Improvement of ammonia removal by glutamine synthesis is associated with attenuation of encephalopathy in acute liver failure: protective effects of the NMDA receptor antagonist memantine

J. Heins[1,2], W. Jiang[2], S. Gottschalk[2], D. Leibfritz[1], R. F. Butterworth[2], and C. Zwingmann[1,2]

[1]Department of Organic Chemistry, University of Bremen, Bremen, Germany, [2]Centre de Recherche, Hospital Saint-Luc, Montreal, Quebec, Canada

Introduction:

Brain edema is a major cause of death of patients with Hepatic Encephalopathy (HE) due to acute liver failure (ALF). Hyperammonemia is considered to be a key factor leading to brain edema in ALF. In the brain, the astrocytic enzyme glutamine synthetase (GS) is uniquely responsible for converting ammonia into glutamine. It has been the prevailing hypothesis that the osmotic disturbance induced by accumulation of glutamine leads to brain edema. However, recent studies point to a negative modulation of GS. As a consequence, ammonia concentrations rise to levels known to lead to disturbed brain energy metabolism and neurotransmission. In particular, a disturbed glutamatergic neurotransmission due to overactivation of N-methyl-D-aspartate receptors (NMDAR`s) is a major factor underlying acute ammonia neurotoxicity. Considerable evidence by us and by others indicates that the nitration of GS by nitric oxide (NO), formed after NMDAR-mediated stimulation of the constitutive neuronal NO synthase (nNOS), is the most likely explanation for the limited capacity for brain glutamine synthesis.

Aims:

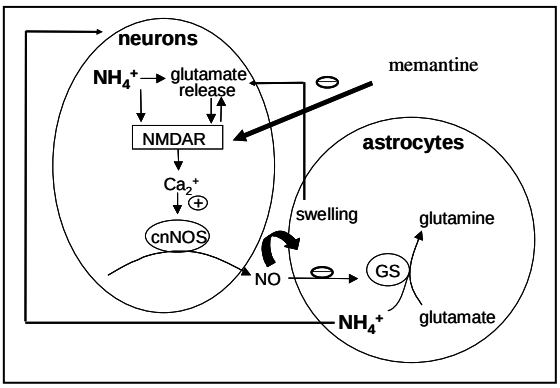
Our aim was to determine in rats with ALF 1) maximal glutamine synthesis in relation to encephalopathy, 2) whether prevention of neuronal NMDA receptor overactivation by the NMDAR antagonist memantine is related to increased glutamine synthesis, and 3) if blocking of the AMPA/KA receptor by 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX) has similar effects.

Methods:

We used the well-validated hepatic devascularized rat model of ALF. In this model, liver failure was induced in rats (approx. 200 g) by an end-to-side portacaval anastomosis (PCA) followed 48 h later by hepatic artery ligation (HAL). We investigated ALF rats early after HAL, at precoma-stages (6-9 h), when they had lost their righting reflexes, and at coma-stages (11-13 h), when rats had lost their corneal reflexes and had developed brain edema. Rats with ALF were administered memantine or CNQX in three single doses of 7 mg/kg (i.p.) 1.5, 3 and 5 h after HAL. At the end of the experiments, all animals received an i.p. administration of [U-^{13}C]glucose (500 mg/kg). The rats were killed 20 min later by decapitation, and the brain tissue samples were immediately snap-frozen in liquid nitrogen. Tissue samples were powdered over liquid nitrogen and homogenized in perchloric acid at 0°C. After lyophilization, the samples were redissolved in 0.5 ml D_2O and centrifuged. ^{1}H- and ^{13}C-NMR spectra were recorded on a Bruker DRX 600 spectrometer. Metabolite concentrations were calculated from ^{1}H-NMR spectra; the percentage ^{13}C-enrichments in metabolites were calculated from ^{13}C-NMR spectra. The NMR studies were complemented by neurological investigations (righting- and corneal reflexes), biochemical analysis (blood ammonia) and molecular biological methods (Western blotting).

Results:

Brain glutamine increased to 431±41.6%, 472±57.2% and 444 ±50.1% at 2.5 h (no encephalopathy), at precoma stages (no brain edema) and at coma stages (brain edema), respectively. Glutamine synthesis via the astrocyte-specific enzyme pyruvate carboxylase (PC) increased >4-fold at all stages and was maximal 2.5 h after HAL ($p<0.001$). Glutamine synthesis via PC was slightly lower at coma stages compared to precoma stages (0.77±0.09 compared to 0.91±0.13 µmol/g ww; $p<0.05$). Glutamine synthesis via PDH (in astrocytes or through neuronal glutamate) increased from 0.18±0.02 to 1.51±0.19 and 1.17±0.11 µmol/g ww ($p<0.05$) at precoma- and coma stages, respectively. Lactate synthesis was unchanged up to 5 hours after HAL, and increased at precoma and coma stages to 221±28.7 and 401±59.2% of controls, respectively.



Treatment of ALF rats with memantine resulted in a delay to the onset of coma (14-17 h compared to 9–11 h; $p<0.001$), concomitant to 32% lower plasma ammonia (increase from 67±9.0 to 457± 82 compared to 673±78 µg/dl in rats with ALF without treatment). Furthermore, astrocytic glutamine synthesis via PC was 1.5-fold higher compared to ALF rats at coma stages treated with saline ($p<0.001$). Also glutamine synthesis via PDH increased considerably (2-fold) compared to rats with ALF without treatment. Increased lactate synthesis at coma stages was largely prevented by memantine treatment (165±27.3% compared to controls).

Treatment of ALF rats with CNQX had no effect on encephalopathy, onset, plasma ammonia, or time to coma stages. In contrast to memantine, CNQX decreased brain glutamine synthesis to 76±11% compared to ALF rats without treatment. The increased synthesis of lactate was partially attenuated by CNQX ($p<0.01$), while it had no effect on glutamine synthesis through PDH.

Conclusions:

The data demonstrate that memantine delays the time to coma in rats with ALF. It is suggested that memantine improves ammonia removal by glutamine synthesis, and support that memantine or other NMDAR antagonists would be useful in the restoration of brain ammonia removal and prevention of cerebral consequences of ALF [funded by CIHR].