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Expression of glutamine transporter isoforms in cerebral cortex of rats with chronic hepatic encephalopathy

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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).