MRI assessment of status epilepticus induced hippocampal plasticity after systemic Mn administration

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Introduction

Mn^{2+}-enhanced magnetic resonance imaging (MEMRI) has potential to reveal functional, structural and connectional alterations in high spatial resolution [1]. Intracerebral Mn injection to entorhinal cortex has been recently used to characterize activity-dependent plasticity in the mossy fiber pathway after status epilepticus in rats [2]. Accumulation of Mn into to dentate gyrus and CA3 of rat hippocampus was attributed to structural plasticity of mossy fibers i.e axonal sprouting, although contribution of activity dependent Mn accumulation could not be completely ruled out. It has been recently shown that Mn^{2+} enters hippocampus also from ventricle after systemic administration [3]. This is likely to be independent of brain activity and we hypothesized that systemic Mn injection may provide a robust MEMRI approach for detection of mossy fiber sprouting. The present work was designed to test this hypothesis, and to assess the possible accumulation of systemically injected Mn into hippocampus during status epilepticus.

Methods

The systemic manganese injection ($MnCl_2$ in bicine buffer, 45mg/kg, i.p.) was given to 10 Spraque Dawley –rats. After 12 hours, 6 of the rats (Mn+SE–group) received kainic acid (KA) injections (10 mg/kg, i.p.) which induced status epilepticus (SE) (Fig 1). MRI was performed under 1% halothane anaesthesia 3 and 25 hours after KA injection. Two months after KA injection, follow-up MRI was performed before and 24 hours after systemic Mn injection. MRI data were acquired at 4.7T using Varian Inova console and a quadratur half volume rf-coil. T1-wt 3D images were collected using a gradient echo –sequence (TE=2.7 ms, TR=120 ms,) incorporating an adiabatic 70-degree BIR-4 excitation pulse to reduce influence of B1 inhomogeneity. Volume of 25*25*35 mm^3 was covered with 192*64*256 points, with 2 averages a phase encoding step. Data for T1-maps were acquired from a single slice using IR-fast spin echo -sequence (TR=4s, echo spacing=13ms, 8 echoes, 128*256pts, FOV=2.0*2.56 cm^2, thk=0.7mm; TI=10, 400, 1000, 1600 ms). All results are presented as mean ± SEM. The statistical analysis is performed using Student's t-test.

Results

In the acute phase, 15h after KA injection, T_1 was slightly increased in hippocampus of KA injected animals if compared to control animals (T_1= 1241±12 ms vs. T1 = 1169±25ms, in KA injected and control animals, respectively, $p<0.05$) which is attributed to oedema formation after status epilepticus. No significant T1 differences between groups were detected in other brain areas, indicating that activity dependent Mn accumulation during generalized seizures must have been negligible.

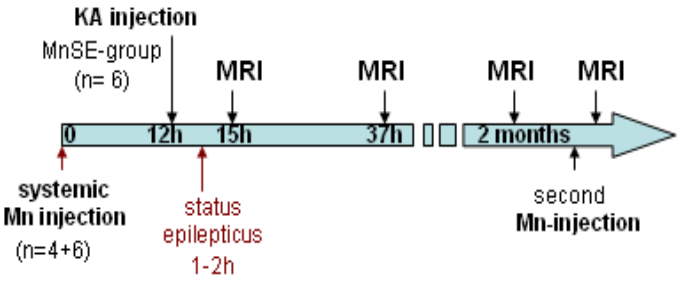

Fig.1. Study design.

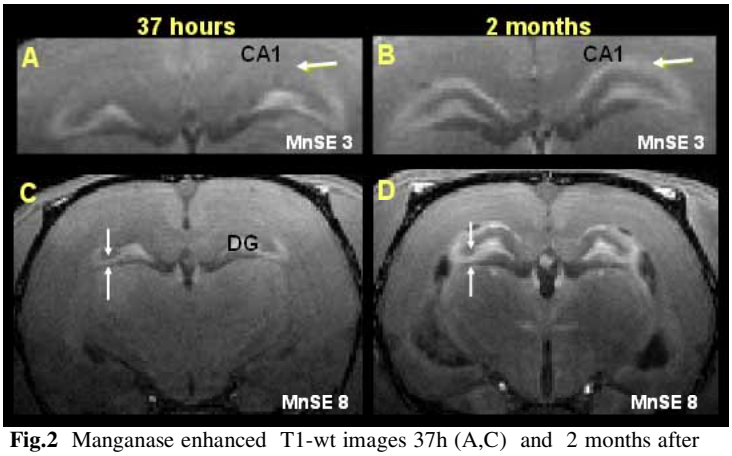

Fig.2 Manganase enhanced T1-wt images 37h (A,C) and 2 months after KA injection from two representative animals.

Two months after KA administration, there were clearly visible differences in the shape and size of the Mn-enhanced structures in the hippocampus (Fig 2.). This was accompanied by significant atrophy and enlarged ventricles leading in some cases to deformation of the hippocampal structures which is typical long term consequences of the KA injection[4]. The most evident alterations were thickening of the DG - CA3 region, and increased signal intensity in CA1 region. As an initial quantitative analysis the thickness of the Mn-enhanced CA3 region was measured as this region is only slightly affected by the deformation of the hippocampus. In Mn-SE group the thickness was 260±40 % higher than in the control group ($p<0.01$). The signal intensity ratios of CA1 and adjacent corpus callosum in T1-weighted images were 1.37±0.03 and 1.22±0.03 in Mn-SE and control group, respectively ($p<0.05$) confirming the increased accumulation of Mn in the CA1 region of the KA-injected animals.

Conclusions

Kainic acid induced status epilepticus did not lead to markedly increased uptake of the Mn into cells 12-15 hours after systemic Mn injection. Part of the T1 lowering effect of Mn may have been masked by T1 increase associated with edema formation, still it seems evident that after systemic Mn injection MEMRI contrast is rather associated with changes in cell density or structural plasticity than brain activation. Significant differences were detected between KA-treated and control animals two months after induction of status epilepticus. Alterations in thickness and shape of the DG and CA3 region of hippocampus can be attributed to axonal growth (i.e mossy fiber sprouting), as recently shown after intracerebral Mn injection in the same animal model [2]. The increased MEMRI contrast in CA1of KA injected animals was unexpected. Accumulation due to spontaneous seizures or increased interictal brain activity seems unlikely as even status epilepticus caused by KA did not lead accumulation of Mn in the CA1. Interestingly, also CA1 has been shown to have abnormal axonal growth after status epilepticus [5], and MEMRI may have potential to reveal this less studied phenomenon.

References: [1] Silva, Korezky review, 2004, [2] Nairismägi *et al.*, 2005, [3] Lee *et al.* 2005, [4] Pirttilä *et al.*, 2001, [5] Smith and Dudek, 2001

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