Hippocampal neurotransmitter imbalance and the role of GS in epileptogenesis; A longitudinal *in vivo* MRS and histochemical study in a juvenile model of temporal lobe epilepsy.

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Introduction

The pathophysiology of temporal lobe epilepsy (TLE) is poorly understood. Proposedly, childhood status epilepticus (SE) initiates an epileptogenic process, which, after a latent phase of several years, leads to spontaneous seizures originating in the hippocampus. Surgical specimens from the hippocampus show sclerosis, characterized by neuronal loss and gliosis. A change in the balance between excitatory (glutamatergic) and inhibitory (GABAergic) neurotransmission is also considered to be important in this process[1]. In addition, glutamine synthetase (GS), an important glial enzyme in the metabolism of glutamate and GABA, is reduced in patients and animals suffering from TLE[2]. How all these factors develop during epileptogenesis and what they contribute to this process, can be studied in the latent phase of the juvenile lithium pilocarpine model. In this model SE is induced in juvenile rats, which develop spontaneous seizures after 8-12 weeks. In this study we compared changes in neurochemical and transmitter related amino acid levels using ^{1}H MRS with changes in neuronal and glial markers during pilocarpine-induced epileptogenesis in the rat.

Materials and Methods

Twenty-day old male Wistar rats were injected with 3 mEq/kg of lithium i.p., followed 20 hours later by an injection of 1 mg/kg scopolamine and 40 mg/kg pilocarpine to induce SE[3]. After one hour of SE, animals were sedated with diazepam (4 mg/kg i.p.). Age-matched controls were injected with saline. MRS and MRI were performed at 4 weeks (n=5) and 8 weeks (n=5) after induction of SE using a 9.4 T Magnex magnet equipped with a 9 cm diameter gradient insert (500 mT/m, 165 µs). Rats were anesthetized and ventilated with halothane in N_2O/O_2 (70:30). A hippocampal voxel (10x2x5 mm^3) was selected in multislice EPI images (Fig. 1A). Volume selection was achieved using ISIS with slice-selective excitation and outer volume suppression. Four CHESS pulses were used for water suppression. GABA was selectively detected using a double spin-echo sequence (TR 4 s, TE = $1/2J_{GABA}$ = 68ms, 64 scans) with editing pulses selective for inversion of GABA 3-CH_2 (1.9 ppm) while detecting GABA 4-CH_2 (2.99 ppm). Spectra were quantified using a dedicated version of LCModel.

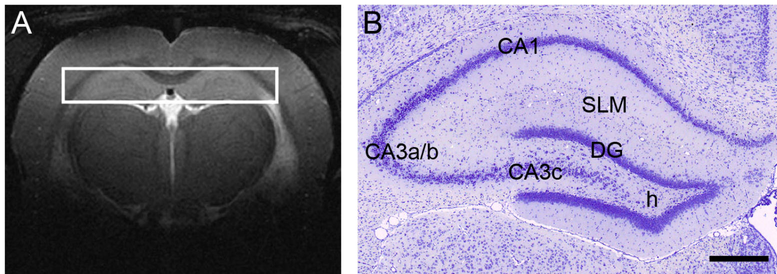

Figure 1: A. T_2 Weighted image of a healthy saline-injected control animal at the 8-week time point with the MRS voxel indicated. B. Nissl stain of the left hippocampus of a control animal at the 8-week time point. Structures most affected in pilocarpine treated rats are indicated: DG = dentate gyrus, h = hilus and SLM = Stratum Lacunosum-Moleculare. Bar = 500µm

For histology, animals were sacrificed with 300 mg/kg pentobarbital i.p. and perfused with paraformaldehyde at 4 (n=6) and 8 weeks (n=6). Brains were dissected, embedded in paraffin and sectioned transversally at 7 µm. General histology was assessed after Nissl staining (Figure 1B). FluoroJade (FJ) staining was performed to analyze neuronal damage. Immunohistochemical staining was performed using specific antibodies against vimentin, to detect reactive glia, against parvalbumine (PV) to detect a subpopulation of GABAergic interneurons, and against GS. Data were analyzed visually by two independent observers. Vimentin staining was scored as present or absent. PV positive neurons were counted in the dentate gyrus (DG) and hilus of the right hippocampus of each animal. Semi-quantification of GS immunoreactivity (IR) was performed using computerized densitometry. No seizures were detected before the MR and histochemical evaluations. All experimental procedures were approved by the local institutional animal care committees.

Results

Results are summarized in Table 1. Typical unedited ^{1}H MR spectra are shown in Figure 2. In the pilocarpine injected animals levels of NAA, glutamate (Glu) and GABA were decreased, while total choline (Cho) was increased compared to controls. GABA levels decreased progressively over time and were more severely affected at 8 weeks. Glutamine (Gln) was significantly increased at 4-weeks but levels were normalized at 8 weeks. FluoroJade (FJ) staining of epileptogenic hippocampi showed damaged neurons, with reactive glia, demonstrated by vimentin (Vim) immunohistochemistry. Both parvalbumin (PV) and GS staining decreased progressively with time, most prominently in the hilus of the DG.

MRS	4w	8w
NAA	-12%	-12%
Cho	15%	15%
Gln	36%	=
Glu	-15%	-17%
GABA	-29%	-41%
Stain		
FJ	+	+
Vim-IR	++	+
PV-IR	-46%	-68%
GS-IR	-11%	-32%

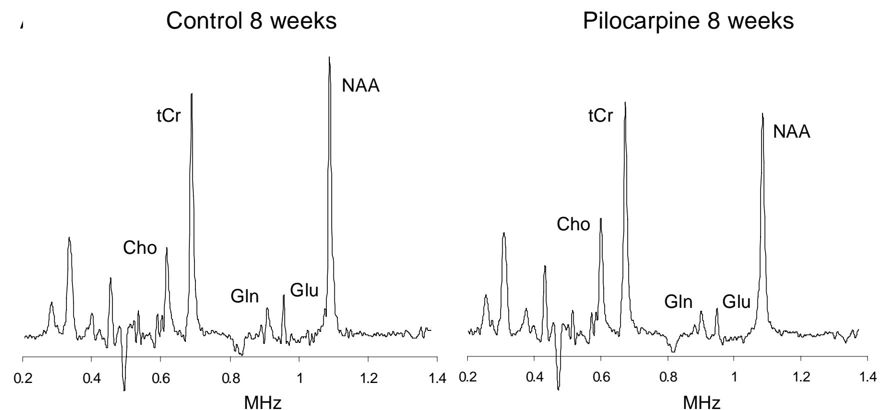

Figure 2: Typical unedited spectra, normalized for creatine, for a control and experimental animal 8 weeks after injection of pilocarpine. Note the decreased level of NAA in the epileptic animal compared to control..

Discussion

The decrease in NAA and increase in Cho suggests neuronal death and gliosis, which was confirmed by the positive FJ and Vim stain. The decreased levels of glutamate and GABA suggested that both glutamatergic and GABAergic neurons were affected, although the greater reduction in GABA is consistent with the hyper-excitable state. We hypothesize that the rise and subsequent normalization of glutamine levels may reflect reduced synthesis of GABA and glutamate in conjunction with adaptative changes in GS.

Conclusion

In this rat model of TLE we detected changes in relevant ^{1}H MRS markers, before spontaneous seizures occurred. These findings were validated with dedicated immunohistological experiments and represent early indicators of imminent epilepsy. The current availability of these MRS techniques in the clinical settings may be useful in the pre-symptomatic diagnosis of human TLE.

References: **1.** Sagar,H.J. *et al.* Ann. Neurol., 22 (1987) 334-340, **2.** Van der Hel W.S. *et al.* Neurology, 64 (2005) 326-33, **3.** Turski,L., *et al,* Synapse, 3 (1989) 154-171.