Imbalance between cortical and subcortical cellular metabolic activity during chronic liver failure induced by portacaval shunting

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Introduction and Aim

Cognitive impairments characteristic of early Hepatic Encephalopathy (HE) in chronic liver failure have classically been attributed to cerebral cortical dysfunction. Positron emission tomography (PET), a technique used to examine basic metabolic brain processes, showed significantly decreased glucose utilization in the cerebral cortex and concomitant increased utilization in the thalamus [1]. These findings suggested that hypometabolism in the frontal cortex in the brain of patients with chronic liver disease could explain the neuropsychiatric abnormalities characteristic of HE. In order to further evaluate brain-region-specific metabolism of glucose in experimental chronic liver failure, high resolution ^{1}H- and ^{13}C-NMR spectroscopy was used to measure metabolite concentrations as well as glucose metabolism via cell-specific pathways in frontal cortex compared to thalamus of rats following end-to-side portacaval anastomosis (PCA), a well characterized model of mild HE [2].

Methods

Animal model. 1) To induce chronic liver failure, an end-to-side portacaval anastomosis was performed in adult male Sprague-Dawley rats under isoflurane anaesthesia [2,3]. 1 day, 4 weeks and 3 months later, the rats were injected with [1-^{13}C]glucose (500 mg/kg; i.p.). 60 min after administration of ^{13}C-labelled glucose, the rats were killed by decapitation. The brains were immediately snap-frozen in liquid nitrogen, and dissected over dry ice for the frontal cortex and the thalamus. ***Extraction.*** Tissue samples were powdered over liquid nitrogen and homogenized in 5% perchloric acid (PCA) at 0°C [3]. Blood (taken from the neck) was immediately mixed with PCA, dual-extracted, and used for ^{1}H-NMR analysis of the blood. ***NMR analysis***. After lyophilization, the samples were redissolved in 0.5 ml D_2O and centrifuged. ^{1}H-, ^{13}C-NMR spectra were recorded on Bruker spectrometers DRX 600 or AVANCE-NB/WB 360. Metabolite concentrations were calculated from ^{1}H-NMR spectra; the flux of ^{13}C through metabolic pathways was followed up by ^{13}C-isotopomer analysis [3].

Results and Discussion

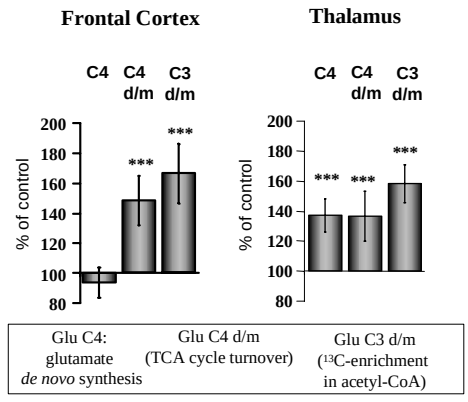
1) PCA leads to accumulation of glutamine and depletion of myo-inositol in frontal Cortex and thalamus. ^{1}H-NMR analysis revealed increased glutamine concentrations in thalamus (4.8-fold) compared to frontal cortex (3.0-fold, $p<0.01$) 4 weeks after PCA. 3 months after PCA, the elevation in glutamine concentration was similar to that observed 4 weeks after PCA in frontal cortex. However, in thalamus, increases in glutamine levels were much less after 3 months PCA compared to 4 weeks of PCA and similar to those observed in frontal cortex. 4 weeks after PCA, increased glutamine was accompanied by equimolar decreases of *myo*-inositol, and astrocyte-specific organic osmolyte, to 76% and 49% of controls in frontal cortex and thalamus, respectively. Similarly, taurine, another organic osmolyte, decreased to 79% and 53% of controls, in frontal cortex and thalamus, respectively.

2) PCA leads to selective stimulation of pyruvate carboxylase in thalamus. ^{13}C-isotopomer analysis showed that the stimulation of glutamine synthesis from glucose was sustained for 3 months after PCA in both frontal cortex and thalamus. Furthermore, the increased thalamic glutamine content 4 weeks after PCA was principally due to stimulation of the pyruvate carboxylase pathway ($p<0.001$), an anaplerotic pathway expressed only in astrocytes. These findings demonstrate that, following PCA, the thalamus has increased ammonia-detoxification capacity compared to frontal cortex, which is consistent with decreased glutamine synthetase activities and disproportionately high ammonia levels in frontal cortex [4].

3) PCA leads to decreased glutamate concentrations in frontal cortex. ^{1}H-NMR analysis showed that PCA (4 weeks) led to a selective 39% ($p<0.01$) decrease of glutamate concentrations in frontal cortex. Glutamate concentrations in thalamus, on the other hand, were not affected by PCA.

4) PCA leads to increased TCA-cycle flux in frontal cortex and thalamus. ^{13}C-isotopomer analysis showed that the decrease in frontal cortex glutamate concentrations can not be attributed to a decreased glucose metabolism. Rather, PCA led to a stimulated flux of carbon through the mitochondrial TCA-cycle in both brain regions, which was calculated from the ratio of the double/mono (d/m) labelled isotopomers of glutamate (Fig. 1). These data indicate that the reduction in frontal cortex glutamate occurs from the glutamatergic neurotransmitter pool and not from the metabolic pool and/or astrocytic glutamate pool.

Fig. 1. Changes in glutamate synthesis and TCA-cycle turnover



Conclusions

The present study suggests that the selective reduction of frontal cortical glutamate does not reflect its loss from metabolic or astrocytic pools; rather, the data suggest a loss from glutamatergic nerve terminals leading to decreased glutamatergic neuronal activity and consequently hypo-excitability in this region. Furthermore, the capacity of the frontal cortex to synthesize glutamine is decreased relative to thalamus. These data provide a plausible explanation for previous observations of selective, relative decreases of cerebral cortical glucose utilization and blood flow and underscore the selective vulnerability of cerebral cortical structures in experimental and human chronic liver failure.

References

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