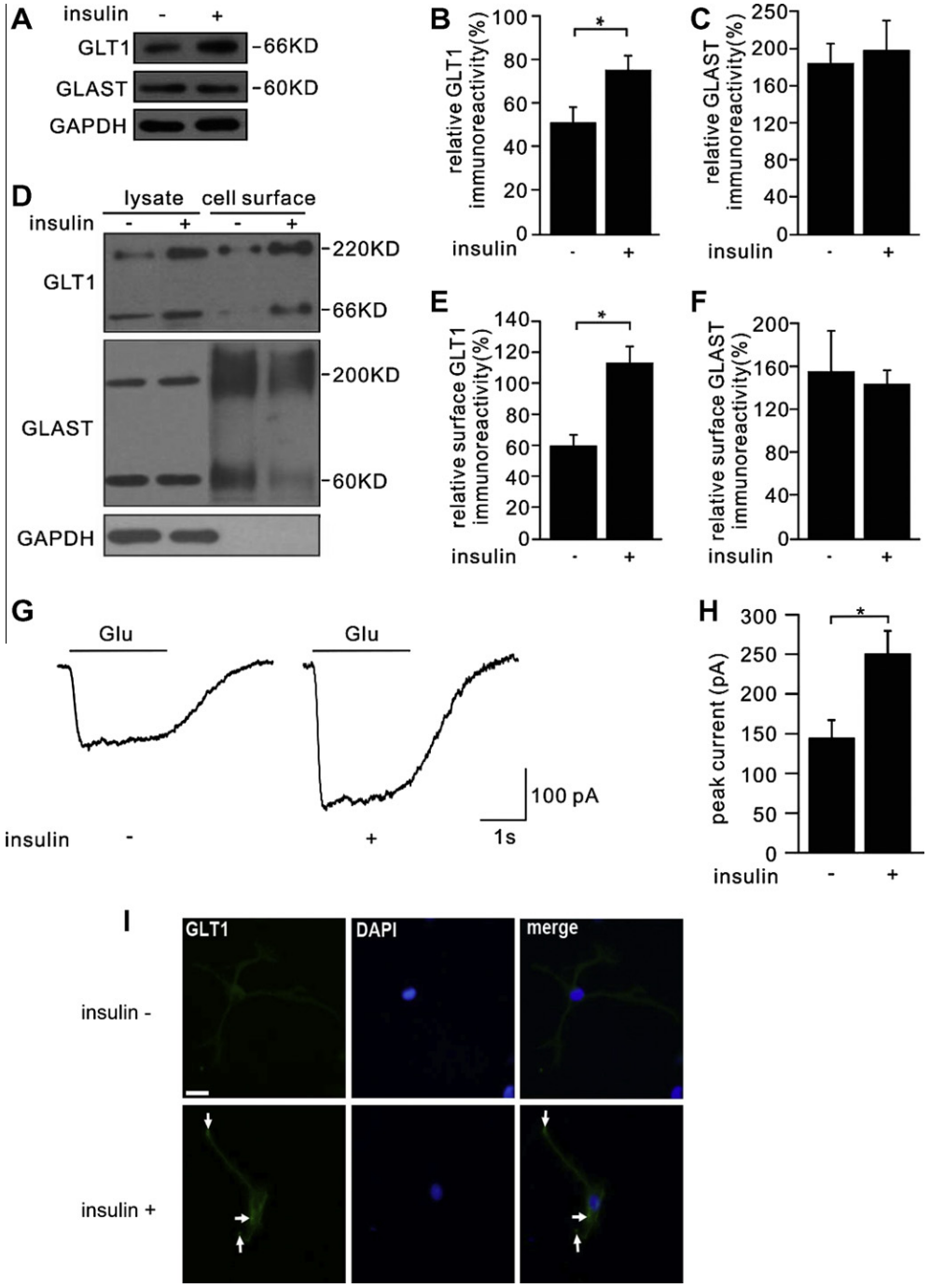

Fig. 1. Insulin increases GLT1 expression in cultured cortical astrocytes. Astrocytes were starved for 16 h and subsequently incubated with 200 nM insulin for 1 h. Immunoblot signals were quantified using ImageJ software. (A) Insulin treatment increased total GLT1, but not GLAST. The lysates were probed by immunoblotting with antibodies to GLT1, GLAST, and GAPDH. (B, C) Signal intensity ratios of GLT1 and GLAST to GAPDH: GLT1, 51 ± 12% (−) and 76 ± 14% (+); GLAST, 183 ± 19% (−) and 196 ± 29% (+). (D) Insulin increased the surface expression level of GLT1, but not that of GLAST. Astrocytic plasma membranes were biotinylated. Lysate (left) and surface (cell surface, right) fractions were probed with both anti-GLT1 and anti-GLAST antibodies. In this experiment, GLT1 migrated as a multimer. (E, F) Surface signal intensity ratios of GLT1 and GLAST to GAPDH: GLT1, 60 ± 5% (−) and 113 ± 7% (+); GLAST, 161 ± 33% (−) and 135 ± 22% (+). (G) Representative whole-cell currents in response to 1 mM glutamate in cultured astrocytes. The peak current after insulin exposure (+) was higher than that in the control group (−). Statistics are shown in (H). (I) Localization of GLT1 at the surface of astrocytes. Cultured astrocytes were incubated with 200 nM insulin for 15 h and immunostained with rabbit polyclonal GLT1 antibody and Alexa Fluor-conjugated secondary antibody. After fixation and permeabilization, nuclei were stained with DAPI. White arrows show the clusters of GLT1 at the surface of cell bodies and processes. The scale bar represents 20 μm. Results are expressed as the mean ± SE of four experiments performed using independent astrocyte cultures. $^{*}P < 0.05$ relative to control (Student's *t*-test).

currents. Second, the cells were stained with DAPI to visualize nuclei and with anti-GLT1 to observe the surface distribution of GLT1. Fluorescent microscopy scanning indicated that incubation with insulin caused significant transporter clustering (Fig. 1I; $n = 20$) compared with the control, in which no transporter clustering was observed ($n = 20$). These data demonstrate that insulin treatment increases both total and surface expressions of GLT1 in cultured astrocytes.

3.2. KBBP and GLT1 mRNA levels are increased by insulin stimulation

KBBP was recently shown to be an important downstream astroglial nuclear factor for GLT1 transcription and expression [18]. We next tested whether insulin treatment altered KBBP expression. Fig. 2A shows that KBBP immunoreactivity was significantly enhanced after insulin treatment, accompanied by increased GLT1 as well (statistics are shown in Fig. 2B and C). Because KBBP was reported to increase GLT1 transcription [18], we investigated the GLT1 mRNA level by real-time PCR. Our results indicated that insulin exposure increased the GLT1 mRNA levels (Fig. 2D). Akt is a positive regulator of the Rheb/mammalian target of rapamycin (mTOR) pathway [26]; thus, we examined total expression and phosphorylation of S6, the substrate of mTOR. Our results demonstrated that 200 nM insulin significantly increased the phosphorylation of S6, while total S6 was unaltered (Fig. 2E). These results suggest that the insulin-induced upregulation of GLT1 correlates with the increased levels of KBBP and GLT1 mRNA.

3.3. Akt is required for insulin-induced GLT1 upregulation

Previous work found that constitutively active Akt significantly enhances the GLT1 expression [6]. We therefore examined whether insulin could concomitantly alter the Akt expression and phosphorylation in the astrocyte cultures. Fig. 3A shows that the phosphorylation, but not the total expression, of Akt increased after insulin treatment. To evaluate whether Akt is required for this insulin-induced GLT1 expression, we pretreated the astrocytes with 10 nM TCBN, a specific Akt inhibitor, for 48 h and then stimulated them with 200 nM insulin. We found that TCBN was sufficient to inhibit the effect of insulin on GLT1 expression (see "TCBN +" in Fig. 3A and B). Biotinylation assay also demonstrated that the application of TCBN inhibited GLT1 surface expression (Fig. 3E). Interestingly, total GLT1 and surface GLT1 were largely reduced in the presence of TCBN compared with those in the controls (Fig. 3A and E), in which the cultured astrocytes had received starvation stimulation. We also tested the actions of TCBN on Akt and mTOR, and TCBN almost blocked pAkt and pS6, indicating that the application of TCBN was effective in this work (Fig. 3C and D). These data suggest that Akt is required for insulin-induced GLT1 upregulation and imply that starvation-induced GLT1 expression might be dependent on Akt.

4. Discussion

In the present work, we found that insulin stimulation is able to elevate both total and surface expressions of GLT1 in cultured astrocytes, which require Akt phosphorylation and correlate with KBBP expression and GLT1 mRNA transcription.

Wu et al. [19] showed that the GLT1 level increased in astrocytes treated using an ADM that may contain EGF, basic fibroblast growth factor, and insulin [20,21]. This work extends previous studies by showing that short-term treatment with 200 nM insulin is sufficient to modulate GLT1 expression in cultured astrocytes. We also found that this insulin treatment increased the phosphorylation of Akt and S6, leading to speculations that insulin receptor activation might increase KBBP and modulate GLT1 expression through the Akt/Rheb/mTOR pathway. Future work should elucidate how KBBP is modulated by this pathway. Interestingly, no obvious changes in GLAST were observed after insulin treatment, suggesting that the modulatory mechanism of GLAST may be different from that of GLT1. This finding is similar to a previous study, in which glutamate stimulation causes the upregulation of GLAST but not that of GLT1 [27].

The factors affecting the expression of astroglial glutamate transporters remain ambiguous, although some investigations have suggested that these factors might be secreted from neurons

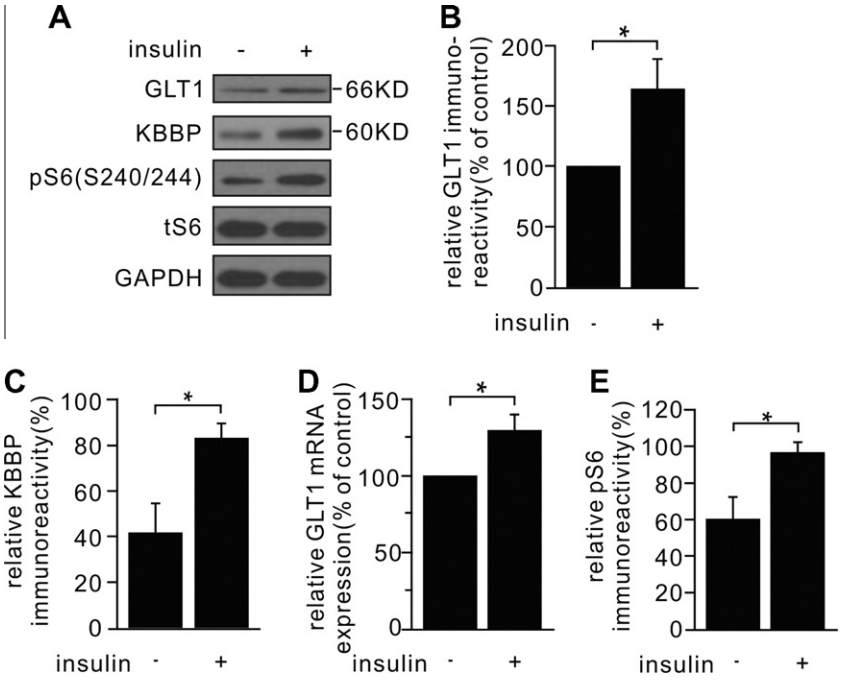

Fig. 2. Insulin treatment increased KBBP and GLT1 mRNA levels. Chemical treatments are listed as "+" or "−". (A) After starvation, astrocytes were incubated with 200 nM insulin for 1 h. The lysates were probed by immunoblotting with antibodies to GLT1, KBBP, pS6 (Ser240/Ser244), S6, and GAPDH. (B) GLT1/GAPDH signal intensity ratio. GLT1 (+) is 165 ± 28% of control (−). (C) KBBP/GAPDH signal intensity ratios: 42 ± 13% (−) and 82 ± 7% (+). (D) β-Actin and GLT1 mRNA levels were quantified using the comparative cycle threshold method. The ratios of GLT1 mRNA to β-actin mRNA in control (insulin −) and insulin stimulation (insulin +) conditions were then calculated. The percentage change in ratios is shown. GLT1 mRNA is 130 ± 5% of control. (E) pS6 (Ser240/Ser244)/GAPDH signal intensity ratios: 61 ± 12% (−) and 97 ± 5% (+). Results are expressed as the mean ± SE of four experiments performed using independent astrocyte cultures. *$P < 0.05$ relative to control (Student's *t*-test).

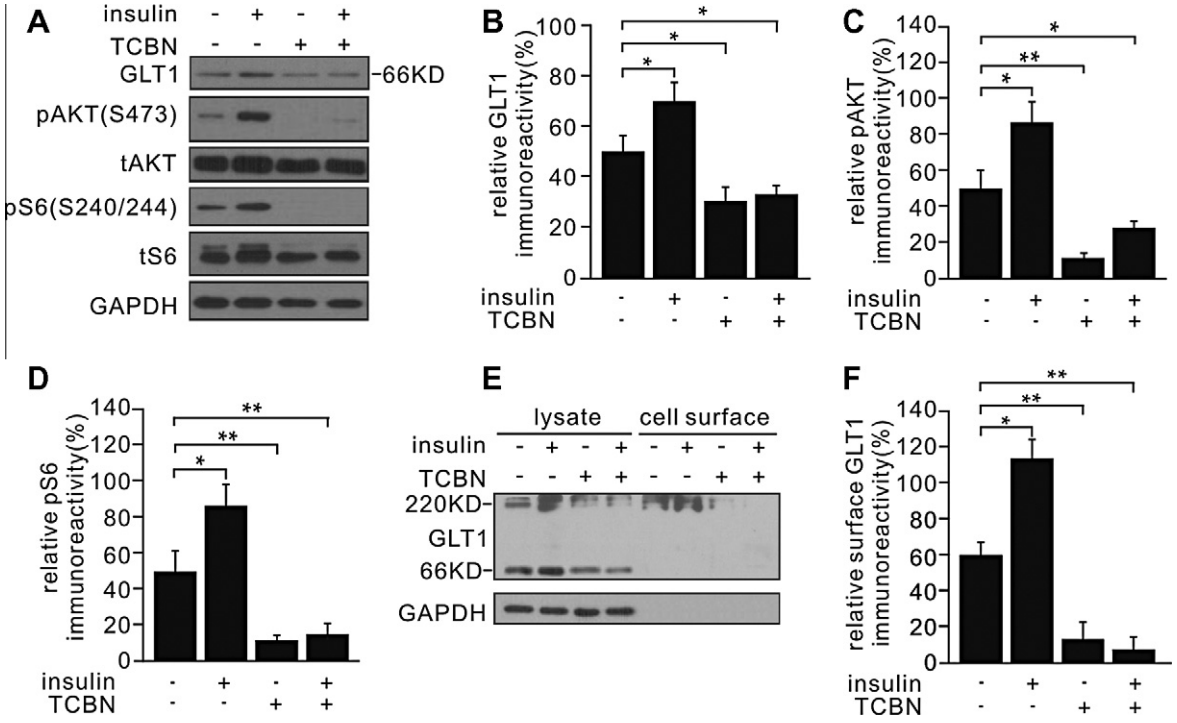

Fig. 3. Insulin-induced GLT1 expression requires the activation of Akt. After starvation, astrocytes were preincubated with 10 μM TCBN for 48 h, followed by incubation with 200 nM insulin for 1 h. Chemical treatments are listed as "+" or "−". (A) The lysates were probed by immunoblotting with antibodies to GLT1, pAkt (Ser473), total Akt (tAkt), pS6 (Ser240/Ser244), total S6 (tS6), and GAPDH. Chemical treatments are listed as "+" or "−". (B) GLT1/GAPDH signal intensity ratios: insulin −/TCBN −, 49 ± 6%; insulin +/TCBN −, 68 ± 7%; insulin −/TCBN +, 30 ± 6%; insulin +/TCBN +, 32 ± 3%. (C) pAkt/GAPDH signal intensity ratios: insulin −/TCBN −, 49 ± 4%; insulin +/TCBN −, 85 ± 4%; insulin −/TCBN +, 11 ± 2%; insulin +/TCBN +, 28 ± 3%. (D) pS6/GAPDH signal intensity ratios: insulin −/TCBN −, 79 ± 4%; insulin +/TCBN −, 124 ± 1%; insulin −/TCBN +, 12 ± 3%; insulin +/TCBN +, 18 ± 5%. (E) Astrocytic plasma membranes were biotinylated. Lysate (left) and surface (cell surface, right) fractions were probed with anti-GLT1 and anti-GAPDH antibodies. The bar graphs in the right panel show the surface GLT1/GAPDH signal intensity ratios in insulin: insulin −/TCBN −, 59 ± 4%; insulin +/TCBN −, 113 ± 8%; insulin −/TCBN +, 14 ± 5%; insulin +/TCBN +, 9 ± 5%. Results are expressed as the mean ± SE of four experiments performed using independent astrocyte cultures. $^{*}P < 0.05$, $^{**}P < 0.01$, and $^{***}P < 0.005$ relative to control (ANOVA).

[12–17]. For example, Yang et al. [18] indicated that excited presynaptic terminals can release unknown soluble factors (other than glutamate) and stimulate the expression of GLT1 under pathophysiological conditions. Interestingly, growth factors have been implicated in the transformation of quiescent astrocytes into reactive astrocytes. In particular, the expression and activation of EGF receptors have been reported to be upregulated in astrocytes after injury [28]. Because insulin and EGF receptors resemble each other in structure and antigenicity [29,30], it will be interesting to investigate whether insulin is the major factor released after injury and whether it participates in neuron–glial interaction-mediated GLT1 expression.

Using the transfection of the dominant-negative and constitutively active Akt, Li et al. [6] were able to determine that Akt is involved in the regulation of GLT1. Wu et al. [19] showed that Akt is activated upon ADM stimulation. In the present work, we found that insulin-stimulated GLT1 expression was dependent on Akt activity, which recruits KBBP expression by unknown mechanisms. All these results suggest that Akt may play critical functions in growth factor-induced GLT1 upregulation.

In summary, we have demonstrated that short-term stimulation of insulin led to the upregulation of both total and surface expressions of GLT1. In correlation with the elevated GLT1, insulin also increased the KBBP expression and mRNA levels of GLT1, as shown by Western blotting and real-time PCR. The phosphorylation of Akt increased after insulin treatment, and triciribine, the inhibitor of Akt phosphorylation, significantly inhibited the effects of insulin. Thus, our study suggests that insulin can actively modulate the expression of astrocytic GLT1, which might occur in reactive astrocytes after CNS injuries.

Acknowledgments

We are grateful for helpful comments from members of Shen laboratory. This work was supported by grants from the National Foundation of Natural Science of China (No. 30600168, to Y.S.), Zhejiang Provincial Foundation of Natural Science (No. R206018, to Y.S.), the National Foundation of Natural Science of China/Hong Kong Research Grants Council JRS fund (No. 30731160616, to Y.S.), the New Century Talent Award of the Ministry of Education of China (No. NCET-07-0751, to Y.S.) and the National Basic Research Program (Project 973) of the Ministry of Science and Technology of China (No. 2009CB94140 and 2011CB5044000).

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bbrc.2011.01.105.

References

[1] B. Billups, D. Rossi, T. Oshima, O. Warr, M. Takahashi, M. Sarantis, M. Szatkowski, D. Attwell, Physiological and pathological operation of glutamate transporters, Prog. Brain Res. 116 (1998) 45–57.
[2] J. Yi, A. Hazell, Excitotoxic mechanisms and the role of astrocytic glutamate transporters in traumatic brain injury, Neurochem. Int. 48 (2006) 394–403.
[3] N. Danbolt, Glutamate uptake, Prog. Neurobiol. 65 (2001) 1–105.
[4] Y. Huang, D. Bergles, Glutamate transporters bring competition to the synapse, Curr. Opin. Neurobiol. 14 (2004) 346–352.
[5] M. Regan, Y. Huang, Y. Kim, M. Dykes-Hoberg, L. Jin, M. Watkins, D. Bergles, J. Rothstein, Variations in promoter activity reveal a differential expression and physiology of glutamate transporters by glia in the developing and mature CNS, J. Neurosci. 27 (2007) 6607–6619.

[6] L. Li, S. Toan, O. Zelenaia, D. Watson, J. Wolfe, J. Rothstein, M. Robinson, Regulation of astrocytic glutamate transporter expression by Akt: evidence for a selective transcriptional effect on the GLT-1/EAAT2 subtype, J. Neurochem. 97 (2006) 759–771.
[7] M. González, M. Robinson, Neurotransmitter transporters: why dance with so many partners?, Curr Opin. Pharmacol. 4 (2004) 30–35.
[8] M. González, B. Susarla, M. Robinson, Evidence that protein kinase Calpha interacts with and regulates the glial glutamate transporter GLT-1, J. Neurochem. 94 (2005) 1180–1188.
[9] A. Sheldon, M. González, E. Krizman-Genda, B. Susarla, M. Robinson, Ubiquitination-mediated internalization and degradation of the astroglial glutamate transporter, GLT-1, Neurochem. Int. 53 (2008) 296–308.
[10] B. Susarla, M. Robinson, Internalization and degradation of the glutamate transporter GLT-1 in response to phorbol ester, Neurochem. Int. 52 (2008) 709–722.
[11] M. Bassan, H. Liu, K. Madsen, W. Armsen, J. Zhou, T. DeSilva, W. Chen, A. Paradise, M. Brasch, J. Staudinger, U. Gether, N. Irwin, P. Rosenberg, Interaction between the glutamate transporter GLT1b and the synaptic PDZ domain protein PICK1, Eur. J. Neurosci. 27 (2008) 66–82.
[12] R. Swanson, J. Liu, J. Miller, J. Rothstein, K. Farrell, B. Stein, M. Longuemare, Neuronal regulation of glutamate transporter subtype expression in astrocytes, J. Neurosci. 17 (1997) 932–940.
[13] B. Schlag, J. Vondrasek, M. Munir, A. Kalandadze, O. Zelenaia, J. Rothstein, M. Robinson, Regulation of the glial Na^+-dependent glutamate transporters by cyclic AMP analogs and neurons, Mol. Pharmacol. 53 (1998) 355–369.
[14] M. Munir, D. Correale, M. Robinson, Substrate-induced up-regulation of Na^+-dependent glutamate transport activity, Neurochem. Int. 37 (2000) 147–162.
[15] O. Zelenaia, B. Schlag, G. Gochenauer, R. Ganel, W. Song, J. Beesley, J. Grinspan, J. Rothstein, M. Robinson, Epidermal growth factor receptor agonists increase expression of glutamate transporter GLT-1 in astrocytes through pathways dependent on phosphatidylinositol 3-kinase and transcription factor NF-kappaB, Mol. Pharmacol. 57 (2000) 667–678.
[16] T. Nakagawa, Y. Otsubo, Y. Yatani, H. Shirakawa, S. Kaneko, Mechanisms of substrate transport-induced clustering of a glial glutamate transporter GLT-1 in astroglial–neuronal cultures, Eur. J. Neurosci. 28 (2008) 1719–1730.
[17] J. Zhou, M. Sutherland, Glutamate transporter cluster formation in astrocytic processes regulates glutamate uptake activity, J. Neurosci. 24 (2004) 6301–6306.
[18] Y. Yang, O. Gozen, A. Watkins, I. Lorenzini, A. Lepore, Y. Gao, S. Vidensky, J. Brennan, D. Poulsen, J. Park, N. Jeon, M. Robinson, J. Rothstein, Presynaptic regulation of astroglial excitatory neurotransmitter transporter GLT1, Neuron 61 (2009) 880–894.
[19] X. Wu, T. Kihara, A. Akaike, T. Niidome, H. Sugimoto, PI3K/Akt/mTOR signaling regulates glutamate transporter 1 in astrocytes, Biochem. Biophys. Res. Commun. 393 (2010) 514–518.
[20] S. Miller, C. Romano, C. Cotman, Growth factor upregulation of a phosphoinositide-coupled metabotropic glutamate receptor in cortical astrocytes, J. Neurosci. 15 (1995) 6103–6109.
[21] C. Vermeiren, M. Najimi, N. Vanhoutte, S. Tilleux, I. de Hemptinne, J. Maloteaux, E. Hermans, Acute up-regulation of glutamate uptake mediated by mGluR5a in reactive astrocytes, J. Neurochem. 94 (2005) 405–416.
[22] V. Tawfik, M. Lacroix-Fralish, K. Bercury, N. Nutile-McMenemy, B. Harris, J. Deleo, Induction of astrocyte differentiation by propentofylline increases glutamate transporter expression in vitro: heterogeneity of the quiescent phenotype, Glia 54 (2006) 193–203.
[23] Y. Shen, Y. Zhou, X. Yang, Characterization of AMPA receptors on isolated amacrine-like cells in carp retina, Eur. J. Neurosci. 11 (1999) 4233–4240.
[24] D. Guertin, D. Stevens, C. Thoreen, A. Burds, N. Kalaany, J. Moffat, M. Brown, K. Fitzgerald, D. Sabatini, Ablation in mice of the mTORC components raptor, rictor, or mLST8 reveals that mTORC2 is required for signaling to Akt-FOXO and PKCα, but not S6K1, Dev. Cell 11 (2006) 859–871.
[25] K. Martin, B. Merenick, M. Ding, K. Fetalvero, E. Rzucidlo, C. Kozul, D. Brown, H. Chiu, M. Shyu, B. Drapeau, R. Wagner, R. Powell, Rapamycin promotes vascular smooth muscle cell differentiation through insulin receptor substrate-1/phosphatidylinositol 3-kinase/Akt2 feedback signaling, J. Biol. Chem. 282 (2007) 36112–36120.
[26] M. Laplante, D. Sabatini, MTOR signaling at a glance, J. Cell Sci. 122 (2009) 3589–3594.
[27] S. Duan, C. Anderson, B. Stein, R. Swanson, Glutamate induces rapid upregulation of astrocyte glutamate transport and cell-surface expression of GLAST, J. Neurosci. 19 (1999) 10193–10200.
[28] L. Li, A. Lundkvist, D. Andersson, U. Wilhelmsson, N. Nagai, A. Pardo, C. Nodin, A. Ståhlberg, K. Aprico, K. Larsson, T. Yabe, L. Moons, A. Fotheringham, I. Davies, P. Carmeliet, J. Schwartz, M. Pekna, M. Kubista, F. Blomstrand, N. Maragakis, M. Nilsson, M. Pekny, Protective role of reactive astrocytes in brain ischemia, J. Cereb. Blood Flow Metab. 28 (2008) 468–481.
[29] G. Csaba, P. Kovács, EGF receptor induction and insulin-EGF overlap in Tetrahymena, Experientia 47 (1991) 718–721.
[30] C. Ward, M. Lawrence, V. Streltsov, T. Adams, N. McKern, The insulin and EGF receptor structures: new insights into ligand-induced receptor activation, Trends Biochem. Sci. 32 (2007) 129–137.