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Pathophysiology of brain dysfunction in hyperammonemic syndromes: The many faces of glutamine

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ABSTRACT

Ineffective hepatic clearance of excess ammonia in the form of urea, as occurs in urea cycle enzymopathies (UCDs) and in liver failure, leads to increases in circulating and tissue concentrations of glutamine and a positive correlation between brain glutamine and the severity of neurological symptoms. Studies using 1H/13C Nuclear Magnetic Resonance (NMR) spectroscopy reveal increased *de novo* synthesis of glutamine in the brain in acute liver failure (ALF) but increases of synthesis rates *per se* do not correlate with either the severity of encephalopathy or brain edema. Skeletal muscle becomes primarily responsible for removal of excess ammonia in liver failure and in UCDs, an adaptation that results from a post-translational induction of the glutamine synthetase (GS) gene. The importance of muscle in ammonia removal in hyperammonemia accounts for the resurgence of interest in maintaining adequate dietary protein and the use of agents aimed at the stimulation of muscle GS. Alternative or additional metabolic and regulatory pathways that impact on brain glutamine homeostasis in hyperammonemia include (i) glutamine deamination by the two isoforms of glutaminase, (ii) glutamine transamination leading to the production of the putative neurotoxin alpha-ketoglutaramate and (iii) alterations of high affinity astrocytic glutamine transporters (SNATs). Findings of reduced expression of the glutamine transporter SNAT-5 (responsible for glutamine clearance from the astrocyte) in ALF raise the possibility of "glutamine trapping" within these cells. Such a trapping mechanism could contribute to cytotoxic brain edema and to the imbalance between excitatory and inhibitory neurotransmission in this disorder.

1. Introduction

Ammonia detoxification in mammalian systems depends principally on its conversion to urea *via* the urea cycle. A complete urea cycle is only expressed in the liver although other tissues, including the brain, may express some of its constituent enzymes. Inborn errors of each of the constituent enzymes of the urea cycle have been described, the most common one being ornithine transcarbamylase (OTC). Depending upon the extent of the deficiency and hence the residual enzyme activity and degree of hyperammonemia, infants and children with urea cycle enzymopathies present with a wide spectrum of clinical symptoms including lethargy, failure to thrive, hypotonia, seizures and severe encephalopathy progressing to coma. A significant percentage of survivors go on to develop severe developmental disabilities including mental retardation and cerebral palsy [1].

Acquired hyperammonemic syndromes leading to CNS dysfunction include hepatic encephalopathy (HE) resulting from either acute or chronic liver failure and patients with these disorders share many of the clinical symptoms encountered in inherited hyperammonemias including lethargy progressing to stupor and coma; seizures have been described in ALF.

Neuropathologic studies in material from patients with inherited or acquired hyperammonemias, particularly ALF, reveal cytotoxic brain edema which, in extreme cases, results in intracranial hypertension and may result in death by brain herniation. Another feature shared by inherited and acquired hyperammonemias is a characteristic alteration of astrocytes, known as Alzheimer type 2 astrocytosis in which the nuclei of these cells are characterized by swelling, glycogen deposition and margination of the normal chromatin pattern. Neuronal cell death has been reported in both inherited and acquired hyperammonemias but is much more common in the former group of disorders consistent with the notion of increased vulnerability of the developing brain to the neurotoxic effects of ammonia.

By definition, circulating ammonia concentrations are significantly increased in these disorders reaching high micromolar or even millimolar concentrations in some UCDs [1] and in ALF [2]. In both types of disorders, blood and brain concentrations of glutamine (GLN) are increased consistent with the conversion of ammonia into GLN, the only significant alternate pathway available in conditions in which hepatic urea synthesis is seriously impaired.

The purpose of the present review is to summarize the evidence in support of a key role of GLN in the pathogenesis of the neurological

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consequences of hyperammonemias, to review the proposed mechanisms implicated and to highlight areas of research in need of further investigation.

2. Brain glutamine accumulation in hyperammonemic disorders

Both classical biochemical investigations in autopsied brain tissue samples as well as neuroimaging approaches in living patients demonstrate that brain glutamine concentrations are significantly increased in both inherited [3], (Fig. 1) and acquired [4] hyperammonemias. In general, the magnitude of the increase in GLN is significantly correlated with the severity of encephalopathy and with the presence of brain edema and intracranial hypertension in patients with ALF [5] and 1H NMR studies in patients with decompensated liver cirrhosis reveal a significant correlation between brain GLN and severity of encephalopathy [6] (Fig. 2). Such findings have led to the suggestion that accumulation of the GLN molecule *per se* is somehow causally related to the neurological symptoms associated with hyperammonemia whatever the aetiology. In support of this notion, early studies in experimental animals repeatedly showed that inhibition of GLN accumulation in brain protects against the neurological consequences of acquired hyperammonemia [7,8]. Suggested mechanisms implicated in the role of GLN in the pathogenesis of the neurological complications of hyperammonemia include alterations in osmolarity resulting in brain water accumulation and GLN-induced increases of cerebral blood flow (hyperemia) [9].

However, although increases in brain GLN are consistently described in hyperammonemias, direct evidence for a key role of increased brain GLN synthesis rates *per se* in the pathogenesis of encephalopathy and brain edema in these disorders is less compelling.

3. Brain glutamine synthesis rates in hyperammonemic disorders

The astrocyte is the neural cell that "bears the brunt" of ammonia removal by the brain since the enzyme responsible, glutamine synthesis (GS) has a predominantly, and perhaps exclusively, astrocytic localization. Many studies have addressed either directly or indirectly the subject of GLN synthesis in the brain in hyperammonemias. In pioneering studies by Cooper in New York, it was shown that ammonia was incorporated almost exclusively into GLN *via* GS, rather than to glutamate *via* glutamate dehydrogenase and that, under normal physiological conditions, brain contained very little excess GS capacity over that required to maintain normal metabolic flux [10]. Subsequent direct measurements of GS in autopsied brain tissue from patients who died in hepatic coma revealed a small but significant loss of enzyme activity [11] rather than the anticipated increase consistent with increased ammonia flux and studies in an experimental animal model of chronic acquired hyperammonemia resulting from end-to-side portacaval anastomosis showed a region-selective pattern of GS distribution with losses of activity again being reported in several brain regions [12].

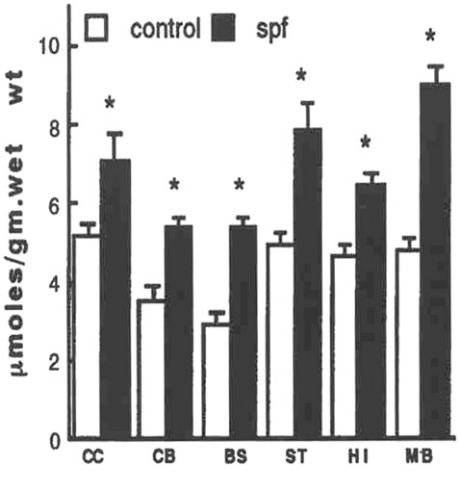

Fig. 1. Region-selective increases of brain glutamine in brains of spf/Y mice (spf) with congenital ornithine transcarbamylase deficiency compared to control. CC; cerebral cortex; CB: cerebellum; BS: brainstem; ST: striatum; HI: Hippocampus; MB: midbrain. Values significantly different from control are indicated by $^{*}p < 0.01$ by Student's t test. Data are from Ref. [3].

One explanation that has been proposed to explain this apparent limitation on GS activity in the brain under conditions of hyperammonemia involves stimulation of post-synaptic glutamate (NMDA) receptors in the brain leading to the formation of nitric oxide (NO) which then goes on to inactivate GS in the neighbouring astrocytes by a process involving protein tyrosine nitration [13]. In these studies, exposure to astrocytes to ammonia *in vitro* or *in vivo* was found to result in GS nitration and a consequent reduction in GS enzyme activity.

In order to directly address the issue of GLN synthesis rates *per se* as the major source of the increased GLN content of the brain in hyperammonemia, the technique of 1H/13C NMR spectroscopy was employed [14]. Using this technique, it was shown unequivocally that the *de novo* synthesis of GLN in the brain was increased, as expected, in an experimental animal model of ALF. However, the study showed that neither the increased brain GLN concentrations nor the magnitude of the increase in *de novo* GLN synthesis rates was significantly correlated with the severity of encephalopathy or with the occurrence of brain edema in these animals (Fig. 3). This does not mean to imply that there is no glutamine synthesis in the brain in hyperammonemia (indeed there is, as shown in Fig. 3) but it does suggest that the capacity for ammonia removal by GS in the brain in hyperammonemia is insufficient to prevent the rise in ammonia which may reach levels that are toxic to brain function.

Moreover mild hypothermia sufficient to prevent coma and brain edema in these animals had no significant effect on the rate of GLN synthesis in the brain [14] leaving open the possibility that the increase in brain GLN reported in hyperammonemic syndromes results primarily from alternative mechanisms such as the inhibition of GLN degradation.

A more detailed assessment of the evidence for and against the role of GLN synthesis as a key factor in the pathogenesis of hepatic encephalopathy and brain edema in liver failure (as well as the mechanisms implicated) has been the subject of a review [15].

4. Glutaminase: more than a neuronal mitochondrial enzyme?

The glutamate–glutamine cycle whereby glutamate carbon is shuttled between astrocytes and neurons is predicated on the assumption that glutaminase (GLNase) is localized exclusively in neurons and this was shown to be the case [16]. However, the results of recent studies suggest that such a simplistic model may need to be adjusted. At least two GLNase isoforms are expressed in mammalian brain, namely the K-type and the L-type with different molecular structures and regulatory properties that are found to co-localize in cells throughout the brain. Moreover, L-type GLNase is localized to both neuronal mitochondria and nuclei [17,18].

Whether or not a fully functional GLNase is expressed in astrocytes remains a matter of debate. Immunohistochemical studies show that the K-type protein is expressed in rat brain astrocytes [19]. On the other hand, no GLNase signal was observed in astrocytes of rat cerebellum [16].

The notion of a possible astrocytic localization for one of the GLNase isoforms led to the theory currently known as the "*Trojan Horse*" *theory* of hepatic encephalopathy. According to this theory, glutamine synthesized in the astrocyte in hyperammonemic conditions such as liver failure serves as a "Trojan horse" and indirect carrier of ammonia into the mitochondrion of the astrocyte where the action of purported astrocytic GLNase rapidly regenerates ammonia with cytotoxic consequences [20]. This ammonia-induced toxicity is predicated on the results of studies of