

Protective actions of L-carnitine in ammonia-precipitated Hepatic Encephalopathy

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Introduction:

Hepatic Encephalopathy (HE) is associated with hyperammonemia and energetic changes in brain. While ammonia is detoxified in the astrocytes, the cellular localization of energetic impairments is unresolved. In ammonia-precipitated encephalopathy, L-carnitine (LC) has been shown to counteract some of the ammoniotoxic effects (O'Connor et al., 1983; Therrien et al., 1997). Furthermore, a protective action of LC against disordered mental function (Malaguarnera et al., 2003) and against ammonia-precipitated HE was observed in cirrhotic patients (Malaguarnera et al., 2003). The mechanism of its action as well as the cellular localization are not known and have not yet been systematically investigated.

Aim:

In order to investigate the protective effect of LC on energy metabolism, ¹H- and ¹³C-Nuclear Magnetic Resonance (NMR) spectroscopy was used to analyze metabolic pathways in brain after administration of [U-¹³C]glucose in rats with ammonia-precipitated encephalopathy.

Methods:

For the model of ammonia-precipitated HE, mild HE was induced in adult rats by portacaval anastomosis (PCA) and administration of ammonium acetate (2.8 mmol/kg, i.p.) four weeks after. Sham-operated rats were time-matched to the rats with PCA and used as controls. LC was applied in a single dose (1.6 mmol/kg) simultaneously to ammonia treatment. Control rats received equivalent volumes of saline. Rats were administered with [U-¹³C]glucose (500 mg/kg, i.p.) 10 min after the administration of ammonia (with or without LC) and sacrificed 20 min later. Immediately snap-frozen brain was powdered over liquid nitrogen and homogenized in perchloric acid on ice. The extracts were processed as described in (Zwingmann et al., 2004) and lyophilized. The lyophilized samples were dissolved in 600 µl D₂O, centrifuged and neutralized. ¹H- and ¹³C-NMR spectra were recorded on Bruker DRX 600 and Bruker WB 360 MHz spectrometers with a 5-mm H,C,N inverse triple resonance probe and a 5-mm ¹³C-Dual probe as described in (Zwingmann et al., 2003). Additional investigations were performed using ammonia-treated (5 mM, 12 h) primary astrocytes in culture.

Results:

Ammonia-precipitated encephalopathy in rats was attenuated by LC as observed by the prolongation of the time to onset to coma. LC slightly increased glutamine but not glutamate concentrations in hyperammonemic rats. The de novo synthesis of glutamine via pyruvate carboxylase (PC) showed a 27.7 ± 11.7% increase after LC treatment. On the other hand, ¹³C-enrichment in glutamate formed after glucose flux through pyruvate dehydrogenase (PDH) decreased after ammonia treatment by 27% which was deteriorated by LC by further 19% (p<0.05). Interestingly, selective and dominant changes occurred in brain lactate concentrations which were significantly increased by 147.5 ± 12.3% in rats with ammonia-precipitated encephalopathy. The administration of LC to rats with ammonia-precipitated HE resulted in decreased lactate concentrations, i.e. from 28.9 µmol/g wet weight to 21.5 µmol/g wet weight (Fig.1). The ammonia-induced increased lactate de novo synthesis (by 56%) was almost completely prevented by LC.

Conclusions:

These results demonstrate that LC prevents increased lactate synthesis in ammonia-precipitated encephalopathy which parallels a significant time lag in the time to coma. The increased de novo synthesis of glutamine via astrocytic PC indicates to a favored ammonia detoxification capacity. Concomitantly, the decrease of brain lactate indicates that LC treatment attenuates ammonia-induced energy failure. This takes presumably place in the astrocytes since flux through PDH, which is mainly neuronal, is not activated by LC. Preliminary experiments using ammonia-treated primary astrocytes confirmed a major beneficial effect of LC on astrocytic energy metabolism and lactate synthesis. Altogether, these data are consistent with LC's therapeutic benefit in the prevention of ammonia-precipitated HE in cirrhotic patients. The data also confirm ammonia-induced cell-specific changes in astrocytes which can not be put solely down to the known effect of LC on fatty acid mobilization to form acetyl-CoA, and point to further investigations on the mechanisms of LC's action in hyperammonemia.

References:

1) Malaguarnera et al. (2003) Dig Dis 21:271-275; 2) O'Connor et al. (1983) Neurochem Res 9:563-570; 3) Therrien et al. (1997) Hepatology 25:551-556; 4) Zwingmann et al. (2003) J Cereb Blood Flow and Met 23:756-771; 5) Zwingmann et al. (2004) NeuroToxicology 25:573-587

