RESEARCH COMMUNICATION

Decrease in cerebral inositols in rats and humans

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Rats with portacaval shunts and humans with hepatic encephalopathy show severe *myo*-inositol depletion in the brain. The portacaval-shunted rat may therefore be a useful model for the

investigation of neurochemical pathways containing *myo*-inositol, which are modulated not only in hepatic encephalopathy but also in diabetes mellitus and Alzheimer's disease.

INTRODUCTION

myo-Inositol, an intermediate in the cerebral inositol polyphosphate (IPP) cascade [1,2], is normally present at about 5 mM in the human brain and has recently been detected in non-invasive proton [3,4] and ^{13}C magnetic resonance (m.r.) spectra [5]. In humans, *in vivo* proton magnetic resonance spectroscopy (^1H m.r.s.) of the brain shows that a significant increase in the *myo*-inositol content is a feature of Alzheimer's disease [6] and diabetes mellitus [7] and, in the neonatal brain, the *myo*-inositol concentration is 250 % of the normal adult level [8,9]. Conversely, a significant reduction in cerebral *myo*-inositol is observed in *in vivo* ^1H m.r.s. in patients with chronic hepatic encephalopathy (HE) [10]. In Alzheimer's disease, it has been postulated that phosphatidylinositol depletion [11] and abnormalities in inositol phosphokinase activity [12] are part of a general neurochemical disturbance of the IPP pathway. An animal model for the study of the factors that modulate cerebral *myo*-inositol metabolism, therefore, is of interest. In the past, rats with portacaval shunts (PCS rats) have served as a model for HE in humans, but a deficiency in cerebral *myo*-inositol has not been reported [13,14] in these animals.

EXPERIMENTAL

Our present study used ^1H m.r.s. and h.p.l.c. to identify the nature of the metabolites affected in brain extracts from rats with PCS and from patients who had died of hepatic coma.

Animals

Male rats (Sprague–Dawley), given free access to food and drink, and weighing 200–250 g, were used. The whole forebrains of ten PCS rats (Zivic Miller) and ten control rats, which had been anaesthetized with intraperitoneal Nembutal (50 mg/kg) and killed 6–8 weeks after surgery, were used in this study. Two additional controls were: (1) a PCS rat which had undergone surgery but in which the shunt had been closed before the animal was killed; and (2) a normal rat which underwent anaesthesia and surgery, but was then allowed to recover, until being killed 6 weeks later. To obtain the brain in as near an *in vivo* state as possible, animals were anaesthetized with intraperitoneal

pentobarbitone and the skull was opened. The whole brain was rapidly excised and immersed in liquid nitrogen.

Patients

The brains from two cirrhotic patients who had died in hepatic coma and from three patients without liver disease who had died of pulmonary embolism, respiratory collapse and myocardial infarction respectively were obtained at autopsy. Samples from the frontal, parietal and occipital lobes were dissected, frozen in liquid nitrogen and stored at -70°C . The interval from death of the subject to freezing of dissected autopsy material was between 12 and 24 h.

For both animal and patient studies, the weighed cerebral tissue was homogenized in 6 % perchloric acid, neutralized with 30 % KOH, but without addition of pH indicator, which interferes with both h.p.l.c. and m.r. assays, and passed through a Sep-Pak C18 cartridge (Millipore, Milford, MA, U.S.A.) H.p.l.c. was performed as described previously by Lien et al. [15].

M.r.s.

^1H m.r.s. was performed on rat and human brain HClO_4 extracts using a Bruker AMX-500 n.m.r. spectrometer. HClO_4 extracts were either directly analysed after the addition of $^2\text{H}_2\text{O}$ containing 5 mM 3-(trimethylsilyl)-1-propanesulphonic acid (DSS) or evaporated to dryness and dissolved in $^2\text{H}_2\text{O}$ containing 5 mM DSS. The acquisition, with presaturation to suppress the water resonance when needed, consisted of 128 scans with a 5.0 s relaxation delay and a 16 K data set. The data processing consisted of an exponential line-broadening of 0.05 Hz using the program Felix 2.05 (Hare Software, Inc.) with a baseline correction using a spline fitting procedure.

RESULTS

Figure 1(a) shows representative h.p.l.c. chromatograms of brain extracts from a PCS rat and a normal rat. The peak areas of *myo*-inositol and glycerophosphocholine (GPC) were decreased, and that of glutamine was increased in the PCS rat compared with the control. In 10 PCS rats, *myo*-inositol and GPC decreased by an average of 40 % and 33 % respectively, and the brain glutamine content increased by 98 % when compared with 10 normal rats

Abbreviations used: IPP, inositol polyphosphate; m.r.s., magnetic resonance spectroscopy; HE, hepatic encephalopathy; PCS rats, rats with portacaval shunts; DSS, 3-(trimethylsilyl)-1-propanesulphonic acid; GPC, glycerophosphocholine.

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Figure 1 Representative h.p.l.c. chromatograms of brain extracts from rats (a) and humans (b)

The upper traces are of a PCS rat (a) and a hepatic coma patient (b); the lower traces are from their respective controls. The peaks of *myo*-inositol (MI), taurine (Tau), GPC, glutamine (Gln), glutamate (Glu), glycine (Gly) and creatine (Cr) are indicated.

($P < 0.01$) (Table 1). The elevation of cerebral glutamine is well known from prior work [16]. Concentrations of other metabolites, including taurine, urea, creatine and glutamate, were not statistically different between the two groups. In the surgical controls ($n = 2$), metabolite concentrations were indistinguishable from those in the normal rats. In particular, *myo*-inositol and glutamine were within the normal range (first control: Gln = 5.62 mM, GPC = 0.69 mM, *myo*-inositol = 5.31 mM; second control: Gln = 3.58 mM, GPC = 0.47 mM, *myo*-inositol = 5.40 mM).

Figure 1(b) shows representative h.p.l.c. chromatograms of human brain extracts from a patient with hepatic coma and a control patient. In patients with hepatic coma, the peak areas of *myo*-inositol and GPC were decreased, and that of glutamine was increased compared with the control. The peak areas of taurine, urea and creatine were also increased in the hepatic coma patients (not confirmed by m.r.s.). The chemical changes were comparable between frontal, parietal and occipital cortex (results not shown) from the same patient. The results of six measure-

Table 1 Brain contents of organic metabolites measured by h.p.l.c.

Glutamate was measured enzymically. Numbers of rats or patients (n), or numbers of brain sections in humans (s) is shown. Values are means $\pm$ S.D. * $P < 0.01$ versus control rats. See Figure 1 for explanation of extraction procedures.

Metabolite	Content (mmol/kg wet wt.)			
	Rats		Humans	
	Control ($n = 10$)	PCS ($n = 10$)	Control ($n = 3$; $s = 6$)	Hepatic coma ($n = 2$; $s = 6$)
<i>myo</i> -Inositol	5.8 ± 0.7	$3.5 \pm 0.7^*$	6.0 ± 0.9	$3.3 \pm 0.4^*$
Taurine	8.3 ± 0.5	7.5 ± 1.3	4.4 ± 0.8	$5.8 \pm 0.9^*$
GPC	0.6 ± 0.1	$0.4 \pm 0.1^*$	0.7 ± 0.2	$0.2 \pm 0.2^*$
Urea	2.6 ± 0.5	2.2 ± 0.9	2.9 ± 0.4	$16.9 \pm 5.1^*$
Glycine	0.7 ± 0.2	0.8 ± 0.2	1.0 ± 0.6	0.8 ± 0.3
Glutamine	4.2 ± 0.4	$8.3 \pm 2.5^*$	4.1 ± 1.5	$14.7 \pm 1.9^*$
Creatine	7.0 ± 0.9	6.4 ± 1.0	6.6 ± 1.0	$12.4 \pm 1.8^{\dagger}$
Glutamate	7.5 ± 0.7	7.6 ± 1.5	8.6 ± 0.8	8.0 ± 0.9

$\dagger$ The creatine findings were not confirmed by m.r.s.

ments from two hepatic coma patients (three brain sections each) and from three control patients (two sections each) are summarized in Table 1. In patients who had died in hepatic coma, cerebral *myo*-inositol and GPC contents were decreased by 45 % and 71 % respectively, and glutamine and taurine concentrations were increased 259 % and 32 % respectively. Glycine, which contributes to the *myo*-inositol peak in *in vivo* ^1H m.r.s., was not decreased in PCS rats (or significantly, in patients who had died in hepatic coma), and this cannot, therefore, be an explanation of the reduced *myo*-inositol peak intensity found *in vivo* [10].

Representative ^1H m.r. spectra of brain extracts from a PCS rat and a normal rat are shown in Figure 2(a). In addition to the dramatic increase in glutamine peak areas, the choline region and *myo*-inositol peaks were decreased in PCS rats compared with controls. The ^1H m.r. spectra from the occipital lobe of an HE patient and a 'control' are distinguished by decreased *myo*-inositol peaks, a decrease in the choline region and an increase in glutamine peaks (Figure 2b). Because the occipital cortex is a location that we have analysed by *in vivo* ^1H m.r.s. in chronic HE patients, these spectra directly confirm our *in vivo* results.

DISCUSSION

The role of *myo*-inositol in the central nervous system is rather complex. First, *myo*-inositol is the precursor of IPPs, which are important secondary messengers involved in a large number of hormonal systems in the central nervous system [17]. Secondly, *myo*-inositol content in nerve cells is related to sodium pump activity and nerve conduction velocity, as shown in diabetic peripheral neuropathy [18]. *myo*-Inositol depletion may contribute to the neurological manifestation of HE. In human subjects, *myo*-inositol depletion is reversed by liver transplantation [10], and neurological status is also improved.

The mechanisms of *myo*-inositol depletion in patients with chronic HE, or in PCS rats, are largely unknown. *myo*-Inositol can be synthesized in the brain from glucose or transported into the brain via specific transporters [19,20]. In HE, hyperammonaemia is associated with decreased glucose utilization in the brain [21], and may therefore cause *myo*-inositol depletion by reducing *myo*-inositol synthesis. Alternatively, since *myo*-inositol is the precursor of glucuronic acid via *myo*-inositol oxygenase, its

Figure 2 Representative ¹H m.r. spectra of brain extracts from rats (a) and humans (b)

The lower spectra are of a PCS rat (a) and the occipital lobe of a hepatic coma patient (b); the upper spectra are their respective controls. The extracts are the same as those described in the legend to Figure 1 (five PCS rats and five controls were used in the m.r.s. study). Resonances of *myo*-inositol (ml), taurine (Tau); choline region (Cho; which includes choline, GPC and diglycerophosphocholine), glutamine (Gln), glutamate (Glu), glycine (Gly), creatine (Cr) and *scyllo*-inositol (sl) are indicated. NAA, *N*-acetylaspartate. (a) The spectra are plotted by normalizing the intensity of the Cr peak at 3.02 p.p.m. with a small correction needed to compensate for extract dilution factors. (b) The spectra are plotted by normalizing the intensity of the peak at 2.9 p.p.m. and correction for extract dilution factors. Note the separation of the glycine and *myo*-inositol resonances, with the area of the relatively small glycine singlet clearly only slightly decreased in the patient compared with the control, and therefore this cannot be responsible for the decrease in this region observed in the *in vivo* spectra of HE patients.

depletion might be the result of the excessive cerebral detoxification that is necessary in patients or experimental animals with long-term portasystemic shunts. This hypothesis also implies that *myo*-inositol depletion contributes to the observed sensitization of the brain to other neurotoxins in HE. Because both glutamine and *myo*-inositol can be viewed as contributing to intracellular osmotic pressure, the reduced *myo*-inositol concentration might simply result from the increased intracellular osmolarity due to accumulation of glutamine observed in the presence of hyperammonaemia [15]. Such a simple explanation was considered unlikely in the setting of human HE (see Fig. 5 in ref. [10]). In Alzheimer's disease, the concomitant alteration in inositol phosphokinase and depletion of inositol phosphate may indicate altered regulation of the biosynthetic pathway of *myo*-inositol.

We have demonstrated cerebral *myo*-inositol and GPC depletion in both PCS rats and patients with hepatic coma. These findings are consistent with our previous *in vivo* m.r.s. studies in

patients with chronic HE. Any hypothesis for the pathogenesis of HE must take into account this decrease in *myo*-inositol. The PCS rat model can be used to explore the role of *myo*-inositol (and IPPs) in neurology and, while we do not suggest any aetiological link between Alzheimer's disease and chronic HE, it may also provide a model for pharmacological manipulation that targets *myo*-inositol in Alzheimer's disease [6].

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