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ABSTRACT

Hepatic encephalopathy (HE) is a neuropsychiatric disorder that occurs due to acute and chronic liver diseases, the hallmark of which is the increased levels of ammonia and subsequent alterations in glutamine synthesis, i.e. conditions associated with the pathophysiology of HE. Under physiological conditions, glutamine is fundamental for replenishment of the neurotransmitter pools of glutamate and GABA. The different isoforms of glutamine transporters play an important role in the transfer of this amino acid between astrocytes and neurons. A disturbance in the GABA biosynthetic pathways has been described in bile duct ligated (BDL) rats, a well characterized model of chronic HE. Considering that glutamine is important for GABA biosynthesis, altered glutamine transport and the subsequent glutamate/GABA–glutamine cycle efficacy might influence these pathways. Given this potential outcome, the aim of the present study was to investigate whether the expression of the glutamine transporters SAT1, SAT2, SN1 and SN2 would be affected in chronic HE. We verified that mRNA expression of the neuronal glutamine transporters SAT1 and SAT2 was found unaltered in the cerebral cortex of BDL rats. Similarly, no changes were found in the mRNA level for the astrocytic transporter SN1, whereas the gene expression of SN2 was increased by two-fold in animals with chronic HE. However, SN2 protein immuno-reactivity did not correspond with the increase in gene transcription since it remained unaltered. These data indicate that the expression of the glutamine transporter isoforms is unchanged during chronic HE, and thus likely not to participate in the pathological mechanisms related to the imbalance in the GABAergic neurotransmitter system observed in this neurologic condition.

1. Introduction

Acute and chronic liver failure result in hepatic encephalopathy (HE), a neurological condition leading to deleterious effects in the central nervous system (CNS), such as motor and cognitive impairments, as well as psychiatric disturbances (Albrecht and Jones, 1999; Ferenci et al., 2002). Hyperammonemia, which occurs due to the significantly reduced capacity of the liver to synthetize urea, is considered one, if not the main pathophysiologic mechanism resulting in HE (Butterworth, 2002). In the CNS, ammonia is mainly detoxified by the activity of glutamine synthetase (GS), an enzyme predominantly, if not exclusively, expressed in astrocytes (Norenberg and Martinez-Hernandez, 1979). This leads to disturbances in glutamine synthesis, which is found increased in the acute form of the disease and has been implicated in the pathophysiology of this neurologic condition (Albrecht et al., 2010; Swain et al., 1992; Tofteng et al., 2006; Zwingmann and Butterworth, 2005).

The mechanisms behind the deleterious effects of glutamine are related to the fact that this amino acid acts as an ammonia carrier into mitochondria where ammonia is generated by the activity of phosphate activated glutaminase (PAG), an enzyme expressed in both neurons and astrocytes (for review, see Schousboe et al., 2013). Consequently, the ammonia concentration increases inside this organelle leading to the production of reactive oxygen and nitrosative species, as well as inducing the mitochondrial permeability transition (mTP), ultimately resulting in mitochondrial dysfunction (Jayakumar et al., 2004; Rama Rao et al., 2005). These observations have led to the

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formulation of the *Trojan horse* hypothesis, which explains many of the events observed during hyperammonemia, e.g. astrocytic swelling (Albrecht and Norenberg, 2006; Rama Rao and Norenberg, 2014; Rama Rao et al., 2012). In addition, some studies have proposed that altered levels of glutamine lead to astrocytic swelling due to the osmotic characteristics of the amino acid (Blei, 1991; Blei et al., 1994; Brusilow and Traystman, 1986). However, the role of glutamine as an osmolyte has been contradicted by the demonstration of a negative time correlation between astrocyte swelling and the increase in glutamine concentration in astrocyte cultures treated with ammonia, while the PAG inhibitor diazo-5-oxo-L-norleucine (DON) led to a significant reduction of ammonia-induced astrocyte swelling suggesting that astrocyte swelling is related to glutamine deamidation (Jayakumar et al., 2006).

Under physiological conditions, glutamine exerts an important role in the so-called glutamate/GABA-glutamine cycle. In this cycle, the transfer of glutamine to the neuronal compartment gives rise to the production of glutamate due to PAG activity, a process fundamental for restoring the neurotransmitter pool since neurons are unable to perform de novo synthesis of glutamate due to the absence of pyruvate carboxylation (Hertz et al., 1999; Schousboe et al., 1997; Yu et al., 1983). Concerning the GABAergic synapses, the majority of this neurotransmitter is recycled in neurons, but part of the released GABA is taken up by surrounding astrocytes and therefore lost from the transmitter pool (Schousboe et al., 2013) . The latter event is compensated by glutamine acting to replenish the neurotransmitter pool of GABA (Liang et al., 2006; Patel et al., 2001; Schousboe, 2003).

In the context of glutamine transfer between neurons and astrocytes, the small neutral amino acid transporters play an important role in the communication between these cells and thus, they are fundamental for the maintenance of the glutamate/GABA–glutamine cycle (Nissen-Meyer and Chaudhry, 2013). The SN1 and SN2 isoforms, also known as SNAT3 and SNAT5, are electroneutral transporters functioning as a Na^+–glutamine symporter and H^+ anti-porter related to glutamine efflux from astrocytes (Chaudhry et al., 1999). SN1 was the first isoform characterized and it has been described as the primary transporter responsible for the release of glutamine from astrocytes. It preferentially transports glutamine, promoting its efflux under physiological concentrations of this amino acid (~400 μM) (Boulland et al., 2002; Chaudhry et al., 1999). One important property of this amino acid transporter is its capacity of flux reversal, which is dependent on pH and also on the extracellular Na^+ concentration, i.e. a scenario in which the flux direction will depend on the microenvironment surrounding the cells (Chaudhry et al., 1999). The importance of this transporter in relation to the glutamate/GABA–glutamine cycle is highlighted by the observation that exogenous glutamate mediates a decrease in the K_m for SN1 in astrocyte cultures (Bröer et al., 2004). Similarly, the SN2 isoform is also functionally related to glutamine efflux from astrocytes, although it also releases glycine serving as a co-transmitter at NMDA receptors (Cubelos et al., 2005; Hamdani et al., 2012).With respect to the CNS localization of these transporters, SN1 has been shown to be confined to astroglial processes ensheathing glutamatergic and GABAergic synapses in different brain structures (Boulland et al., 2002; Chaudhry et al., 1999), while SN2 has also been found in astrocytes predominantly close to glutamatergic synapses (Cubelos et al., 2005).

The transporters responsible for glutamine uptake in the neuronal compartment are known as system A, which is comprised of the SAT1 and SAT2 transporter isoforms, also named SNAT1/SA2 and SNAT2/SA1. These transporters are Na^+–glutamine symporters, i.e. electrogenic in nature constituting the driving force for the amino acid uptake (Chaudhry et al., 2002). SAT1 is expressed in the somatodendrites of neurons in brain regions enriched in GABAergic neurons, and its proximity to VGAT, the vesicular GABA transporter indicates that it might be related to glutamine uptake as a prerequisite to replenish the GABA neurotransmitter pool (Solbu et al., 2010; Varoqui et al., 2000). SAT2 is more ubiquitously expressed, being found localized mainly in somatodendrites and axons of glutamatergic neurons (González-González et al., 2005; Jenstad et al., 2009). The importance of this transporter has been demonstrated by employing MeAIB (methylamino-iso-butyric acid), an inhibitor of system A, able to reduce glutamine uptake, which consequently diminishes the intracellular concentration of glutamate (Jenstad et al., 2009).

The role of neural glutamine transporters during HE has been investigated in different experimental conditions and it remains controversial whether their expression is altered in this neurologic disorder (Desjardins et al., 2012; Rama Rao and Norenberg, 2014). Moreover, most studies have investigated their expression during acute hyperammonemia and HE and it remains unknown whether glutamine transporters are also involved in the pathologic mechanism of chronic HE. In this context it is of interest that using bile-duct ligated rats (BDL), an experimental model of chronic HE, it has been demonstrated that the biosynthetic pathway for GABA was altered to occur preferentially via the tricarboxylic acid (TCA) cycle relative to the direct decarboxylation of glutamate not involving the TCA cycle (Leke et al., 2011a). However, no differences in the gene expression were found for the glutamate decarboxylase (GAD) enzyme isoforms GAD65 and GAD67 (Leke et al., 2014), which have different roles in the two GABA biosynthetic pathways mentioned earlier (Waagepetersen et al., 1999, 2001; Walls et al., 2011). Therefore, it can be hypothesized that glutamine transfer between astrocytes and neurons may be altered leading to changes in the biosynthesis of neurotransmitter GABA. Hence, the aim of the present study was to examine the expression of the different glutamine transporter isoforms in the CNS of rats with bile duct ligation.

2. Materials and methods

2.1. Experimental model of HE

Adult male Wistar rats (n = 20, weight 361.21 ± 28.19 g, 70–80 days old) were obtained from the Experimental Animal Unit of Research Center of Hospital de Clínicas de Porto Alegre, maintained in a controlled environment (20 °C ± 2 °C, 12 h light/dark cycle) with standard food and water *ad libitum*. The surgical procedure of bile duct-ligation, serving as a model of chronic HE, was performed as previously described (Bak et al., 2009). Rats were anaesthetized (ketamine 90 mg/kg, xylazine 12 mg/kg, i.p.); a middle abdominal incision was performed; the hepatic ligament exposed and the common bile duct was double ligated and resected in between the ligatures. The control group rats underwent the same surgical procedure, with exception that the bile duct was not ligated. All animals were maintained in the animal colony room for 6 weeks post-surgery. The handling and care of the animals were conducted according to the National Guidelines on Animal Care, and all experiments were approved by the Ethics Committee of the Hospital de Clínicas de Porto Alegre.

2.2. Sample collection

Rats were anesthetized as previously described and transcardially perfused with 50 mL of cold phosphate buffer (0.1 M phosphate buffer containing 23 mM Na_2HPO_4 and 77 mM NaH_2PO_4, pH 7.4) to remove residual blood cells in brain. Thereafter, rats were decapitated and brains were dissected in order to isolate cortices. This tissue was immediately frozen in liquid nitrogen and stored at –80 °C. Liver samples were also dissected to document the presence of the chronic liver disease, as previously described (Leke et al., 2014).

2.3. Gene expression assays

Total mRNA was isolated from the cerebral cortices of control and BDL rats employing the Trizol reagent (Ambion®, Life Technologies™, Carlsbad, CA, USA). Thereafter, 1.25 µg of total RNA was treated with deoxyribonuclease I (Invitrogen) and cDNA was synthesized by RT-PCR (High Capacity cDNA reverse Transcription Kit, Invitrogen) according to the manufacturer's protocols. Relative quantitation of mRNA expression was performed employing Taqman MGB Probes (Invitrogen) for SAT1 (Slc38a1, Rn00593696_m1), SAT2 (Slc38a2, Rn00710421_m1), SN1 (Slc38a3, Rn01447660_m1), SN2 (Slc38a5, Rn00684896_m1) and GAPDH (Rn01775763_g1) as an endogenous control by real-time PCR (qPCR) as a multiplex assay in 48 well StepOne™ system (Applied Biosystems®, Life Technologies™, Carlsbad, CA, USA). Each reaction contained 5 µL of TaqMan® Gene Expression Master Mix (Life Technologies™, CA, USA), 0.5 µL of Taqman probe for each target gene and for the endogenous control and 2 µL of cDNA which was previously diluted 2 times, for a total reaction volume of 10 µL. All samples were run in triplicate and the results were calculated by the equation $2^{-\Delta\Delta Ct}$, which gives the fold change of the target gene expression, normalized by an endogenous control and relative to a calibrator. The calibrator in this study was the control group (Livak and Schmittgen, 2001).

2.4. Western blot assays

Cortices were homogenized in lysis buffer containing: 5 mM Tris base, 1 mM EDTA, 1 mM EGTA, 1% SDS, 1% Triton x-100, protease inhibitor cocktail (Roche Molecular Systems Inc, Branchburg, NJ), pH 7.4, and subsequently centrifuged at 10,000 × *g* for 10 min at 4 °C and the supernatant was collected. Protein concentration was determined by the Bradford method (Bio-Rad, Hercules, CA) and sample concentrations were adjusted and diluted 1:1 proportion with Laemmli Buffer (0.01 g % Bromophenol Blue, 60 mM Tris base, 20% glycerol, SDS 2% and 2-b-mercaptoethanol 5%; pH 6.8) to reach a final concentration of 2 mg/mL. Equal amounts of protein (40 µg protein per lane) were employed for the determination of SN2 protein. Cortical lysates were subjected to SDS–PAGE using 12% gels and electrophoretically transferred to PVDF membranes (EMD Millipore Inc, Temecula, CA). Blots were blocked with 5% non-fat dry milk in TBS-T (TBS; 20 mM Tris–HCl, 150 mM NaCl, pH 7.4, and 0.05% Tween 20) for 1.5 h at room temperature and then incubated with goat anti-SN2 (1:1000, Santa Cruz, Biotechnology Inc, CA), over-night at 4 °C. Subsequently, membranes were washed with TBS-T and incubated with HRP-conjugated secondary antibody (1:1000, bovine anti-goat HRP, Santa Cruz, Biotechnology Inc, CA) for 1 h at room temperature. The proteins were visualized using enhanced chemiluminescence (ECL, GE Healthcare, USA), densities of the bands were detected by autoradiography and the results were quantified with the Sigma Scan Pro program. Samples were run in duplicate and were calculated as intensity of protein expression, normalized by endogenous control (1:1000 mouse anti-α-tubulin, Santa Cruz, Biotechnology Inc, CA) as percentage of the control group.

2.5. Statistical analysis

Since all data exhibited normal distribution, as verified by Shapiro–Wilk test (data not shown), the results are expressed as mean ± standard error of the mean (SEM) and were analyzed by unpaired two-tailed Student's t-test. For all parameters a $p < 0.05$ was considered statistically significant. Data analysis was performed using Microsoft Excel 2010 and GraphPad Prism 5.0 softwares and figures were created using the image manipulation program Gimp 2.8.

3. Results

3.1. Experimental model of chronic liver disease

From an initial 10 BDL rats, three rats died prior to sample collection and one rat had the extrahepatic bile duct regenerated and was therefore excluded from the study. As described previously (Leke et al., 2013, 2014), BDL rats exhibited the manifestations of chronic liver disease and HE, including hepatomegaly and ascites and short term memory deficits. Histological examination of the livers showed disturbed cytoarchitecture with pronounced bile duct proliferation and fibrosis, while control rats exhibited normal liver parenchyma. A three-fold increase in ammonia levels was also found in the BDL rats.

3.2. Glutamine transporter mRNA expression

No differences in mRNA expression for the neuronal glutamine transporters SAT1 (1.002 ± 0.0205 control, 0.9405 ± 0.02783 BDL, Fig. 1a) and SAT2 (1.007 ± 0.04161 control, 0.9380 ± 0.1244 BDL, Fig. 1b) were observed between control and BDL rats in the cerebral cortex samples. Likewise, gene expression for the astrocytic transporter SN1 was found unchanged between control and BDL rats (1.008 ± 0.04476 control, 1.064 ± 0.07210 BDL, Fig. 1c), However, SN2 mRNA expression was found significantly increased in cortex of BDL rats, when compared to the control group (1.0201 ± 0.0721 control; 2.3267 ± 0.3204 BDL, $p < 0.001$, Fig. 1d).

3.3. Glutamine transporter protein expression

Since the mRNA expression for SN2 was found to be altered in the BDL rats, the protein expression for this transporter was investigated by Western blot analysis. As shown in Fig. 2 no difference

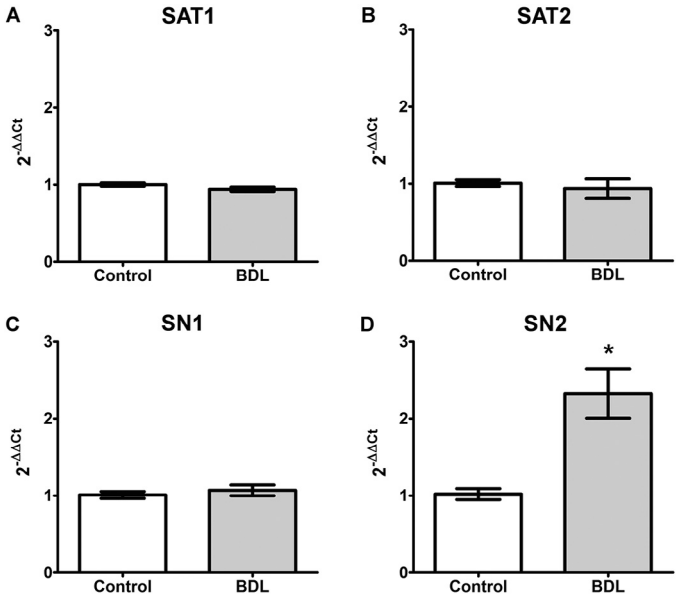

Fig. 1. Glutamine transporter isoform gene expression in cerebral cortex. Total RNA was isolated from cortices from control and BDL rats, and the RNA subsequently underwent reverse transcription for the synthesis of cDNA. Gene expression for SAT1(1A), SAT2 (1B), SN1 (1C), SN2 (1D) was performed by a qPCR multiplex assay and analyzed by the comparative method employing GAPDH as endogenous control and in relation to the control group (for more details see Section 2.3). White bars represent the control group (n = 9) and gray bars the BDL group (n = 6). Results are presented as mean ± SEM. Asterisk indicate a statistically significant difference between control and BDL groups for SN2 mRNA expression by Student's t-test ($p < 0.0001$).

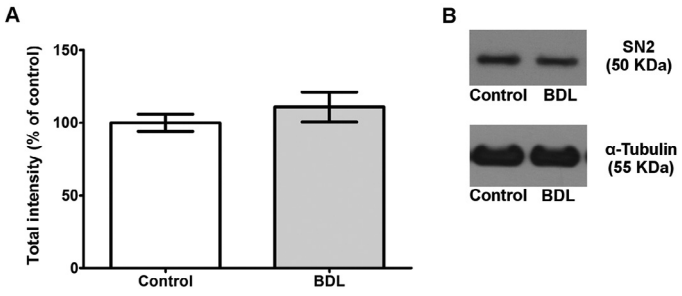

Fig. 2. Glutamine SN2 protein content in cerebral cortex. Protein from cortices from control and BDL rats were subjected to SDS–PAGE, electrophoretically transferred to PVDF membranes, blocked and incubated with goat anti-SN2 (1:1000). Subsequently, blots were incubated with HRP-conjugated secondary antibody (1:1000, bovine anti-goat HRP). The proteins were visualized using ECL and densities of the bands were detected by autoradiography. Results were quantified as a proportion of the signal of α-tubulin (1:1000) (for more details see Section 2.4). A. Quantification of SN2 protein densities presented as mean ± SEM, white bars represent the control group (n = 9) and gray bars the BDL group (n = 6). B. Representative immunoblot of SN2 and α-tubulin protein densities.

in cerebral cortex SN2 protein expression was observed between control and BDL rats (100.00% ± 5.81% control, 110.90% ± 10.18% BDL).

4. Discussion

The pathophysiology of HE, the hallmark of which is a rise in the CNS ammonia concentration, has repeatedly been associated with disturbances in brain energy metabolism and in various neurotransmitter systems, particularly those using GABA and glutamate as transmitters (Albrecht and Jones, 1999; Butterworth, 2002; Schousboe et al., 2014). In line with these findings, we have previously reported that the synthesis of neurotransmitter GABA is altered during chronic HE (Leke et al., 2011a), albeit no change was observed in the expression of GAD65, which is the GAD isoform closely associated with the biosynthesis of GABA in the vesicular neurotransmitter pool (Leke et al., 2014; Waagepetersen et al., 1999, 2001; Walls et al., 2011). Considering that glutamine is important in the restoration of GABA pools and that synthesis of this amino acid is disturbed during HE, it is possible that during this neurologic disorder the expression of the glutamine transporter isoforms might be altered which would consequently affect the function of the glutamate/GABA–glutamine cycle and thus likely contribute to the observed disturbance of GABA neurotransmitter synthesis (for further discussion, see Walls et al., 2015).

Here we demonstrate that the gene expression for the neuronal SAT1 and SAT2 transporter isoforms was unchanged in cerebral cortex of BDL rats. Likewise, a study employing the rat model of hepatic devascularization for the investigation of acute HE demonstrated unaltered cerebral mRNA levels for SAT1 (Desjardins et al., 2012). In accordance with this finding, a study of the effect of ammonia on GABA biosynthesis in co-cultures of cortical neurons and astrocytes showed that the average incorporation of ^{13}C from labeled glutamine into glutamate and GABA was dependent on the exogenous glutamine concentration. Exposure to ammonia, however, decreased the ^{13}C incorporation in both amino acids, which could be due to increased synthesis of unlabeled glutamine, as previously verified by increased pyruvate carboxylation (Leke et al., 2011b). These results from metabolic studies, together with the reports of no changes in gene expression, indicate that the expression of neuronal glutamine transporters is likely to be unaltered in HE. Since astrocytes are the major target in HE (Norenberg, 1998), it is possible that an altered glutamine transporter expression is to be found in these cells.

In line with this possibility, the mRNA expression for the astrocytic glutamine transporter SN2 exhibited an increased level in cerebral cortex of BDL rats with chronic HE, while that of SN1 was unaltered. Considering that SN2 was the only transporter isoform that exhibited changes in mRNA levels, indicating that it might also exhibit increased protein translation, we further investigated the protein content of this specific isoform. Surprisingly, no changes in the immuno-reactivity for this transporter were found in the cerebral cortex of BDL rats. Desjardins et al. (2012) also studied the effect of acute HE on SN2 gene expression and described diminished mRNA levels of this transporter. Based on mRNA data, these authors speculated that reduced expression of SN2 would lead to glutamine accumulation in astrocytes and consequently cellular osmotic swelling (Desjardins et al., 2012). On the contrary, Rama Rao and Norenberg (2014) demonstrated that the protein levels of SN2 were unchanged in the cerebral cortex of mice with acute HE induced by the hepatotoxin thioacetamide (TAA). This finding was confirmed in cultures of astrocytes acutely exposed to 5 mM ammonia, which exhibited no changes in SN2 protein levels (Rama Rao and Norenberg, 2014). Similar results were observed by Zielińska et al. (2014), who described decreased gene expression but no changes at the protein level for SN2 in cerebral cortices of rats with acute HE induced with TAA, as well as unchanged gene and protein expression in hyperammonemic rats. As discussed by Rama Rao and Norenberg (2014) a decrease in SN2 expression is not in agreement with the fact that acute HE is characterized by increased extracellular concentrations of glutamine in the brain. With respect to SN1 expression, Zielińska et al. (2014) described both decreased gene expression and protein levels in TAA-induced HE in rats, whereas no differences in mRNA and protein levels were observed in hyperammonemic rats. It should be taken into account that the contradictory results regarding SN1 and SN2 expression might be due to the fact that different experimental models were used for studying HE. In this regard, it is important to emphasize that the present study aimed at verifying the expression of glutamine transporters during chronic HE, and although acute and chronic HE share common pathologic mechanisms, e.g., hyperammonemia, it is well recognized that these conditions exhibit different features due to the rate and extent of the metabolic liver dysfunction (Weissenborn et al., 2005).

Of note, ammonia levels reached in chronic HE are much lower than those observed in the acute form of the disease (Felipo and Butterworth, 2002). As a consequence, the literature is conflicting regarding the glutamine concentration in the CNS during chronic HE, with no changes observed in BDL rats and increased levels observed in rats that underwent portacaval anastomosis (PCA) to induce HE (Cordoba et al., 1996; Fries et al., 2014; Jover et al., 2006; Wright et al., 2007). However, analyzing the glutamine concentration in whole cerebral cortex tissue or CSF does not accurately predict the alterations that might occur during the ammonia detoxification process, and thus it is necessary to consider that the levels of amino acids vary in cellular and subcellular compartments and micro domains. It is also possible that during chronic HE, glutamine levels are unchanged due to an adaptive process, e.g., increase in clearance from the brain. Nonetheless, altered glutamine transport might be independent of glutamine concentrations, which would interfere with the operation of the glutamate/GABA–glutamine cycle, as well as the synthesis and replenishment of neurotransmitter glutamate and GABA.

It is interesting, however, that the transcription for SN2 was found increased at the same time frame that the protein translation remained unaltered, indicating that during chronic HE there might be post transcriptional events influencing mRNA stability. In line with this possibility, it has been demonstrated that RNA is a target for oxidation during acute HE (Görg et al., 2008). These authors observed that 7% of total RNA was oxidized in cultures of astrocytes

exposed to 1 mM ammonia, while 6% of total RNA was found oxidized in cerebral cortex of hyperammonemic rats (Görg et al., 2008). Oxidized mRNA has been related to abnormal protein level and function (Nunomura et al., 2006). In agreement with that, oxidized mRNA and decreased ribosomal rRNA and tRNA levels due to oxidation have been related to abnormal protein synthesis in post mortem cerebral tissue samples of patients with Alzheimer disease (Ding et al., 2005; Shan et al., 2003). Considering that increased oxidative stress is also a feature of chronic HE (Carbonero-Aguilar et al., 2011; Seyan et al., 2010), it is possible that part of the mRNA pool is oxidized in the cerebral cortex of BDL rats. It should also be considered that the protein levels of the astrocytic glutamine transporter might change depending on the cellular and cerebral structures. Therefore, although our data provide important evidence regarding the total expression profile of the transporters, it would be of interest studying them by immunohistochemistry assays, which could provide additional information to the results described.

Although gene and protein expression for SN1 and SN2 remained the same during chronic HE, respectively, it is possible that these transporters undergo other regulatory modifications. For that reason it should be considered that these transporters may be subject to post translational regulation, a mechanism that has been broadly described for SN1 (Karinch et al., 2002; Nissen-Meyer and Chaudhry, 2013). Accordingly, different studies observed that SN1 is directly phosphorylated by the enzymes PKCα, PKCγ, and PKCδ (Nissen-Meyer et al., 2011; Sidoryk-Wegrzynowicz et al., 2011). More specifically, PKC phosphorylates SN1 in astrocytes at one single serine residue at the N-terminal of the transporter, which consequently targets the transporter for intracellular reservoirs and therefore regulates the transporter activity (Nissen-Meyer et al., 2011). Also, the prolonged activation of PKC results in the degradation of SN1 transporter. Concerning the SN2 isoform, it is still unknown if post translational mechanisms modulate this transporter but they might undergo similar processes mediated by PKC, since they possess 50% homology (Reimer et al., 2000). Additionally, SN2 activity is regulated by pH, having its activity decreased with lower pH, partially due to an action at the histidine residues found in the transporter (Baird et al., 2006). During HE, it has been described that ammonia leads to intracellular astrocytic alkalinization, as demonstrated in both cell cultures and astrocytes from portacaval-shunted rats (Rose et al., 2005; Swain et al., 1991). Therefore, an increase in pH might interfere with glutamine transporter activity in the astrocytic compartment.

The neuronal transporters also undergo translational regulation, as it has been demonstrated that leucine enhanced System A activity by activation of phosphatidylinositol 3-kinase (PI3K) in L6 muscle cells (Peyrollier et al., 2000). In addition, SAT2 is pH sensitive by the same mechanisms as those observed for SN2 as previously mentioned (Baird et al., 2006). Whether these mechanisms are occurring in neurons remains to be determined.

5. Conclusion

The astrocytic SN1 and the neuronal SAT1 and SAT2 isoforms were expressed at unaltered mRNA levels in chronic HE, while SN2 mRNA was found to be increased. However, its protein immunoreactivity was found unchanged. These results indicate that the glutamine transporter isoforms studied here possibly do not participate in the pathological mechanisms related to the imbalance in the GABAergic neurotransmitter system observed in chronic HE. However, post-translational protein modifications might alter the activity of these transporters in this neurological condition and additional investigations are necessary to better understand the involvement of such regulatory mechanisms.

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