Published in final edited form as:
J Neurochem. 2011 April ; 117(1): 82–90. doi:10.1111/j.1471-4159.2011.07174.x.

Hippocampal Betaine/GABA Transporter mRNA Expression is not Regulated by Inflammation or Dehydration Post-Status Epilepticus

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Abstract

Seizure activity can alter GABA transporter and osmoprotective gene expression, which may be involved in the pathogenesis of epilepsy. However, the response of the betaine/GABA transporter is unknown. The goal of the present study was to compare the expression of betaine/GABA transporter mRNA to that of other osmoprotective genes and GABA transporters following status epilepticus (SE). The possible contributory role of dehydration and inflammation was also investigated because both have been shown to be involved in the regulation of GABA transporter and/or osmoprotective gene expression. BGT1 mRNA was increased 24 h post-SE, as were osmoprotective genes. BGT1 was decreased 72 h and 4 weeks post-SE, as were the GABA transporter mRNAs. The mRNA values for osmoprotective genes following 24-hour water withdrawal were significantly lower than the values obtained 24 h post-SE despite similarities in their plasma osmolality values. Betaine/GABA transporter mRNA was not altered by lipopolysaccharide-induced inflammation while the transcription factor TonEBP and the GABA transporters (GAT1 and 3) were. These results suggest that neither plasma osmolality nor inflammation fully account for the changes seen in betaine/GABA transporter mRNA expression post-SE. However, it is evident that BGT1 mRNA expression is altered by SE and displays a temporal pattern with similarities to both GABA and osmolyte transporters. Further investigation of BGT1 regulation in the brain is warranted.

Keywords

betaine/GABA transporter; status epilepticus; GABA transporters; osmolyte transporters; epilepsy; SLC6a12

Introduction

GABA is the primary inhibitory neurotransmitter in the mammalian CNS and after its release from interneurons, GABAergic signaling is terminated via reuptake by GABA transporters (Madsen et al. 2010) The betaine/GABA transporter (BGT1) is able to transport the organic osmolyte betaine as well as GABA, and is therefore both an osmolyte transporter and a GABA transporter (GAT). A variety of GAT alterations have been shown to exist in

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Authors state no conflict of interest.

epilepsy. GAT1 and GAT3 expression are altered in a time-dependent manner following seizure activity; GAT1 may transiently increase post-seizure but is consistently downregulated chronically. Similarly, GAT3 has been reported to both increase and to decrease following seizure activity (Hirao et al. 1998; Ueda and Willmore 2000; Andre et al. 2001; Patrylo et al. 2001; Sperk et al. 2003; Zhu and Ong 2004). GABA transporters display altered distribution and decreased function in tissue from humans with epilepsy (During et al. 1995; Mathern et al. 1999; Patrylo et al. 2001). In the rat kainic acid model of status epilepticus (SE), BGT1 expression was reported to increase post-SE (Zhu and Ong 2004). GAT downregulation in epilepsy may serve to increase tonic inhibitory conductance by increasing the extracellular GABA concentration. However, these transporters are able to reverse and release GABA under certain conditions such as elevated extracellular K^+ and glutamate (Gaspary et al. 1998; Heja et al. 2009), which is known to occur during seizures (Krnjevic et al. 1982; Somjen and Giacchino 1985; Liu et al. 1997). Downregulation of these transporters, then, may exacerbate seizure activity because transporter reversal may be important in seizure cessation or in preventing seizure spread (Patrylo et al. 2001). Indeed, it has been shown that glutamate causes a significant increase in extracellular GABA in non-epileptogenic hippocampi, but not in epileptogenic hippocampi from patients with refractory epilepsy (During et al. 1995). However, GAT inhibition remains a viable anticonvulsant strategy despite seizure-related downregulation as demonstrated by the clinical efficacy of the specific GAT1 inhibitor, tiagabine (Madsen et al. 2010). The role of BGT1 in controlling neuronal excitability is unknown. However, it is evident that regulation of GAT1 by tiagabine can control seizures and that seizures can alter the expression of GAT1 and GAT3.

The role of osmolyte transporters in epilepsy is less clear, but several associations exist. Organic osmolytes accumulate in cells and cause water retention to avoid cell shrinkage in hyperosmotic solutions without disturbing cellular function (Yancey et al. 1982; Lang et al. 1998; Wehner et al. 2003; Strange 2004). Dehydration causes the upregulation of genes responsible for the synthesis of osmolyte transporters or biosynthetic enzymes of organic osmolytes (Burg et al. 1997; Waldegger and Lang 1998; Handler and Kwon 2001). Through this mechanism, osmolyte transporters modulate the size of the extracellular space (ECS) by exerting control over cell size. Excessive neuronal activity, such as that associated with epileptiform bursts, causes cell swelling and ECS reduction (Dietzel et al. 1982). A reduced ECS enhances neuronal excitability, while an increased ECS dampens excitability (Dudek et al. 1990; Schwartzkroin et al. 1998). Therefore, osmotic homeostasis is important in controlling neuronal excitability. In addition, seizure-induced alterations to osmotic stress have been described. The Na^+/myo-inositol co-transporter (SMIT) is significantly increased post-SE (Nonaka et al. 1999). Furthermore, levels of the organic osmolyte taurine, are altered by seizures (Lehmann et al. 1985; Baran 2006), and BGT1 expression is reportedly increased following kainic acid-induced SE (Zhu and Ong 2004). In addition betaine, myo-inositol and taurine all display anticonvulsant activity, suggesting a direct interaction between osmotic status and seizure susceptibility (Freed et al. 1979; French et al. 1986; Patishi et al. 1996; Solomonia et al. 2007; Junyent et al. 2009).

Transcription plays an essential role in the upregulation and insertion of BGT1 into the cell membrane. Increased transcription, rather than increased mRNA stability, is responsible for a significant proportion of the hypertonicity-induced increase in BGT1 (Uchida et al. 1993; Lammers et al. 2005) and AR (Garcia-Perez 1995). There is also extensive post-transcriptional control over the surface expression of BGT1. De novo synthesis and membrane trafficking of cytosolic pools were shown to contribute to hypertonicity-induced BGT1 upregulation in the kidney (Basham et al. 2001; Kempson et al. 2003). In the absence of a specific antibody that can reliably detect BGT1 in brain tissue, mRNA studies remain able to produce meaningful results since de novo synthesis is known to contribute to BGT1 upregulation in other tissues.

The known transcription factor for BGT1 is the tonicity-responsive enhancer binding protein (TonEBP). TonEBP expression increases and it is translocated to the nucleus in response to dehydration in the kidney (Woo et al. 2000b) to promote the expression of osmoprotective genes: the osmolyte transporters BGT1, the taurine transporter (TauT), and SMIT, as well as the intracellular enzyme responsible for the production of the osmolyte sorbitol, aldose reductase (AR) (for review see (Woo et al. 2002)). The osmoprotective genes are able to increase in the brain in response to hypertonicity and cause alterations in osmolyte tissue content (Heilig et al. 1989; Lien et al. 1990; Verbalis and Gullans 1991; Ibsen and Strange 1996; Minami et al. 1996; Bitoun and Tappaz 2000b). While TonEBP is believed to be the major transcriptional regulator of the osmoprotective genes in the kidney and is able to perform bi-directional regulation of the osmoprotective genes (Burg et al. 1996; Miyakawa et al. 1999; Woo et al. 2000a; Na et al. 2003; Lopez-Rodriguez et al. 2004), this does not appear to be true in the brain. For example, TonEBP is expressed differentially from its downstream osmoprotective genes in the brain with regard to cell type, region, and expression level (Maallem et al. 2006b). Furthermore, the expression of TonEBP and of each osmoprotective gene varies by tissue (Zhang et al. 2003). Finally, TonEBP is only appreciably present and hypertonic-inducible in neurons in the brain (Loyher et al. 2004; Maallem et al. 2006a), yet other non-neuronal cells, such as astrocytes and endothelial cells, can upregulate the osmoprotective genes in response to osmotic stress, suggesting a TonEBP-independent mechanism (Isaacks et al. 1994; Strange et al. 1994; Wiese et al. 1996; Bitoun and Tappaz 2000a; Petronini et al. 2000; Maallem et al. 2006b).

Recently, it has been suggested that an endogenous inhibitory GABAergic system exists within the immune system, which includes the presence of GATs on T cells and macrophages (Bhat et al. 2010). Furthermore, inflammation has been shown to modulate the expression of GATs. GAT1 and GAT3 are increased in the spinal trigeminal nucleus following carrageenan injection, a response likely to contribute to hyperalgesia (Ng and Ong 2001). Following an encephalitic insult, GAT1 is significantly downregulated in the spinal cord of WT mice, and the GAT1 KO mouse has an exacerbated response to an encephalitic insult, suggesting that GAT1 is an important modulator of Ag-specific T cell responses (Wang et al. 2008). TonEBP is also known as nuclear factor of activated T-cells 5 (NFAT5), an inflammatory-mediated transcription factor. Despite a significant increase in TonEBP in response to an inflammatory stimulus in T lymphocytes, AR did not increase (Trama et al. 2000). Furthermore, immunosuppressant drugs do not alter the hypertonicity-induced upregulation of BGT1, and different immunosuppressants display divergent effects regarding SMIT activity (Atta et al. 1999). The available data indicate that 1) TonEBP may regulate the osmoprotective genes only in response to osmotic stimuli, not to inflammatory stimuli, and 2) TonEBP regulation of osmoprotective genes is only relevant in select tissues, but not in the brain. As discussed above, this expression pattern was compared to that of other GABA transporters as well as osmoprotective genes.

The current study was performed to determine if BGT1 mRNA (SLC6a12) expression is altered in the hippocampus following SE, and to investigate whether inflammation and/or dehydration may contribute to the BGT1 response pattern in the brain.

Methods

Animals

Adult male C57/B6 mice weighing a minimum of 18 grams (Charles River, Kingston, WA) were used. Animals were housed in a temperature-, humidity-, and light-controlled (12h light: dark cycle) facility. Mice were group-housed and permitted free access to food and water except during the dehydration protocol. All experimental procedures were performed

in accordance with the guidelines established by the National Institutes of Health (NIH) and received approval from the University of Utah's Animal Care and Use Committee (IACUC).

Pilocarpine-induced status epilepticus (SE)

Pilocarpine SE was induced by injection of 330 mg/kg (i.p.) pilocarpine hydrochloride in 0.9% saline (Mazarati et al. 2004) (Sigma, St. Louis, MO). −/− scopolamine methyl-bromide (1 mg/kg, Sigma-Aldrich, St. Louis, MO) was administered 30 minutes prior to pilocarpine injection to reduce peripheral cholinergic effects (Shibley and Smith 2002). Mice were observed for SE; animals displaying at least three stage 3-5 seizures according to a modified Racine scale (Racine 1972), or at least one stage 5 seizure accompanied by continuous clonus were considered to have undergone SE. The motor component of SE was abolished after 60 minutes by an injection of diazepam (10 mg/kg, i.p., Sigma-Aldrich, St. Louis, MO). Mice in the control group received 0.9% saline in place of pilocarpine; otherwise they were treated identically to experimental animals.

Induction of Inflammation and Dehydration

mRNA levels of SE animals were compared to levels from animals 24 h following LPS injection (4 mg/kg, i.p., strain 0111:B4, Sigma-Aldrich, St. Louis, MO) or after 24 h of water withdrawal (n=10 per group). The presence of an inflammatory response was confirmed utilizing core body temperature and weight measurements. Body temperatures were determined at baseline and hourly for 4 h post-injection, then again at 8 h and 24 h. The peak body temperature reached by each mouse was compared to its individual baseline temperature, and the temperature change was determined for each mouse in order to normalize for mice with low or high basal temperature. Dehydration was determined by plasma osmolality values. Blood was obtained from mice by decapitation and centrifuged for 3 min at >3,000 g to separate plasma. Plasma osmolality was determined utilizing an osmometer (Advanced Instruments, Inc., Model 3320 Osmometer, Norwood, MA).

RNA extraction and cDNA synthesis

Eight, 24, and 72 h, one week and four weeks post-SE, animals were sacrificed by cervical dislocation and hippocampi were dissected out for gene expression determination (n=6-9 per group). RNA was extracted and processed with RNeasy miniprep kits (QIAGEN, Valencia, CA) according to the manufacturer's instructions. A NanoDrop 3300 Spectrophotometer (Thermo Fisher Scientific, Pittsburgh, PA) was utilized to calculate RNA concentrations based on spectral absorbance at 260/280 nm. A standard RT reaction was run to produce cDNA. Two μg of total RNA, 1 μl of 250 ng/μl random hexamers, 1 μl of 10 mM dNTPs, and water to a volume of 13 μl were heated to 65 °C for 5 minutes. Four μl of 5x First Strand buffer, 2 μl of 0.1 M DTT, and 1 μl of SuperScript II (Invitrogen, Carlsbad, CA) were added and the reaction run at 42 °C for 50 minutes then 70 °C for 15 minutes. Fifty μl of purified water was added and samples stored at -20°C until use.

Quantitative PCR

Osmolyte-related, GAT1 and GAT3 internal standards for PCR were cloned, while the standards and primers for BGT1 were purchased from Origene (Rockville, MD). The University of Utah DNA Sequencing Core Facility confirmed the cloned DNA sequences and a BLAST search was performed to confirm gene specificity. Primer sequences and product sizes are presented in Table I. The quantitative PCR reaction contained 12.5 μl of RT^2 Real-Time SYBR Green PCR Master Mix (SA Biosciences, Frederick, MD), 10.5 μl of purified water, 1 μl of 10 μM primer set, and 1 μl of cDNA template. Quantitative PCR was performed using a PTC-200 Peltier Thermal Cycler with a Chromo4 Continuous Fluorescence Detector (MJ Research Inc., Waltham, MA). Data was analyzed with MJ

Opticon Monitor Analysis Software, Version 3.00 (Bio-Rad Laboratories, Hercules, CA). All values were normalized to Proteosome subunit β (Psmb6), a housekeeping gene with minimal variability (Rubie et al. 2005). Primer sequences, annealing temperatures, and product sizes are presented in Table I. Each gene was amplified for 35 cycles.

Analysis and Statistics

The copy number of a specific gene in a sample was normalized to the value of the housekeeping gene, Psmb6, for that sample. Results are expressed relative to a mean control value of 100%; i.e. each experimental group was normalized to a mean control value of 100% by dividing each experimental value by the average control value. Results are presented as mean ± SEM. A two-tail Student's t-test was utilized prior to normalization to determine significant differences between control and experimental groups. Differences were considered significant at $p < 0.05$.

Results

The mRNA expression levels of BGT1, GAT1, GAT3, TauT, SMIT, AR, and TonEBP were determined 8 h, 24h, 72h and 1 and 4 weeks following pilocarpine-induced SE. Figure 1 displays the temporal expression pattern of the genes following SE. Eight h following SE, SMIT mRNA was increased significantly, while GAT1 mRNA was decreased compared to control values. At 24 h post-SE, BGT1 mRNA expression was significantly increased, as were the osmolyte transporters SMIT and TauT. GAT3 mRNA was decreased whereas GAT1 mRNA had returned to control values. At the 72 h time-point BGT1 mRNA expression was significantly decreased along with GAT1 and GAT3, while SMIT remained increased. Hence, the expression of BGT1 switched from being similar to that of the osmolyte transporters early on post-SE (24 h) to being more like that of the GABA transporters at a later time-point (72 h). This pattern was again observed 4 weeks post-SE, when BGT1, GAT1, and GAT3 mRNA were downregulated, while SMIT was upregulated. AR mRNA expression was not affected by SE at any of the time-points examined. The transcription factor of the osmolyte-related genes, TonEBP, was downregulated 72 h post-SE, and was not altered at other time-points. As such, there is no apparent relationship between the expression of the osmolyte-related genes and TonEBP post-SE. Interestingly, no gene investigated was different from control values one week following SE.

BGT1 mRNA expression appears to be regulated similarly to the osmolyte transporters 24 h post-SE. Since osmolyte transporters may be subject to regulation by the systemic osmotic state, dehydration following SE could contribute to the observed upregulation of TauT, SMIT, and BGT1. Animals were significantly dehydrated following both SE and 24 h of water withdrawal compared to controls ($p<0.01$ for both groups; control = 313.3 ± 0.99 mOsm/kg; 24 h post-SE = 326.4 ± 3.1 mOsm/kg; 24 h water withdrawal = 327.7 ± 1.3 mOsm/kg). Plasma osmolality values between experimental groups were strikingly similar. Therefore, the expression profiles of these two groups were compared to determine the impact of dehydration on the expression patterns seen post-SE. As shown in Figure 2, SE animals experienced significantly greater alterations in osmoprotective genes than that those observed in dehydrated animals. In fact, only SMIT was significantly increased following 24 h of water withdrawal. This is not surprising, because a mild dehydration protocol was chosen for this study to produce similar plasma osmolality values to those seen 24 h post-SE. These results suggest that the alterations in osmolyte transporter mRNA expression following SE are not accounted for by dehydration.

BGT1 mRNA expression is downregulated 72 h and 4 weeks post-SE, similarly to the GABA transporters, GAT1 and GAT3. The effect of an inflammatory insult on the downregulation of these genes was subsequently investigated. LPS-induced inflammation

was confirmed by a significant increase in body temperature ($p<0.01$; control= -0.01 ± 0.07 °C; LPS= 1.21 ± 0.29 °C) and weight loss ($p<0.01$; control= -0.1 ± 0.1 g; LPS= -3.3 ± 0.1 g) compared to controls. Plasma osmolality was not different between groups. As shown in Figure 3, GAT1 and GAT3 mRNA expression were decreased in response to inflammation, while BGT1 mRNA expression was not altered. Inflammation may therefore contribute to the regulation of GAT1 and GAT3. As seen in Figure 4, BGT1, TauT, SMIT, and AR mRNA levels were not altered post-LPS. TonEBP, however, was significantly increased by exposure to LPS. These results illustrate that, despite being a known transcription factor of the investigated osmoprotective genes, TonEBP does not activate these genes in response to an inflammatory stimulus.

Discussion

Osmoprotective gene expression is altered by tonicity due to promotion by the transcription factor TonEBP. Under osmotic stress, TonEBP expression can increase and alter the expression of BGT1, TauT, SMIT, and AR (Woo et al. 2002). SE results in dehydration, and it is possible that the changes in gene expression observed in osmoprotective genes following SE were due to dehydration. Following SE, animals were dehydrated to a similar extent to animals that had water withheld for 24 h as determined by plasma osmolality values. However, SE produced upregulation of BGT1, TauT, and SMIT mRNA, while 24 h water withdrawal caused exclusive upregulation of SMIT mRNA. SMIT, TauT, and BGT1 mRNA values following 24 h water withdrawal were significantly lower than the expression values following SE. This result indicates that dehydration does not account for the upregulation of the osmolyte transporters following SE.

BGT1 expression has been shown to closely mimic TonEBP expression in the kidney, but an apparent disparity exists between their expression patterns in the brain (Zhang et al. 2003; Maallem et al. 2006b). In agreement with these previously reported trends, the current study also found that osmolyte-related genes do not mimic TonEBP expression in the brain following SE, mild dehydration, or inflammation. While SMIT, TauT, and BGT1 mRNA were increased 24 h post-SE, TonEBP mRNA was unaltered at this time-point. TonEBP and BGT1 mRNA were significantly downregulated at 72 h post-SE, while SMIT was upregulated and TauT was not different from control values. At four weeks post-SE, SMIT was increased and BGT1 was decreased, while TonEBP and TauT were not different from controls. Thus, it appears that the mRNA expression patterns of the osmoprotective genes are not following those of TonEBP in the brain. Furthermore, they also appear to be regulated differentially from each other. In contrast to TonEBP, AR mRNA was not altered by any manipulation performed in the current study, i.e. SE, mild dehydration, or inflammation. This is not surprising because of the determined discrepancy between expression of these genes in the brain, and because a mild dehydration protocol was utilized. From the currently available data, it appears that the osmolyte transporters are not exclusively regulated by TonEBP in the brain.

Inflammation was thought to contribute to the alterations in GATs and osmoprotective genes because both GAT1 (Wang et al. 2008) and TonEBP (Trama et al. 2000) have been previously associated with inflammation. Furthermore, seizures induce rapid yet persistent CNS inflammation which can enhance neuronal excitability, reduce cell survival, and increase blood-brain barrier permeability, resulting in a predisposition to seizure activity (see (Vezzani and Granata 2005) and (Choi and Koh 2008) for reviews). Therefore, mRNA values observed post-SE were compared to those obtained from mice that had been subjected to an inflammatory insult to determine whether inflammation could contribute to the downregulation seen post-SE. GAT1 and GAT3 values were decreased post-LPS compared to controls, and were not significantly different from SE animals. This finding

supports the hypothesis that inflammation may contribute to GAT downregulation post-SE; however, it appears that GAT downregulation post-SE is more severe than post-LPS, suggesting that other mechanisms contribute to the observed post-SE GAT downregulation. Further studies will be necessary to determine the nature and extent of inflammation post-SE, and to determine the degree to which inflammation may actually contribute to the observed GAT downregulation.

TonEBP (NFAT5) is induced by T-cell activation, but AR is not increased by this receptor-mediated stimulus (Trama et al. 2000). The current study complements these data, and is the first to investigate inflammatory-mediated regulation of TonEBP in the brain, as well as its downstream targets. The results demonstrate that TonEBP is responsive to neuroinflammation but does not regulate osmotic elements under such conditions. BGT1, TauT, and SMIT were significantly altered post-SE, but were not changed post-LPS. Hence, inflammation does not contribute to the transcriptional response of BGT1 and the osmolyte transporters. Surprisingly, TonEBP was not upregulated following SE, despite the known occurrence of an inflammatory response following seizures. It is possible that TonEBP is regulated by only a subset of inflammatory stimuli; e.g., TonEBP responded to LPS, which signals through Toll-like receptor 4 (Poltorak et al. 1998). The innate and/or adaptive inflammatory mediation following seizures may not activate pathway(s) that activate TonEBP expression although Toll-like receptor 4 has been implicated in ictogenesis (Maroso et al. 2010).

The observed expression patterns closely mirror the phases of the pilocarpine-induced SE model of temporal lobe epilepsy. This model includes an acute insult that produces seizure activity, followed by a recovery period during which animals have a low probability of seizures (Williams et al. 2007), followed by a chronic period when spontaneous seizure activity occurs (Leite et al. 2002). The phase of low seizure probability is referred to as the latent period, and is the stage in which the cellular and molecular alterations occur that underlie the eventual development of spontaneous seizures, i.e. epileptogenesis (Loscher 1998; White 2002). Approximately 2 weeks following SE (13.1 ± 2.4 d in C57/B6 mice, (Shibley and Smith 2002)), animals begin to display recurrent spontaneous seizure activity, although monitoring was not performed in the current study. The observed genes were altered in the acute and long-term phases of the model, but returned to normal during the latent period. The functional significance of the current findings remains to be determined. Since GATs are able to reverse and release GABA into the extracellular environment (Attwell et al. 1993; Levi and Raiteri 1993), the net effect of their upregulation is unclear.

The results obtained in this investigation are not dissimilar from previous studies. SMIT was increased following kainic acid-induced SE, and peaked in CA1 of the hippocampus at 24 h post-SE before returning to normal (Nonaka et al. 1999). The study was only carried out for one week, so a long-term comparison cannot be made with this study. Kainic acid-induced SE also caused a downregulation of GAT1 and GAT3. GAT1 was significantly downregulated at 24 h and 30 d post-SE, but returned to normal at one-week post-SE. These results are in close agreement with those found in the current study. GAT3, however, remained consistently downregulated following kainic acid-induced SE, in contrast to the results obtained with pilocarpine-induced SE in this study (Sperk et al. 2003). As such, it is important to keep in mind that different results may be observed in other epilepsy models compared to those currently observed in the pilocarpine model.

In summary, BGT1 mRNA expression is upregulated early post-SE along with other osmolyte transporters; at later time-points, BGT1 is downregulated along with the GABA transporters. TonEBP does not exert full control over the expression of BGT1 and the other osmoprotective genes in the brain, nor does it induce their expression in response to

inflammation. Neither plasma osmolality nor inflammation appear to account for the changes observed in BGT1/osmolyte transporter mRNA expression post-SE. In contrast, inflammation may contribute to the downregulation of GAT1 and GAT3. The mediator(s) of BGT1/osmoprotective mRNA regulation in the brain post-SE therefore remain(s) unknown. However, it is evident that these genes are altered by seizure activity. Since osmotic balance plays a role in controlling neuronal excitability, further investigation into the regulation of, and the role played by these genes in epilepsy is warranted. BGT1 is of particular interest due to its ability to transport both GABA and the organic osmolyte betaine.

Acknowledgments

We wish to acknowledge Dr. Phillip Moos and Dr. Michael Franklin for allowing the use of equipment and offering technical expertise. This work was supported by NINDS Contract NO1-NS42359.

Glossary

BGT1	betaine/GABA transporter 1
GAT	GABA transporter
SMIT	sodium-myo-inositol co-transporter
TauT	taurine transporter
AR	aldose reductase
TonEBP	tonicity-responsive enhancer binding protein
LPS	lipopolysaccharide
ECS	extracellular space

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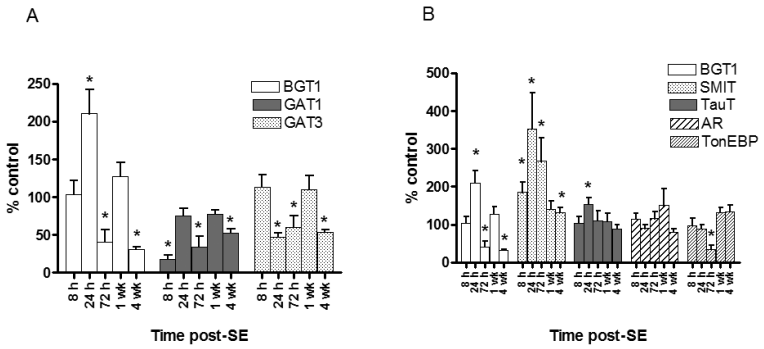

Figure 1.

Alterations in GAT (a) and osmoprotective (b) gene mRNA expression 8 h, 24 h, 72 h, 1 week, and 4 weeks post-SE (means ± SEM). Results are expressed relative to control values (normalized to 100%). *$p < 0.05$ vs. control; $n = 6–10$ per group.

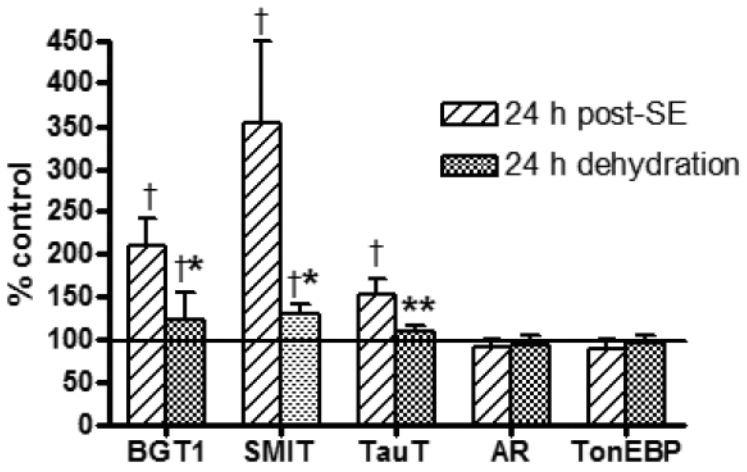

Figure 2.

Effect of SE (24 h post-insult) and 24 h water withdrawal on osmoprotective gene mRNA expression (means ± SEM). Results are expressed relative to control values (normalized to 100%). †$p < 0.05$ vs. control; *$p < 0.05$ vs. post-SE; **$p < 0.01$ vs. post-SE; n = 8–10 per group.

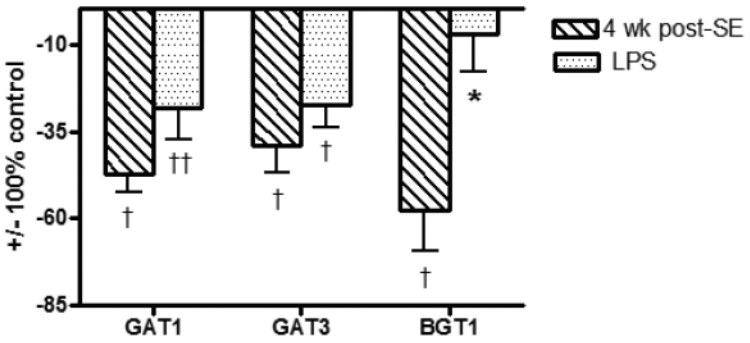

Figure 3.
Effect of SE (4 weeks post-insult) and LPS (24 h post-insult) on GAT mRNA expression (means ± SEM). Results are expressed relative to control values (normalized to 100%). †$p < 0.05$; ††$p < 0.01$ vs. control; *$p < 0.05$ vs. post-SE; n = 6–10 per group.

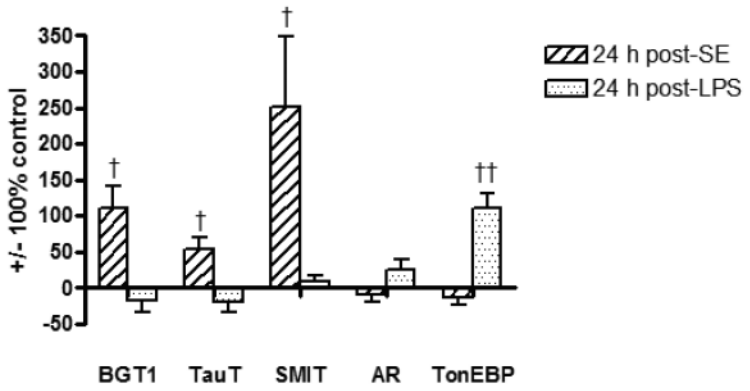

Figure 4.

Effect of SE (24 h post-insult) and LPS (24 h post-insult) on osmoprotective gene mRNA expression (means ± SEM). Results are expressed relative to vehicle controls (normalized to 100%). †$p < 0.05$; ††$p < 0.01$ vs. control; n = 8–10 per group.

Table I

Primers for PCR amplification

Gene	Forward Primer	Reverse Primer	AT	PS (bp)
GAT1	taacaacaacagcccatcca	ggagtaaccctgctccatga	60°C	326
GAT3	tttggtcttccccttttcct	aagactccactcaaccccct	60°C	214
SMIT	cactctgagtggatacttcc	tctcttaacttcctcaaacc	52°C	544
TauT	tccacaaagacatcctgaagc	ggtgaagttggcagtgctaag	60°C	539
AR	ttgactgcgcccaggtgtac	tatatgctgtcaccacgatgc	60°C	504
TonEBP	atgcaatttcagaatcagcc	gcatttgctgagaaagaag	60°C	514
Psmb6	tctgatggcaggaatcat	acatagccatagatgtacga	57°C	137

GAT, GABA transporter; SMIT, sodium/myo-inositol cotransporter; TauT, taurine transporter; AR, aldose reductase; TonEBP, tonicity-responsive enhancer binding protein; Psmb6, proteosome subunit beta 6; AT, annealing temperature; PS, expected produce size of the PCR amplicon; bp, base pairs.