Assessment of the Protective Effect of L-Carnitine in Hepatic: Improvements of Muscle and Brain Mitochondrial and Fatty Acid Metabolism

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Introduction

Hepatic Encephalopathy (HE) in both chronic and acute liver failure is associated with hyperammonemia and energetic changes in brain and peripheral organs. In hyperammonemic animal models and in ammonia-treated cultured neurons, L-Carnitine has been shown to counteract some of the toxic effects of ammonia (Minana et al., 1996; Therrien et al., 1997). Furthermore, a protective effect of L-Carnitine against disordered mental function (Malaguarnera et al., 2003) and against ammonia-precipitated encephalopathy was observed in cirrhotic patients with HE (Malaguarnera et al., 2003). The mechanisms of its action as well as their cellular localization in HE are not known and have not yet been systematically investigated.

Objective

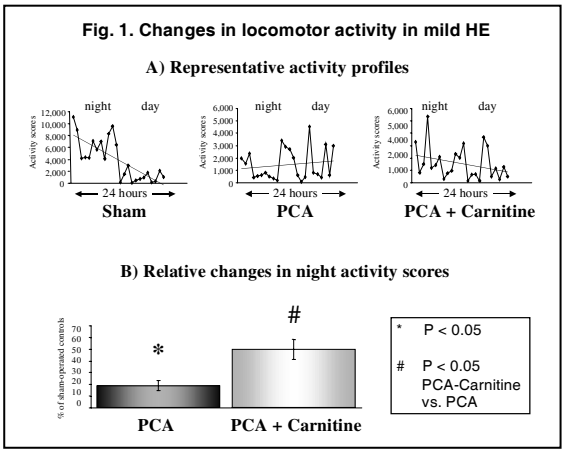
It is known that L-Carnitine can facilitate the entry of fatty acids into mitochondria. These fatty acids can be used to produce energy. In particular, activated fatty acids in form of acyl-CoA are carried across the mitochondrial membrane by L-carnitine. In order to investigate the effect of L-carnitine on energy- and fatty acid metabolism, ^{1}H- and ^{13}C- Nuclear Magnetic Resonance (NMR) spectroscopy was used to measure metabolic pathways in brain and muscle following administration of [U-^{13}C]glucose to rats four weeks after end-to-side portacaval anastomosis (PCA) and in rats with ammonia-precipitated encephalopathy.

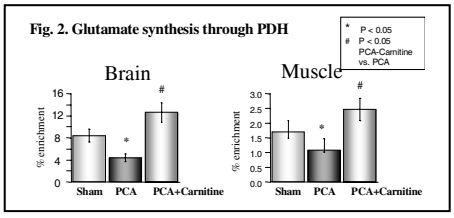
Methods

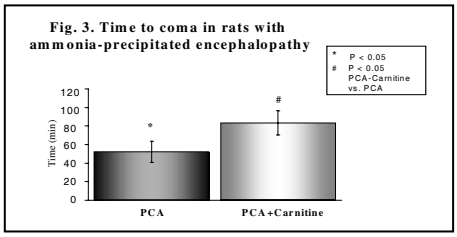
As a model of mild HE, we investigated rats four weeks after an end-to-side PCA. Sham-operated rats were time-matched to the rats with PCA and were used as controls. During the last seven days, rats with PCA and sham-operated controls were daily administered with either L-carnitine (0.8 mmol/kg; i.p.) or equivalent volumes of saline. Another group of rats received ammonium acetate (3.8 mmol/kg; i.p.) four weeks after PCA to precipitate encephalopathy; L-carnitine was administered in a single dose (1.6 mmol/kg concomitantly with ammonium acetate treatment. All rats were administered with [U-^{13}C]glucose (500 mg/kg; i.p.; 30 – 60 minutes) 12 hours after the last administration of L-carnitine or saline. The tissues from brain, liver, and muscle were quickly snap-frozen in liquid nitrogen and subjected to a dual extraction method using perchloric acid and methanol/chloroform to obtain water-soluble metabolites and membrane/fatty acid components. Metabolite concentrations and metabolic pathways were investigated from high-resolution ^{1}H- and ^{13}C-NMR spectra (Bruker DRX 600 MHz spectrometer). 1D-NMR spectra were recorded on DRX-600 and WB-360 Bruker spectrometers. Fully-relaxed ^{1}H-NMR spectra were recorded using (trimethylsilyl)-propionic-2,2,3,3d$_4$-acid (TSP) as standard. Integrals in ^{13}C-NMR spectra were corrected for nuclear Overhauser enhancement and saturation effects (Aureli *et al.*, 1999).

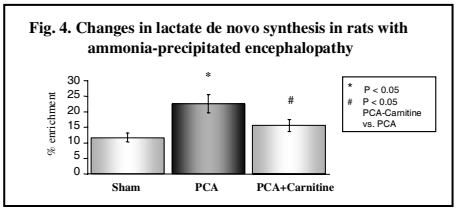
Results

PCA in the rat resulted in a broad spectrum of neurobehavioral changes. The locomotor activity four weeks after PCA was measured for 24 hours after the last administration of L-carnitine or saline (Fig. 1). PCA resulted in a more than 80% impaired locomotor activity. Treatment with L-carnitine resulted already after the first day in a 75% increase of locomotor activity compared to rats with PCA treated with saline ($p<0.001$). urthermore, seven days treatment with L-carnitine stimulated mitochondrial metabolism as evident by a 288 ± 32% and 226 ± 37% increase in the de novo synthesis of glutamate through pyruvate dehydrogenase (PDH) in brain and muscle, respectively (Fig. 2). On the other hand, after L-carnitine treatment, the fractional ^{13}C-enrichment in glutamine decreased to 54 ± 8% and 67 ± 9% in brain and muscle, respectively, compared to PCA rats. L-carnitine had no effect on total glutamine concentrations in muscle and brain. Together with an improved Krebs cycle, the astrocytic marker-molecule and organic osmolyte *myo*-inositol was normalised in L-carnitine treated rats with PCA, while this molecule showed a 66% ± 5% decrease in saline-treated rats with PCA. ^{1}H-NMR spectra of brain lipid extracts showed a 71% ± 4% decrease in the ratio of CH_2- to CH_3 groups of brain fatty acids, which explains the dilution in glucose-derived glutamine by mitochondrial catabolism of long-chain fatty acids in L-carnitine-treated rats.









After administration of ammonium acetate to rats with PCA, L-carnitine significantly prolonged the time until the onset of coma (Fig. 3). Interestingly, selective changes occurred in the synthesis of brain lactate, which was increased by 192% ± 25% in ammonium-treated rats. After a single dose of L-carnitine, however, lactate synthesis increased to a lesser extent of 133% ± 11% of controls (Fig. 4).

Conclusions

These results demonstrate that in chronic HE L-carnitine acts both in the brain and in the muscle not by improvement in ammonia removal but by improvement of mitochondrial metabolism and fatty acid catabolism. The data further demonstrate that L-carnitine prevents increased lactate synthesis in ammonia-precipitated encephalopathy which parallels a significant increase in the time to coma. These mechanisms explain L-carnitine's therapeutic benefit in the prevention of mild HE and ammonia-precipitated encephalopathy in cirrhotic patients. L-carnitine could be further considered as a therapeutic intervention.