Cerebral Metabolic Effect of Acute Inhibition of Aspartate Aminotransferase Measured by ^{1}H MRS

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Introduction

Aspartate aminotransferase (AAT) is a pyridoxal-5'-phosphate dependent enzyme that occurs in virtually all organisms. It catalyzes the ineterconversion of L-aspartate and L-glutamate with the corresponding α-keto acids and plays a key role in intermediary nitrogen metabolism. Hydrazinossuccinate, a hydrazine analog of aspartate, has been shown to be a potent inhibitor of AAT. Previous studies demonstrated that acute administration of L-hydrazinosuccinate at a dose of 0.6 mmol/kg produces a strong, durable and relatively specific inhibition of cytosolic AAT in liver and kidney. Its inhibitory action in brain was also found to be significant although weaker than in liver and kidney (1). In this study, we investigated the changes in brain metabolite concentrations after intraperitoneal injection of L-hydrazinosuccinate in isoflurane anesthetized rats using *in vivo* ^{1}H magnetic resonance spectroscopy at 11.7 Tesla.

Materials and Methods

Male adult Sprague-Dawley rats (160-200 g) were anaesthetized using 1.5% isoflurane mixed with a 1:1:1 O_2/N_2/room air gas mixture. The animals were divided into two groups: control (n = 5) and L-hydrazinosuccinate treated (n = 5) ones. One artery was cannulated for blood sampling to monitor blood gases and physiological variables. One femoral vein was cannulated for administration of 8.4 % sodium bicarbonate, pancuronium, and/or 50% dextrose when necessary for adjusting physiological parameters. L-hydrazinosuccinate (0.3 mmol/kg, i.p., 99%, Rintech, Inc; Gaithersburg, MD) or equal volume of saline was injected before and after surgical preparation, respectively, in either L-hydrazinosuccinate-treated or control rats. All experiments were performed on a Bruker 11.7 T AVANCE spectrometer interfaced to an 89 mm i.d. vertical-bore magnet. The surface proton coil was positioned ~ 0-2 mm posterior to bregma and located close to the gradient isocenter. A single-shot short-TE proton MRS sequence was used for data acquisition from a 4.5 x 2.5 x 4.5 mm^3 voxel placed in the neocortex. Data were acquired three hours after the first injection of L-hydrazinosuccinate or saline. TR/TE = 2000/15 ms. For each spectrum, 256 acquisitions were averaged for a total of 8.5 min. Proton MRS data were fitted using the LCModel package. The concentration of total creatine was assumed to be 8.5 mM and used as an internal concentration reference standard**.** Unpaired two-tail t-test was used for statistical analysis.

Results

Localized *in vivo* short-TE ^{1}H spectra were shown in Fig.1. The short-TE *in vivo* ^{1}H spectrum demonstrates excellent spectral resolution and sensitivity. Using t-test, no statistically significant differences in phosphocreatine (PCr), creatine (Cr), myo-inositol (MI), taurine (Tau), phosphorylethanolamine (PE), γ-aminobutyric acid (GABA), glycerophosphorylcholine (GPC) and phosphorylcholine (PCh) concentration between the control and the L-hydrazinosuccinate treated groups were found. The concentrations of alanine (Ala, control rats: 0.26 ± 0.08 mM, L-hydrazinosuccinate-treated rats: 1.55 ± 0.42 mM, P<0.0001), glutamine (Gln, control rats: 1.86 ± 0.34 mM, L-hydrazinosuccinate-treated rats: 9.25 ± 1.48 mM, P<0.000004), and lactate (Lac, control rats: 0.99 ± 0.40 mM, L-hydrazinosuccinate-treated rats: 3.62 ± 1.24 mM, P<0.002) (Fig.2) were significantly increased resulting from L-hydrazinosuccinate treatment. In comparison, the concentration of glutamate (Glu, control rats: 10.22 ± 0.84 mM, L-hydrazinosuccinate-treated rats: 7.53 ± 1.15 mM, P<0.003) was significantly decreased (Fig.2).

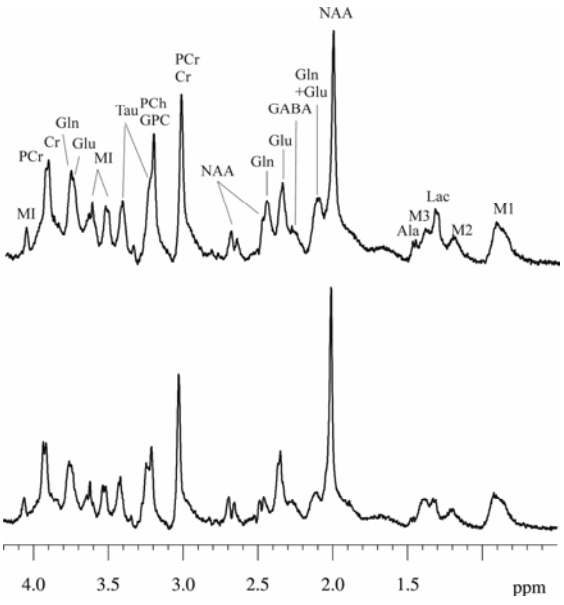

Fig.1. Comparison of localized *in vivo* ^{1}H spectra of a control rat (lower) and a L-hydrazinosuccinate-treated rat (upper).

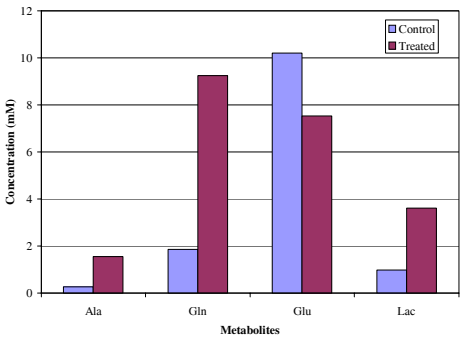

Fig.2. Effect of L-hydrazinosuccinate administration on brain metabolite levels.

Discussion

Acute systemic administration of L-hydrazinosuccinate is known to cause significant inhibition of aspartate aminotransferase in liver and kidney accompanying elevated ammonia in the liver (1). Therefore, the increased glutamine level in the brain should be due to ammonia detoxification as glutamine synthesis is the major pathway for ammonia removal in the brain. Two major sources contribute to the carbon skeleton of elevated glutamine: aneplerosis and glutamate. The decrease in glutamate concentration therefore should be due to increased conversion to glutamine. The reason for elevated alanine and lactate following acute administration of L-hydrazinosuccinate needs further investigation.

References

1. Yamada R *et al*, *Biochim Biophys Acta* 1987; 911 : 372-375.