In vivo GABA edited ^{1}H MRS and dynamic ^{13}C MRS of neurotransmitter metabolism during epileptogenesis in lithium-pilocarpine induced epilepsy in the rat

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Introduction

In the mammalian brain GABA and glutamate are the most abundant inhibitory and excitatory neurotransmitters respectively. Not surprisingly they are involved in epileptogenesis. Injection of lithium and pilocarpine induces status epilepticus (SE) in the rat. This will eventually lead to spontaneous seizures after a latent phase during which animals show no clinical symptoms or signs. *Ex vivo* determination of GABA and glutamate concentration during SE shows that both increase in the hippocampus. During the latent phase GABA shows a substantial decrease, while the increase in glutamate persists. In the chronic phase, an increase in GABA and glutamate was found[1]. The possibility to monitor changes in GABA and glutamate concentration *in vivo* would greatly enhance our ability to longitudinally investigate epileptogenesis in experimental and clinical temporal lobe epilepsy. Also, the metabolic rates of these neurotransmitters in epilepsy have not yet been investigated. Therefore our aim is to measure hippocampal concentrations and turnover of GABA and glutamate during epileptogenesis in the lithium pilocarpine model with *in vivo* GABA edited ^{1}H MRS and indirect ^{13}C MRS after injection of [U-$^{13}C_6$]-glucose.

Methods

21-day-old male wistar rats were injected with lithium (3 meq), 24 hours prior to induction of SE with scopolamine (1 mg/kg) and either pilocarpine (40 mg/kg) or saline. Four and eight weeks after SE, animals were anesthetized, intubated by tracheotomy and ventilated with 0.5% halothane in a mixture of N_2O and O_2 (30/70). The femoral artery and vein were cannulated for monitoring of pCO_2, pO_2, blood pressure and infusion of [U-$^{13}C_6$]-glucose (only after 8 weeks). During MR experiments, animals were restrained in a head holder and immobilized with d-tubocurarine chloride. Experiments were performed on a 9.4 T Magnex magnet and Bruker console equipped with a 9 cm diameter gradient coil insert (500 mT/m, 165 µs). A 14 mm diameter surface coil was used for ^{1}H RF pulse excitation and signal reception, while two orthogonal 21 mm-diameter surface coils were used for ^{13}C spectral editing and decoupling. 3-D localization for an hippocampal voxel (2.5x10x4mm) was achieved by a combination of OVS, ISIS and slice-selective excitation. Adiabatic pulses were used for water suppression. The MRS and infusion protocol has extensively described previously[2]. Selective GABA detection was achieved with a double spin-echo sequence using refocusing pulses selective for GABA-H3 at 1.89 ppm (Fig 1). The GABA H_4 peak was quantified by integration in the edited ^{1}H MR spectra and referenced to total creatine (10mM). All other spectra were analyzed with a dedicated version of LCmodel. Turnover data will be modeled with a four compartment metabolic model, comprising glutamatergic and GABAergic neurons, astroglia and blood compartments.

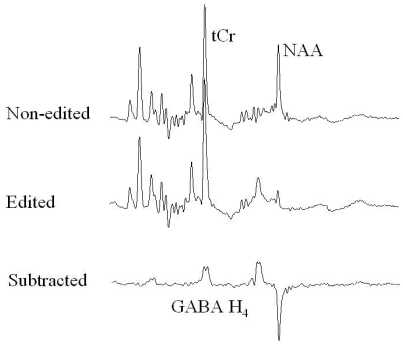

Fig 1: GABA edited spectra

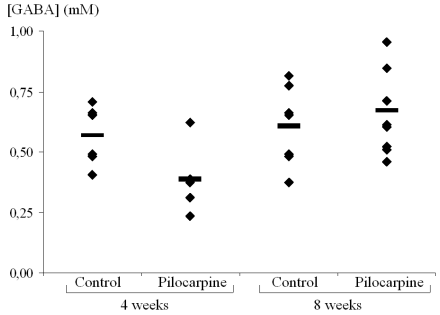

Fig 2: [GABA] in controls and experimental animals After 4 and 8 weeks

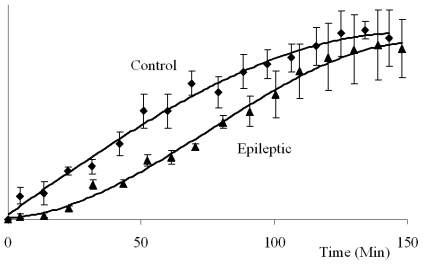

Fig 3: 4-^{13}C GABA turnover for controls and experimental animals

Results

All animals injected with pilocarpine showed SE following a known sequence of pathological symptoms[1], while none of the saline injected animals showed abnormal behavior. Edited ^{1}H MRS showed a 32% decrease (p=0.029) in GABA concentration in the hippocampus four weeks after pilocarpine injection (n=5 vs. 5). Only one animal in the 4 week experimental group is within the range of the control group. After 8 weeks GABA concentrations had returned to normal levels (n=7 vs. 7) (Fig 2). Dynamic indirect ^{13}C detection using ^{1}H-[^{13}C] MRS revealed differences in the [4-^{13}C] GABA turnover curves for controls and experimental animals after 8 weeks (Fig 3). [4-^{13}C] glutamate and [4-^{13}C] glutamine curves were also altered in the epileptic compared to control animals. Results of these experiments are currently being evaluated and analyzed in full detail.

Discussion

The sharp drop in GABA concentration in pilocarpine injected animals and its return to normal values is in agreement with previous *ex vivo* research[1]. This decrease may be due to massive and selective death of GABAergic cells, although changes in GABA synthesis is also likely[3]. During the latent phase compensatory mechanisms develop in reaction to the epileptic damage. These mechanisms may cause GABA to increase again, but are also responsible for the development of the eventual epilepsy, by forming aberrant recurrent loops. Our ^{13}C turnover studies show that despite a normalization of absolute GABA concentrations at 8 weeks after SE, GABA metabolism is by no means normalized.. We have indications also that glutamate and glutamine metabolism are compromised. Detailed results of these studies will be presented and discussed.

Conclusion

We show that changes in inhibitory and excitatory neurotransmitter concentrations and metabolism during epileptogenesis can be probed non-invasively with MRS in an animal model for temporal lobe epilepsy. Even in the silent phase, before actual spontaneous seizures occur, GABA is shown to decrease, with a subsequent normalization, although GABA turnover remains compromised. Furthermore, methods can potentially be used to monitor epileptogenesis in a population at risk, like children with febrile convulsions.

References

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