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Inhibition of glutamine synthesis induces glutamate dehydrogenase-dependent ammonia fixation into alanine in co-cultures of astrocytes and neurons

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ABSTRACT

It has been previously demonstrated that ammonia exposure of neurons and astrocytes in co-culture leads to net synthesis not only of glutamine but also of alanine. The latter process involves the concerted action of glutamate dehydrogenase (GDH) and alanine aminotransferase (ALAT). In the present study it was investigated if the glutamine synthetase (GS) inhibitor methionine sulfoximine (MSO) would enhance alanine synthesis by blocking the GS-dependent ammonia scavenging process. Hence, co-cultures of neurons and astrocytes were incubated for 2.5 h with [U-^{13}C]glucose to monitor de novo synthesis of alanine and glutamine in the absence and presence of 5.0 mM NH_4Cl and 10 mM MSO. Ammonia exposure led to increased incorporation of label but not to a significant increase in the amount of these amino acids. However, in the presence of MSO, glutamine synthesis was blocked and synthesis of alanine increased leading to an elevated content intra- as well as extracellularly of this amino acid. Treatment with MSO led to a dramatic decrease in glutamine content and increased the intracellular contents of glutamate and aspartate. The large increase in alanine during exposure to MSO underlines the importance of the GDH and ALAT biosynthetic pathway for ammonia fixation, and it points to the use of a GS inhibitor to ameliorate the brain toxicity and edema induced by hyperammonemia, events likely related to glutamine synthesis.

1. Introduction

Liver failure and disorders of the urea cycle enzymes may lead to hepatic encephalopathy (HE), a neuropsychiatric syndrome with symptoms ranging from mild cognitive impairment to coma (Albrecht and Jones, 1999; Hazell and Butterworth, 1999; Butterworth, 2002; Muñoz, 2008). Although the exact mechanism of action is unclear, the hyperammonemic condition is essential and a correlation has been demonstrated between the plasma ammonia level and the severity of the disease (Clemmesen et al., 1999; Kramer et al., 2000; Butterworth, 2002; Felipo and Butterworth, 2002). Studies in animal models of acute and chronic liver failure have repeatedly shown that the concentration of glutamine in the brain is increased (Hindfelt et al., 1977; Giguere and Butterworth, 1984; Hawkins and Jessy, 1991; Swain et al., 1992; Dejong et al., 1993; Hilgier and Olson, 1994; Albrecht et al., 2007). Moreover, studies in animal models of HE as well as in patients have found increased levels of alanine in the brain (Swain et al., 1992; Tofteng et al., 2006). Additionally, studies using [^{13}N]ammonia as a tracer have clearly demonstrated its rapid incorporation into the amide group of glutamine in hyperammonemic rats (Cooper et al., 1985). Also in cell culture models of hyperammonemia, an increased glutamine content has been observed (Johansen et al., 2007; Leke et al., 2010). Since the enzyme responsible for synthesis of glutamine from ammonia and glutamate, glutamine synthetase (GS) is located predominantly if not exclusively in astrocytes (Norenberg and Martinez-Hernandez, 1979) this glutamine formation is an astrocytic phenomenon. It has been proposed that such increase in the glutamine level leads to astrocytic swelling and concommittant cerebral edema (Norenberg and Bender, 1994; Zwingmann et al., 2000; Häussinger et al., 2000;

Abbreviations: HE, hepatic encephalopathy; MSO, methionine sulfoximine; GS, glutamine synthetase; GDH, glutamate dehydrogenase; ALAT, alanine aminotransferase; PBS, phosphate buffered saline.

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Pasantes-Morales and Franco, 2004; Albrecht et al., 2007). Interestingly, administration of methionine sulfoximine (MSO) which specifically inhibits GS (Ronzio et al., 1969) prevents astrocytic swelling and brain edema (Takahashi et al., 1990, 1991; Blei et al., 1994; Willard-Mack et al., 1996; Tanigami et al., 2005; Albrecht et al., 2007). It is, however, possible that increased levels of glutamine may have other detrimental effects than cell swelling. Hence, it has been shown that exposure of astrocytes to glutamine induces mitochondrial damage and activation of the mitochondrial permeability transition (Ziemińska et al., 2000; Rama Rao et al., 2003), a process likely involving uptake of glutamine into the mitochondria and subsequent production of ammonia by the action of phosphate-activated glutaminase (Jayakumar et al., 2004). This has lead to the notion of glutamine acting as a "Trojan horse" in ammonia toxicity (Albrecht and Norenberg, 2006).

Recent studies using a cell culture model of neuron-glia interactions in GABAergic neurotransmission developed by Leke et al. (2008) have provided evidence that ammonia may be detoxified not only by incorporation in glutamine but also by the concerted action of glutamate dehydrogenase (GDH) and alanine aminotransferase (ALAT) leading to trapping of ammonia in alanine (Leke et al., 2011) as illustrated in Fig. 1A. In order to further examine this possible role of GDH in addition to that of GS for ammonia detoxification, experiments were performed using the above mentioned cell culture model consisting of astrocytes in co-culture with GABAergic neurons (Leke et al., 2008, 2011) to investigate how addition of MSO may influence the biosynthesis and incorporation of ammonia in alanine and glutamine, respectively. Experiments in rats pretreated with MSO have shown that under such conditions a significant amount of [^{13}N]ammonia could be found in glutamate, i.e., via reductive amination catalyzed by GDH (Cooper et al., 1979). We have recently demonstrated using the above mentioned culture preparation and [^{15}N]ammonia that there is a substantial incorporation of ammonia nitrogen into both alanine and glutamine (Leke et al., 2011). The present study was designed to specifically obtain information about de novo synthesis of the carbon skeleton of glutamate, glutamine, aspartate and alanine using [U-^{13}C]glucose as the substrate (see Fig. 1B) in the presence of NH_4Cl and MSO. Hence, the contents of and incorporation of ^{13}C into the amino acids glutamate, glutamine, aspartate and alanine were determined in the presence or absence of NH_4Cl alone or together with MSO.

2. Materials and methods

2.1. Animals and chemicals

NMRI mice were obtained from Taconic M&B (Ry, Denmark). Plastic tissue culture dishes were purchased from NUNC A/S (Roskilde, Denmark), fetal bovine serum from GIBCO, Invitrogen (Taastrup, Denmark). Culture medium and poly-D-lysine (MW > 300,000) were from Sigma Chemical Co. (St. Louis, MO, U.S.A.). Penicillin was from Leo (Ballerup, Denmark). Isotopically labeled glucose ([U-^{13}C]glucose, 99%) was from Isotec, a subsidiary of Sigma Chemical Co. (St. Louis, MO, USA). All other chemicals used were of the purest grade available from regular commercial sources.

2.2. Co-cultures of cortical neurons and astrocytes

Co-cultures of cortical neurons and astrocytes were obtained as previously described by Leke et al. (2008). Cells were seeded in poly-D-lysine coated 6 well plates (2 ml/well) at a density of 2.75×10^6 cells/ml in a slightly modified (Hertz

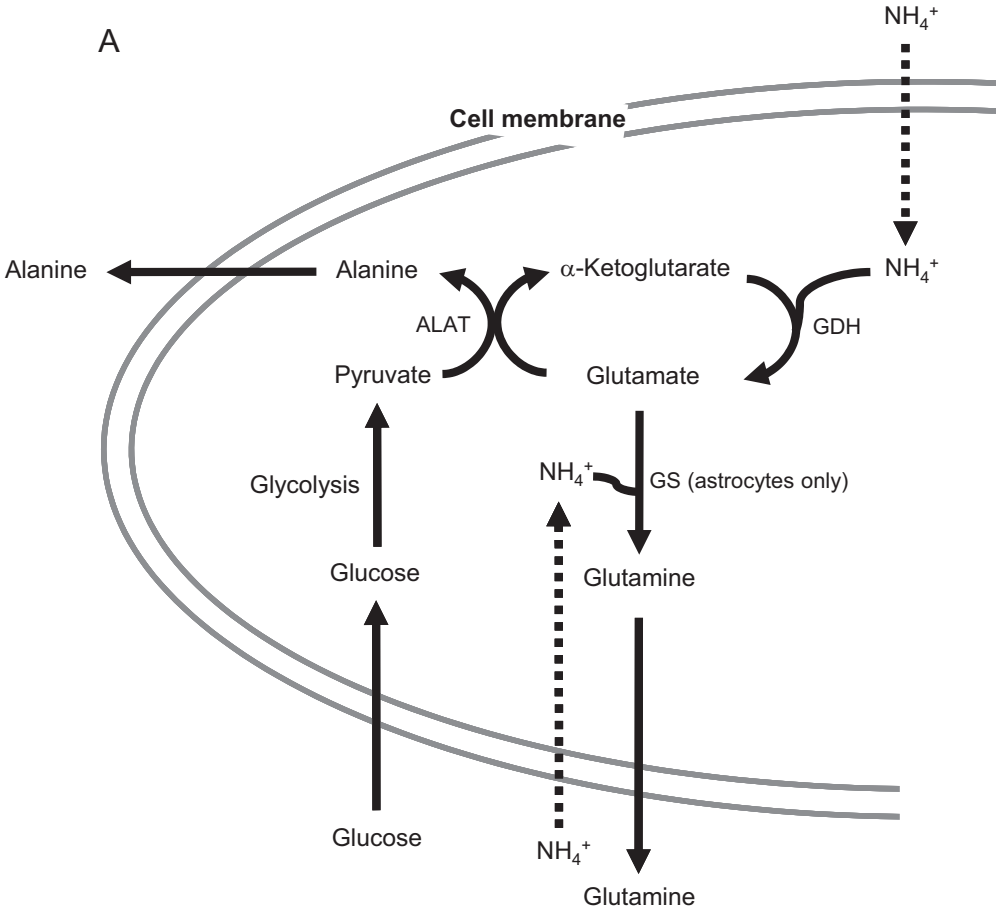

Fig. 1. Schematic representation of the reactions leading to incorporation of ammonia into glutamine and alanine (A) and of the labeling patterns from [U-^{13}C]glucose in amino acids derived from TCA cycle intermediates (B). The black circles in (B) denote labeled carbon atoms. It should be noted that some acetylCoA may be unlabeled as it may originate from other sources than labeled glucose.