Epileptogenesis: Brain Structure and Metabolism during the Latent Period following Kainate-induced Seizures in Rats

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INTRODUCTION

Human temporal lobe epilepsy (TLE) is often characterized by hippocampal sclerosis (HS), consisting of extensive neuronal loss and gliosis. MR imaging is a routine tool in TLE diagnosis[1], but few reports have correlated MRI findings and 13C-NMR-spectroscopy (NMR-S) analysis of intermediary metabolism in the kainite (KA) model of TLE. N-acetylaspartate (NAA) is reduced in epileptogenic hippocampus affected by HS[2], and serves as a marker of either neuronal loss or impaired mitochondrial metabolism. In addition, studies have revealed perturbed glutamate homeostasis, with extracellular glutamate overflow prior to and during seizures[3] and reduced cycling of glutamate and glutamine between neurons and glia in human hippocampus[4]. Animals injected with KA, a glutamate analogue and a potent convulsant, are commonly used as an experimental model of human TLE. At sufficient doses KA induces status epilepticus (SE) in rats, followed by a latent period preceding the onset of spontaneous recurrent seizures. To elucidate the mechanisms underlying epileptogenesis, we investigated intermediary metabolism in the latent period, taking into account the hippocampal structural damage.

METHODS

Male Sprague Dawley rats (~250g) were injected intraperitoneally (i.p.) with KA (10 mg/kg body weight). 14d after KA injection [1-13C]glucose and [1,2-13C]acetate were injected i.p. and the rats killed 15min later. Hippocampus and cortex were dissected and extracted. Magnetic resonance imaging (MRI) scans were obtained 1d before, 1d after and 14d after kainate induced SE (n=3) on a 2.35 T Bruker Biospec (Bruker Biospin, Germany). A T2-weighted MRI sequence (RARE) was used, with consecutive 1mm thick slices, 4.5x4.5cm FOV, 176x176µm in-plane spatial resolution, 65ms effective echo time and a total acquisition time of 33min. Maps of the Apparent Diffusion Coefficient (ADC) were produced from the same image slices, using b-values 0, 25 and 500 s/mm^2, TE=60ms, 704x704µm in-plane resolution, total acquisition time 38min. Analyses of brain extracts were performed using 1H- and 13C-NMR-S according to Kondziella et al[5] (n=6-7). Briefly, proton decoupled 125.5MHz 13C NMR spectra were obtained on a Bruker DRX-500 spectrometer (Bruker). Samples were re-dissolved in D_2O containing ethylene glycol 0.1% as an internal standard. Spectra were accumulated using a 35deg pulse angle, 25kHz spectral width with 64k data points. The acquisition time was 1.3s, and a 2.5s relaxation delay was used. The number of scans was typically 10,000. Correction factors for nuclear Overhauser effects were applied to all spectra.

RESULTS AND DISCUSSION

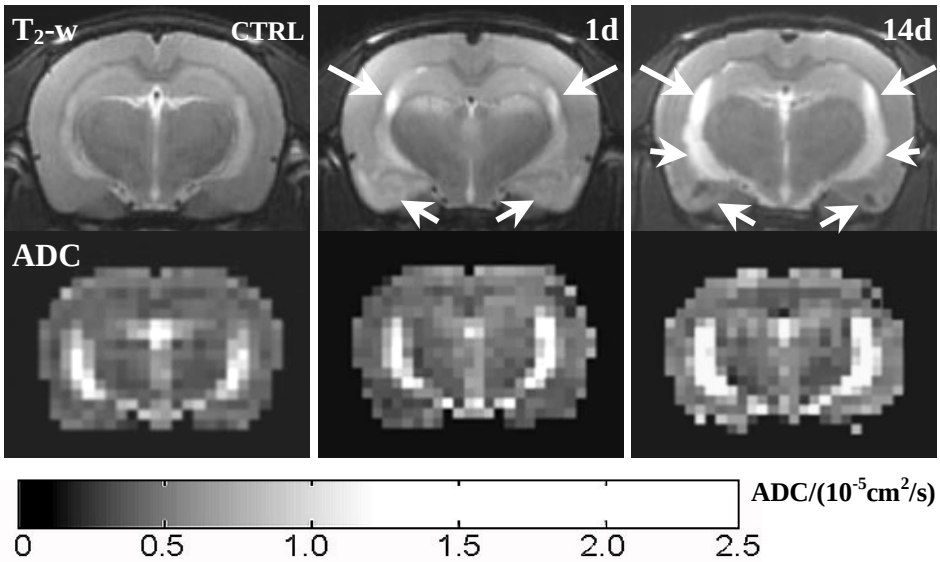

Figure 1. *Top: T2-weighted MR images of rat brain before, 1 day, and 14 days after KA-induced Status Epilepticus (SE). Bottom: ADC-maps of the corresponding slices. Arrows point to areas of structural effects (hippocampi, piriform cortices and amygdalae).*

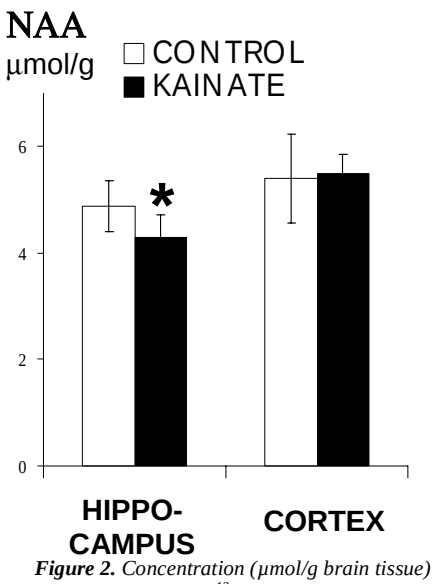

Figure 2. *Concentration (µmol/g brain tissue) of NAA measured by ^{13}C NMR-S. Mean±SD. * p<0.05*

Cortex: (i) Increased signal intensity in T2-weighted MR images bilaterally 1 day after SE in the perform cortices and amygdalae and reduced signal intensity after 14 days (Fig 1); (ii) Unaltered total amount/g tissue of glutamate, glutamine, GABA, succinate, taurine, lactate, alanine and creatine/phosphocreatine; (iii) Increased labeling derived from [1-13C]glucose in glutamate, glutamine, aspartate and succinate (mainly labeled in neurons[6]). [1-13C]glucose concentration also increased; (iv) Increased labeling derived from [1,2-13C]acetate (exclusively entering astrocytes[7]) in glutamate and glutamine.

Hippocampus: (i) Signal hyperintensity in T2-weighted MR images and ADC-maps (Fig 1), suggesting structural changes such as neuronal loss, gliosis or edema; (ii) Unaltered total amount/g tissue of glutamate, glutamine, GABA, succinate, taurine, lactate, alanine and creatine/phosphocreatine; (iii) Visible loss (Nissl-stained sections) of pyramidal neurons (glutamatergic) and gliosis in the hippocampal subregions CA3 and CA1, in addition to cell loss and gliosis in the dentate hilus; (iv) Increased labeling derived from [1-13C]glucose (mainly metabolized in neurons[3]) only in succinate; (v) Unaltered astrocytic metabolism of [1,2-13C]acetate (vi) Decreased total amount of NAA by ~13% (Fig 2).

NAA reduction is a marker of both neuronal death and impaired mitochondrial metabolism. However, the 13C NMR-S results do not indicate mitochondrial impairment. Together with histologically assessed loss of glutamatergic neurons this suggests that the NAA reduction in this study indeed reflects neuronal loss after KA induced seizures. This agrees with earlier studies reporting a good correlation between NAA decrease and neuronal death in the KA TLE model[8]. Taking into account this loss of glutamatergic cells, an unaltered overall hippocampal neuronal metabolism may in fact reflect an up-regulation of metabolism in the remaining glutamatergic neurons, producing overall "normal" amounts of metabolites. One could argue that glutamate producing GABAergic neurons could be responsible for the production of glutamate derived from glucose, but this is unlikely since GABA metabolism is not increased.

CONCLUSIONS

In KA treated rats, structural changes are clearly visible on MR images suggesting neuronal loss and/or edema. The metabolism in hippocampus might be up-regulated in remaining glutamatergic neurons in the latent period (2weeks). In cortex the neuronal and astrocytic metabolism of excitatory glutamate increases, while the metabolism of inhibitory GABA remains unchanged.

REFERENCES

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