

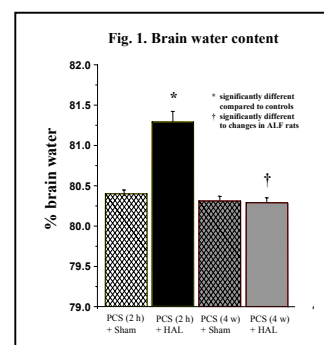
Lack of brain edema in acute on chronic liver failure results from compensatory mechanisms in skeletal muscle and brain

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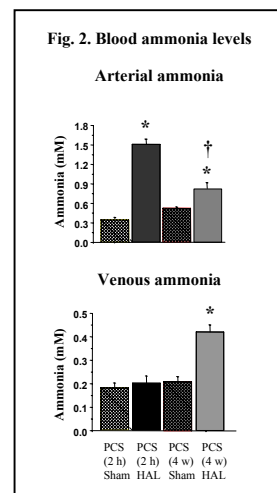
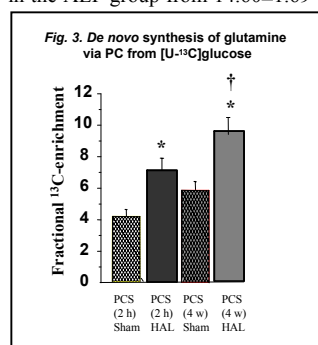
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Introduction. Liver failure invariably results in central nervous dysfunction known as Hepatic Encephalopathy (HE), which is classified into two basic forms: Acute liver failure (ALF) and chronic liver failure (CLF). A key factor responsible for the development of HE is the neurotoxic rise of ammonia. In acute HE, the major cause of death is brain edema (resulting from astrocyte swelling), leading to increased intracranial pressure and brainstem herniation [1]. It is suggested that the osmotic effect of astrocytic glutamine accumulation (the main detoxification product of ammonia in the brain) is responsible for the development of brain edema. It is interesting, that brain edema has been observed rarely in low-grade hyperammonemia associated with CLF, although, like in ALF, brain glutamine concentrations increased several-fold. And, interestingly, brain edema is rarely encountered in acute-on-chronic liver failure (ACLF) [2] – e.g. ALF induced on the top of CLF. This means, an acute insult on a chronically decompensated liver does not lead to brain edema. Therefore, in the present study, we investigated whether devascularization of the liver (HAL) (ALF) following 2 hours or 4 weeks after portacaval anastomosis (PCA) results in differences in relation to the onset of brain edema, blood ammonia and cerebral metabolism using *ex vivo* NMR spectroscopy.

Methods. We used the well-validated hepatic devascularized rat model of ALF. In the ALF 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 at coma stages (11–13 h), when rats had developed brain edema. In the model of ACLF, liver failure was induced in rats by an end-to-side portacaval anastomosis (PCA) followed 4 weeks later by hepatic artery ligation (HAL). The investigations of the ACLF rats were time-matched to the ALF rats at coma stages. Appropriate weight-matched sham-operated control groups were included for both models. Neurological investigations were done at different time-points during the development of HE. At the end of the experiments, all animals received an i.p. administration of [¹³C]glucose (400 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 [2,3]. After lyophilization, the samples were redissolved in 0.5 ml D₂O and centrifuged. ¹H- and ¹³C-NMR spectra were recorded on a Bruker DRX 600 spectrometer. Metabolite concentrations were calculated from ¹H-NMR spectra; the percentage ¹³C-enrichments in metabolites and metabolic pathways were calculated from ¹³C-NMR spectra [2,3]. The NMR studies were complemented by neurological investigations (locomotor activity, righting- and corneal reflexes), biochemical analysis (arterial and venous ammonia and brain water content) and molecular biological methods (Western blotting; RT-PCR).



Results: The onset of encephalopathy was significantly delayed in the ACLF group of animals compared with the ALF group. Brain water content was increased in ALF compared to controls (81.39±0.15% vs. 80.12±0.09%; p<0.01; Fig. 1). Arterial ammonia concentration followed a similar pattern (ALF: 1.51±0.07 mM vs ACLF: 0.52±0.09 mM; p<0.01). However, venous ammonia concentrations increased significantly only in the ACLF group (Fig. 2). In rats with ALF, the activity of the ammonia-metabolizing enzyme glutamine synthetase (GS) in brain was unchanged compared to controls. However, GS activity increased significantly in the ACLF group (1.7-fold; p<0.001). Brain glutamine concentrations increased in the ALF group from 14.60±1.69 to 24.03±2.06 μmol/g ww, and in the ACLF group from 21.22±2.06 to 34.14±3.12 μmol/g ww. The percentage ¹³C enrichment in [4,5-¹³C]glutamine, synthesized from [¹³C]glucose through pyruvate dehydrogenase, increased from 4.71±0.51% to 7.34±0.65% in the ALF group; changes in the ACLF group were not significantly different compared to the ALF group. However, the percentage ¹³C enrichment in [2,3-¹³C]glutamine, synthesized from [¹³C]glucose through pyruvate carboxylase, increased from 4.09±0.55% to 7.08±0.78% in the ALF group, but from 5.74±0.29 to 9.52±1.08% in the ACLF group (p<0.01 compared to ALF; Fig. 3). The percentage ¹³C enrichment in [1,2,3-¹³C]lactate increased from 13.53±1.67% to 34.31±3.79% in the ALF group, but was unchanged in the ACLF group. Furthermore, the percentage ¹³C enrichment in [3,4-¹³C]aspartate decreased from 2.01±0.30% to 1.39±0.20% in rats with ALF (p<0.05), and was unchanged in rats with ACLF.



Summary: The time to the onset of coma (loss of corneal reflex) was significantly delayed in the ACLF group (PCS (4W) + HAL) compared to the ALF group (PCS (2h) + HAL). ALF caused significant changes compared to ACLF: a) increased % brain water (brain edema), 2. increased arterial ammonia concentrations, 3. Decreased brain glutamine synthesis through pyruvate carboxylase, 4. increased brain lactate synthesis, and 5. decreased brain aspartate synthesis.

Conclusions: ALF in rats result in a faster time to the onset of coma, brain edema and arterial ammonia elevations as compared to ACLF. The simultaneous decrease in venous ammonia concentrations in the ACLF group compared to the ALF group suggests that ammonia per se may be responsible for the development of brain edema. Only rats with ALF showed evidence for impaired brain energy metabolism by increased lactate synthesis and decreased aspartate synthesis. Furthermore, in parallel to higher brain glutamine concentrations and attenuation of brain edema and hyperammonemia in rats with ACLF, flux through pyruvate carboxylase contributing to glutamine synthesis increased significantly compared to ALF. All together, these observations strongly suggest an important role of ammonia per se, which is not sufficiently detoxified by brain pyruvate carboxylase and glutamine synthesis, as well as of impaired brain energy metabolism, for the development of brain edema in ALF.

[1] Blei AT. Neurochem Int 2005;47:71-77; [2] Shawcross D and Jalan R. Cell Mol Life Sci 2005;62:2295-2304.