The anaplerotic metabolic flux through pyruvate carboxylase is a prerequisite for ammonia detoxification in acute and chronic liver failure

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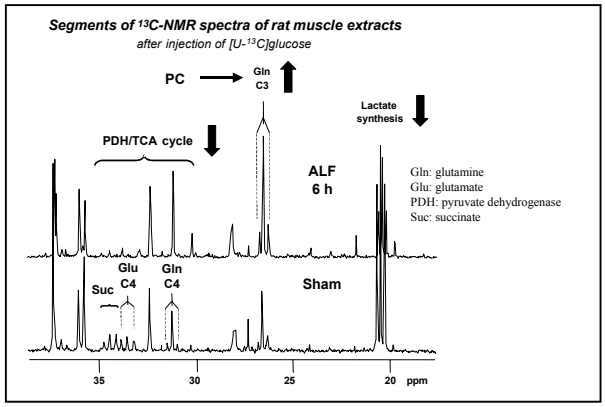
Introduction. Liver failure can result in central nervous dysfunction known as Hepatic Encephalopthay (HE). A key factor responsible for the development of HE in both acute liver failure (ALF) and chronic liver failure (CLF) is the neurotoxic rise of ammonia. In acute HE, the major cause of death is brain edema [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 astrocyte swelling and brain edema. However, recent ^{13}C-NMR studies challenged this view [2,3]. It is interesting, that brain edema has been observed rarely in low-grade hyperammonemia accociated with CLF, although, like in ALF, brain glutamine concentrations increased several-fold. Furthermore, brain edema is rarely encountered in acute-on-chronic liver failure (ACLF) [4]. We suggest that, rather than glutamine accumulation, a limited synthesis of glutamine account for the development of both acute and chronic HE. The continuous drain of Krebs cycle intermediates, and therefore of glutamate as the substrate for GS, must be complemented by an anaplerotic mechanism. A major anaplerotic enzyme is pyruvate carboxylase (PC), and PC is prerequisite for glutamine formation in brain [5]. In order to evaluate the association of PC with glutamine synthesis in HE, high resolution ^{1}H- and ^{13}C-nuclear magnetic resonance (NMR) spectroscopy was used to measure the carbon flux through PC contributing to glutamine *de novo* synthesis in brain, liver and muscle of rats with different forms of experimental HE.

Methods. As the first group, we used the well-validated hepatic devascularized rat model of ALF. In this model, ALF 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 t = 6 h (before precoma stages), at precoma stages (7-9 h), and rats at coma-stages (11-13 h), when rats had developed brain edema. As the second group, we used a model of mild HE, which is induced by PCA; investigations were done 1,4 and 12 weeks after PCA. As the third group, we used a model of ACLF, in which HAL was conducted 4 weeks following PCA. Appropriate sham-operated control groups were included in all 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 [1-^{13}C]- or [U-^{13}C]glucose (200-400 mg/kg). The rats were killed 15-60 min later by decapitation, and the tissue samples from brain, liver and muscle were immediately freeze-clampled or 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 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 and metabolic pathways were calculated from ^{13}C-NMR spectra [2,3]. The NMR studies were complemented by neurological investigations (locomotor activity, righting- and corneal reflexes), biochemical analysis (plasma ammonia and brain water content) and molecular biological methods (Western blotting; RT-PCR). Each experiment was performed on at least 4 animals. The data are expressed as means ± SD. Statistical analysis was performed by ANOVA.

Results. Compared with sham-operated controls, brain glutamine levels increased to an equal extent to 451±5.5% and 434 ±5.8% in ALF rats at precoma stages (no brain edema) and coma stages (brain edema), respectively ($p<0.001$). The precoma- and coma stages of ALF were both associated with a significant and equal 9-fold increase of the total *de novo* synthesis of the glutamine isotopomer synthesized after flux of ^{13}C-labelled glucose through PC ($p<0.001$). In the muscle, severy hyperammonemia already 6 h after HAL was accompanied by an increased amount of the ^{13}C-labelled glutamine isotopomer synthesized through prior PC flux to 269±18.2% of controls ($p<0.01$) (Fig. 1). In contrast to the brain, PC-mediated glutamine synthesis in the muscle increased between precoma- and coma stages in relation to the progression of liver failure ($p<0.001$). Furthermore, in rats with chronic HE, which showed decreased locomotor activities 4 weeks after PCA, increased flux through PC contributing to glutamine synthesis was much higher in the non-vulnerable thalamus (7.8-fold, $p<0.001$) compared to the vulnerable frontal cortex (2.9-fold; $p<0.01$). Furthermore, while PC flux decreased in the liver to 72±12.2% ($p<0.01$) 4 weeks after PCA, the synthesis of glutamine via PC increased in the muscle to 149±19.1% ($p<0.01$). 12 weeks after PCA, on the other hand, when rats have recovered from the neurological symptoms, flux through cerebral PC normalized in thalamus. In the rat model of ACLF, brain water content was unchanged compared to sham-operated controls – in contrast to significant brain edema in the ALF model ($p<0.01$). Concomitant to attenuation of brain edema and increased arterial ammonia, brain glutamine synthesis via PC significantly increased in ACLF rats compared to ALF rats ($p<0.05$).



Summary/Discussion: 1) During the course of ALF, the flux through PC flux contributing to glutamine synthesis is limited and contributes to further increased plasma ammonia concentrations. In this model, the muscle becomes a quantitatively important organ for ammonia clearance, which is due to stimulation of PC. This means that between precoma- and coma stages a limited anaplerotic flux through PC in the brain, but not in the muscle, correlates with the development of brain edema in ALF. Moreover, these data further support that the muscle becomes the major organ for ammonia detoxification. 2) The capacity of the frontal cortex, the vulnerable brain region in chronic HE, to synthesize glutamine is decreased relative to thalamus, which is attributed to limited flux through PC. This means, a limited anaplerotic flux through PC in the frontal cortex correlates with the selective vulnerability of this brain region compared to thalamus. The results also provide an explanation, why, following PCA, thalamus has increased ammonia-detoxification capacity compared to frontal cortex [6], and for the observations in patients with minimal HE of relative decreases in cerebral blood flow [7] and ammonia extraction [8] in frontal cortex compared to thalamus. 3) In parallel to higher brain glutamine concentrations and attenuation of brain edema and hyperammonemia in ALF on top of CLF (ACLF), PC flux contributing to glutamine synthesis increased compared to ALF.

Conclusions. A significant limitation in anaplerotic flux through PC in the brain, but not in the muscle, correlates with the development of hyperammonemia and neurological manifestations in acute and chronic HE. These findings suggest that carbon flux through cerebral PC is essential for ammonia detoxification in HE. Moreover, considering the limited brain capacity for glutamine synthesis, increasing the anaplerotic flux in HE might be of therapeutic value to decrease brain ammonia concentrations; compounds such as ornithine-aspartate would be potential candidates.

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