Hyperammonemia and chronic hepatic encephalopathy: an in vivo PMRS study of the rat brain

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Abstract

The brain energy metabolism of rats affected by chronic hepatic encephalopathy due to portacaval shunting was monitored by in vivo 31P-nuclear magnetic resonance spectroscopy before and after ammonium acetate administration. With respect to healthy unoperated and to sham operated controls, portacaval shunting decreased the levels of the nuclear magnetic resonance (NMR) visible brain phosphocreatine and nucleoside phosphates, and the intracellular [free Mg2+]. Ammonium acetate induced a further decrease of the levels of the NMR detectable phosphocreatine and nucleoside triphosphates and of the [free Mg2+], while the PMR spectra of the brain of non-shunted rats did not show any significant change even after treatment with ammonium acetate. © 2000 Elsevier Science B.V. All rights reserved.

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1. Introduction

Hepatic encephalopathy (HE) is characterized by cerebral disorders and impairment of both brain metabolism and neuronal function.

A high concentration of ammonium has been found in arterial blood and in brain of HE patients and of animal models; therefore, ammonia having a direct effect on neuronal membrane and on energy metabolism [1–6], an increased level of the toxin has been regarded as an important factor in HE onset.

Nevertheless, 31P-nuclear magnetic resonance spectroscopy (PMRS) studies of brain of rats, affected by liver failure caused by acute hepatotoxic agent administration (including ammonium), showed an apparently normal high-energy phosphate profile and intracellular pH [7–9]. The PMR spectra of the brain of patients affected by HE, instead, showed altered ratios between high-energy phosphate levels [10,11].

In our study, we superimposed a high-grade acute ammonia intoxication to a chronic lower grade hyperammonemia in portacaval shunted rats [1,12–14], simulating the effects in patients with liver bypass suffering from ammonium intoxication owing either to acute gastrointestinal bleeding or a sudden increase in protein intake. This animal model and the biochemical and physiological implications for the brain have been well described by Hindfelt et al. [15] and Ehrlich et al. [16]. With this paper, we aim to give some insight into the alterations of the brain energy metabolism as have been observed in living rats by in vivo PMRS.

2. Materials and methods

2.1. Animal model

Twelve adult male Sprague-Dawley rats (weighing ca. 400 g), starved for 24 h and anaesthesitised with 2 ml/kg body weight of fentanyl/droperidol (Leptofen; Carlo Erba, Milano, Italy), underwent an end-to-side porta-caval anastomosis. After surgery, the animals were single caged, fed with standard chow and watered ad libitum.
Eight weeks later, the rats, starved for 12 h, were cannulated intraperitoneally under anaesthesia with the same anaesthetic, scalp and the temporalis muscles retracted to avoid contamination of PMR spectra from unwanted tissues.

To six of them was injected intraperitoneally a bolus (1 ml in 1 min) of 0.52 mmol/kg body weight of ammonium acetate (AmAc) in physiologic saline solution and to the other six, a tenfold higher, near lethal dose of 5.2 mmol/kg body weight of AmAc.

Six healthy rats, scalped, cannulated intraperitoneally, were regarded as absolute controls and an other group of six, scalped, cannulated intraperitoneally and sham-operated, were regarded as specific controls. The rats of the two groups were pooled in the same group of controls (12 rats) since their spectra did not differ. This finding was not unexpected since no alterations were observed in the brain of sham-operated rats [15–17].

Moreover, in the measurements, each rat served as its own control since the data acquired after the injection were compared with the data acquired prior to the injection from the same rat.

To the rats of the control group, when submitted to the PMRS, was injected a bolus of sodium acetate (NaAc) to mimic the experimental stressing conditions of the animals of the shunted-and-treated groups, and then treated with AmAc to check its effect in the non-shunted animals.

The acquisition of rat brain PMRS spectra started 12 min before the bolus injection, immediately after the ammonium (or sodium) acetate injection and every 12 min thereafter. Thus, the spectra were collected 4 min before the AmAc (or NaAc) injection, 8 min after the injection and every 12 min thereafter. Each experiment lasted a total time of 80 min.

An intraperitoneal catheter allowed the bolus infusion without changing the rat positioning during the MRS measurements. The animals, during the spectral acquisition, were spontaneously breathing and their temperature was kept constant with a thermal blanket. At the end of the measurement sessions, the rats were sacrificed with the same anaesthetic used for the sedation.

No blood ammonium level has been measured to avoid an additional stress to already stressed animals and disturbances of the instrumental conditions. On the other hand, the time course of both the blood and brain ammonium levels in the same animal models has been already reported in the literature [15,16].

The experimental protocols were approved by the Institutional Ethic Committee, and the animal handling was in accordance with the recommendations of the Helsinki Declaration and of the WHO Advisory Committee on Medical Research—Council for the International Organisation of Medical Sciences.

2.2. Instrumental set-up

PMRS was performed on a Varian-SIS Imager/Spectrometer (Palo Alto, CA, USA) operating at 200 MHz for proton and 81 MHz for phosphorus resonance. The instrument was equipped with an Oxford 4.7 T, 30 cm bore, horizontal magnet and with a SUN 3/330 GX computer (Sun Microsystems, Mountain View, CA, USA) for the instrumental control and the data acquisition. A 15 mm external diameter home-made two-turn surface coil was placed on the top of the rat exposed skull. The shimming was adjusted on the brain water resonance to a line width of 20–30 Hz. The pulse width, the pulse amplitude (estimated flip angle about 60° at the coil centre) and the interpulse delay (0.9 s, inclusive of the acquisition time) were selected to get the best possible signal intensity without saturation effects due to partial relaxation, excluded by comparison of the actual spectra with the fully relaxed spectra (10 s interpulse delay). The spectra were collected without proton broad band decoupling to avoid intensity bias of the peaks due to NOE. Each spectrum lasted 8 min and 8 s, allowing a time resolution compatible with the experimental time course.

Before every experimental session, the spectrometer was calibrated making use of a vial containing sodium ortho-phosphate (10 mM) and triethylphosphate (10 mM) in physiological saline solution buffered at pH 7.1 (at 25°C) with Tris. Thus, the experimental conditions were kept the same for all measurements, allowing the direct comparison between spectra of the brain of different animals, or run in different experimental sessions, without (or, at least, with negligible) instrumental or methodological error.

The acquired data were monitored on-line with the spectrometer interfaced computer and post-processed off-line on a SUN SPARC 2 and on a SUN ULTRA 5 workstations. The standard VARIAN-SIS software (VNMR 5.2f), running under SUN OS 4.1.3, Solaris 2.5.3 and Solaris 7 were used.

2.3. Spectral manipulation and data handling

The spectra were the result of 512 free induction decays collected with a spectral width of 8 kHz and 8 K points, interpolated to a Fourier number of 16 K. A line broadening of 10 Hz was then applied to the FIDs before the fast Fourier transform.

The large spectral hump, more than 2 kHz, supposedly arising from the immobilised calcium phosphates of the skull bone and from the low mobility brain phospholipids, was removed by interpolation of the base line with a spline function to allow reliable peak integration. The spectra were displayed and evaluated in absolute intensity mode [18], i.e. the spectra were scaled having as intensity reference the noise level, thus allowing direct comparison among spectra.