Inter-organ metabolism in Hepatic Encephalopathy due to acute liver failure

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Introduction

A key factor responsible for the development of Hepatic Encephalopathy (HE) is the neurotoxic rise of circulating and brain ammonia due to acute liver failure (ALF). The major cause of death is brain edema (resulting from astrocyte swelling), leading to increased intracranial pressure and finally brainstem herniation [1,2]. 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. However, recent ^{13}C-NMR studies challenged this view and indicate rather to a direct effect of ammonia resulting in impaired brain energy metabolism and lactate accumulation [3,4]. ALF still represents a major therapeutic challenge due to a disturbed whole body nitrogen homeostasis and its progression to multiorgan failure. To obtain more insight into ammonia detoxification processes and the role of inter-organ metabolism for the development of brain edema, our first aim was to investigate the metabolism of ^{13}C-labelled glucose in the brain and in peripheral organs in relation to the progression of liver failure. Mild hypothermia is a new approach receiving increasing interest to the management of patients with ALF, as it effectively improves outcome by prevention of cerebral edema [5]. But hypothermia provides also an important research tool to study basic mechanisms; and our second aim was to investigate the effect of mild hypothermia on whole-body metabolic pathways in experimental ALF.

Methods

Animal model. We used the well-validated hepatic devascularized rat model of ALF. Briefly, 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). 15-60 min after i.p. administration of [U-^{13}C]glucose or [1,2-^{13}C]acetate (which is taken up by astrocytes only), the rats were killed by decapitation. We investigated sham-operated controls, rats 48 h after PCA (without HAL), as well as rats with ALF at precoma- and coma-stages, which were maintained at 37°C (normothermic) or 35°C (hypothermic). The brain and the peripheral organs (i.e. muscle, kidney, liver, spleen, heart) were rapidly removed and immediately snap-frozen in liquid nitrogen. The blood was taken from the neck and immediately mixed with 20% perchloric acid. ***Extraction.*** Tissue samples were powdered over liquid nitrogen and homogenized in perchloric acid at 0°C [3,4]. After lyophilization, the samples were redissolved in 0.5 ml D_2O and centrifuged. ^{1}H- and ^{13}C-NMR spectra were recorded on Bruker DRX 600 or AVANCE-NB/WB 360 spectrometers. Metabolite concentrations were calculated from ^{1}H-NMR spectra; the percentage ^{13}C-enrichments were calculated from ^{13}C-NMR spectra as described previously [3,4]; the flux of ^{13}C through metabolic pathways was followed up by ^{13}C-isotopomer analysis of the ^{13}C-^{13}C coupling pattern in amio acids. Gradient selected 2D-NMR inverse homonuclear (COSY) and heteronuclear (HSQC) chemical shift correlations were applied to identify unknown resonances. The NMR studies were complemented by molecular biological methods (western blotting; RT-PCR).

Results

In portacaval-shunted rats (after 48 h) without HAL, no abnormal neurological signs were observed. At this stage, brain glutamine synthesis increased 3-fold, while only minor changes were observed in glutamine of muscle and kidney. Glycolytic formed [^{13}C]lactate, on the other hand, was unchanged in brain, but increased up to 2-fold in muscle and kidney. After HAL, normothermic ALF rats were hyperammonemic (10-fold increase of plasma ammonia) and developed encephalopathy consisting of loss of righting ability (precoma) progressing to brain edema and loss of corneal reflex (coma). Analysis of isotopomer patterns revealed that coma stages of encephalopathy in ALF rats were accompanied by a 3-4 fold increase in synthesis of glutamine in brain and skeletal muscle. Furthermore, while the increase of glutamine in the brain was maximal already at precoma stages, it increased in the muscle and the blood as a function of the deterioration of neurological function in ALF. In addition, the majority of circulating glutamine was released from muscle. Parallel studies of the expression of glutamine synthetase using RT-PCR revealed that increased muscle glutamine synthetase (GS) in ALF occured as a result of increased expression of GS mRNA whereas changes in brain and kidney were post-translational in nature. In contrast to brain glutamine, brain lactate synthesis increased in parallel to impaired mitochondrial glucose oxidation (i.e. flux of carbon through pyruvate dehydrogenase) and in relation to the encephalopathy and the development of brain edema in ALF animals. Muscle lactate synthesis, on the other hand, was significantly reduced. Hypothermia (35°C) resulted in prevention of encephalopathy and brain edema and concomitant attenuation of (i) the increase in brain lactate synthesis and, (ii) the increase of circulating lactate, and (iii) the increase in muscle and blood (but not brain) glutamine in ALF rats. Furthermore, the increase of alanine *de novo* synthesis in brain and muscle was much higher compared to glutamine synthesis, and was prevented by hypothermia. Analysis of ^{13}C-NMR spectra showed furthermore, that the coma stages of encephalopathy led to increased brain and muscle formation of 5-phosphoribosyl pyrophosphate, a condensation product between the pentose shunt intermediate ribose-5-phosphate and ammonia indicating the existence of a novel pathway for ammonia removal in ALF. The kidney, which releases ammonia after hydrolyzation of glutamine under normal conditions, showed a controversial pattern at coma stages of ALF in that the concentrations of glutamine, alanine and lactate increased to 620%, 278% and 182%, respectively, which showed an even further increase under hypothermic conditions (to 133%, 174%, and 162% compared to normothermic ALF rats).

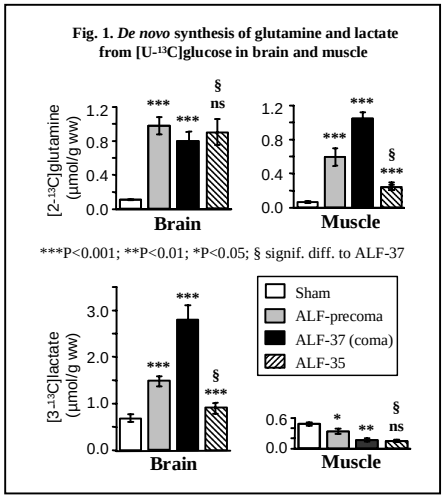

Fig. 1. ***De novo*** **synthesis of glutamine and lactate from [U-^{13}C]glucose in brain and muscle**

Conclusions

The present study demonstrate that the application of *ex vivo* 13C-NMR spectroscopy is a very powerful approach to investigate whole-body metabolic alterations in acute liver failure. Our findings clearly indicate to organ-selective metabolic responses to hyperammonemia and provide direct evidence for the notion that skeletal muscle becomes the major organ responsible for ammonia removal in ALF. The results not only provide some possible metabolic mechanisms associated with a re-distribution of whole body nitrogen metabolism in ALF, but also extend our knockledge about organ-specific ammonia detoxification processes, which may be valuable for the development of therapeutic means.

References

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