Hippocampus is sensitive to hypoxia ischemia in neonatal rat: an *in vivo* magnetic resonance spectroscopy study

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Introduction

Hypoxic-ischemic injury is a common cause of brain injury during the perinatal period, which frequently results in mortality and abnormal neurodevelopmental outcomes that range from mild learning disabilities to profound disability including mental retardation[1,2]. A key requirement for developing efficient therapies is to identify alterations which occur during ongoing damage that continues to develop over hours to weeks after the initial brain insult[3]. The use of animal models of neonatal hypoxia-ischemia (HI) that show injury patterns similar to those seen in human newborns can greatly facilitate the process of identifying promising therapies[4]. To date, there have been no studies using *in vivo* high resolution localized proton MR spectroscopy to follow sequentially the metabolism changes up to 28 days in brain on rat model of neonatal HI. The present ^{1}H MRS study aims to evaluate the brain regional metabolic alterations from the moderate neonatal HI survivors at 24 hours, 72 hours, 7 days, and 28 days of recovery.

Materials and Methods

The Rice-Vannucci model[5] of HI on Sprague–Dawley rat pups was used in the study (Control: 6 male and 6 female; HI: 6 male and 6 female). The model used a combination of unilateral common carotid artery ligation with hypoxia. The right internal carotid artery was ligated and cut while the animals were under isoflurane anesthesia on postnatal day 7. Transient hypoxia consisted of 75 min of 8% O2 in a warmed, humidified chamber as described in the Rice-Vannucci method[5]. The pups then recovered at 37°C in room air for 2 hours prior to return to the dam.

In vivo MRS experiments were performed on a BrukerBioSpec 7T MR scanner. A Bruker four-element ^{1}H surface coil array was used as the receiver and a Bruker 72 mm linear-volume coil as the transmitter. The rat was anesthetized in an animal chamber using medical air (1 L/min) and isoflurane (2.5 - 3 %) then later maintained at 1-1.5% isoflurane during scanning. An MR compatible small-animal monitoring and gating system was used to monitor the animal respiration rate and body temperature. The animal body temperature was maintained at 36-37°C using a warm water circulation. A customer-modified Point-RESolved Spectroscopy (PRESS) pulse sequence[6] (TR/TE = 2500/10 ms, NA = 300) was used for MRS data acquisition from four regions including left (contralateral) and right (ipsilateral) cortex (1.5 x 3.5 x 3.0 mm^3), left and right hippocampus (2.0 x 3.5 x 2.5 mm^3). The unsuppressed water signal from the prescribed voxel was collected as a metabolite concentration reference for each scan. Each rat pup was scanned at 4 time points followingHI procedure: 24 hours (24 hr), 72 hours (72 hr), 7 days (7 d), and 28 days (28 d). All animal procedures were approved by the Institutional Animal Care and Use Committee at the University of Maryland School of Medicine. Quantification of the MRS was based on frequency domain analysis using LCModel package[7]. All concentrations were expressed as mean ± standard error of the mean (SE). The criteria to select the reliable metabolite concentrations were based on the Cramer-Rao lower bounds (CRLB), which are determined by LCModel. The metabolites that had a CRLB ≤ 25 % in more than 90 % of the data sets were included for further statistical analysis, except for Lac which may have high CRLB values. Statistical analyses were performed for each metabolite in each region of the two groups and at four time points using two-way repeated analysis of variance (ANOVA). Post hoc test was done by multiple comparison corrections using Tukey test. Statistical significance was defined as $p < 0.05$.

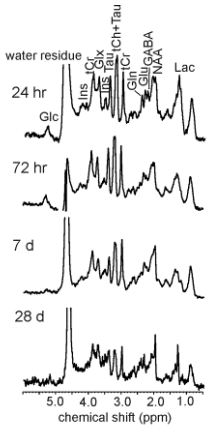

Fig1. In vivo ^{1}H MRS from ipsilateral hippocampus of a HI rat pup at 4 different time points. γ-aminobutyric acid (GABA), glucose (Glc), glutamine (Gln),glutamate (Glu), glutathione (GSH), glutamine+glutamate (Glx), creatine+phosphocreatine (tCr), glycerophosphorylcholine+ phosphorycholine (tCho),lactate (Lac), myo-inositol (Ins), N-acetylaspartate (NAA), and taurine (Tau).

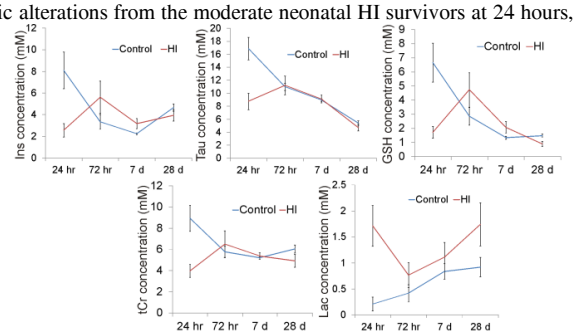

Fig 2. Metabolite concentrations of Ins, Tau, GSH, tCr and Lac measured from ipsilateral hippocampus of control (n=12) and perinatal HI (n=12) rats at 4 differnet time points. All values are expressed as mean± standard error.

	Group x Time		Post hoc test	
	F (6,22)	p	p (24 hr)	p (28 d)
GSH	7.991	**<0.001**	**<0.001**	0.525
Ins	7.411	**<0.001**	**<0.001**	0.364
Lac	2.732	**0.05**	**<0.001**	**0.03**
Tau	9.009	**<0.001**	**<0.001**	0.579
tCr	6.629	**<0.001**	**<0.001**	0.307

Table 1. Statistical comparisons of metabolites in ipsilateralhippocampus of control and neonatal HI rats

Results

Fig 1 shows representative spectra of one HI rat in the ipsilateral hippocampus. Major temporal changes were found in the ipsilateral hippocampus of the HI rats including a 1) severe oxidative stress (significant GSH reduction); 2) severe alterations in brain osmolytes (significant Tau and Ins reductions); 3) impaired oxidative phosphorylation and consequent reliance on anaerobic glycolysis (significant tCr reduction and Lac elevation) (Fig 2 and Table 1). No statistically significant metabolic alterations were found in the other three regions.

Discussion

Hippocampus is a highly plastic structure that is susceptible during various insults, especially when the injury occurs during early stages of development[8]. The first 2 weeks after birth in rats signifies the period of rapid brain growth and correspond to "brain growth spurt" in humans extending through last trimester of pregnancy to early postnatal period[9]. During this period, the developing hippocampus passes simultaneously through the phases of extensive development and maximum differentiation and these ongoing processes influence the vulnerability towards a number of insults[10]. In the rat hippocampus, the pyramidal cells, a main neuronal population in the region, are generated prenatally which undergo differentiation during early postnatal period. In contrast, the granule cells, another main neuronal population in the dentate gyrus of hippocampus, develop during the first three weeks after birth and continue to be generated throughout postnatal and adult life. The results of this study provide evidence that the ipsilateral hippocampus of the neonatal rat is sensitive to HI injury. The severe oxidative and osmotic stress coupled with impaired oxidative phosphorylation and consequent reliance on anaerobic glycolysis in the ipsilateral hippocampus may alter the rate of neuronal generation and/or differentiation inducing a cascade of deficits in learning ability.

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References

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