Connectional Network Changes and Mossy Fiber Sprouting in Rat Brain During Epileptogenesis Revealed by Manganese Enhanced MRI

J. Nairismägi[1], A. Pitkänen[1], S. Narkilahti[1], J. Huttunen[1], R. A. Kauppinen[2], O. H. Gröhn[1]

[1]University of Kuopio, A.I. Virtanen Institute, Kuopio, Finland, [2]University of Manchester, Manchester, United Kingdom

Introduction

Mossy fiber sprouting has been known as one of the hallmark features depicting reorganization of perforant pathway in the pathology of epilepsy. It has been suggested that abnormal axonal growth correlates with cell loss in hippocampal hilar region after seizures [1] and therefore it could potentially be used as a surrogate marker for neuronal death caused by epilepsy. Hence, one can claim that non-invasive detection of mossy fiber sprouting would be of great importance for the asessment of disease progression *in vivo*. In the present study Mn^{2+}-enhanced MRI (MEMRI) was used to image perforant pathway structures during subacute phase of epileptogesis resulting from kainic acid (KA) induced status epilepticus (STE). MEMRI is able to reflect characteristics of neuronal network connections with high anatomical resolution [2]. Mn^{2+} has similar ionic radius as Ca^{2+} and consequently, these ions bind to similar sites *in vivo*. Mn^{2+} is handled similarly to Ca^{2+} by neurons [3]. Mn^{2+} is transported both anterogradely and retrogradely by axons and it can also cross synapses [4]. Mn^{2+} is strongly paramagnetic shortening water T_1.

Methods

KA model of epilepsy was used. Briefly, STE was induced in adult male Wistar rats (14 epileptic and 6 controls, 303.2±24 g) with an intraperitoneal injection of KA (3mmol/ml/kg) in saline. Rats were monitored for 4 hours to confirm criteria of STE. Two weeks later rats weighting 290±26 g received 30-50 nl of $MnCl_2$ (1 M) into the left entorhinal cortex. Coordinates of injection site were set according to the Paxinos rat brain atlas [5]: -8.3mm from bregma, -4.0mm from sagittal suture and -5.3mm from brain surface. Control rats were not injected with KA, but received equivalent intracerebral injection of $MnCl_2$. Animals were anesthetized with 1.0% halothane in N_2O/O_2 for MRI that was acquired 1, 6, 12, 24, 48 hours and 3, 5, 7 and 10 days after $MnCl_2$ injection. A Varian ^{UNITY}INOVA system operating at 4.7 T with a transmit/receive quadrature surface-coil was used. T_1-weighted (TE=2.7 ms, TR=120 ms) 3D gradient echo method incorporating a BIR-4 excitation pulse to reduce influence of B1 inhomogeneity was used for MEMRI. Volume of 25x 25*35 mm was covered with 192*64*256 points, with 2 averages a phase encoding step, leading to a total acquisition time of 49 min per animal. Signal intensities in ROIs were normalised for that determined in the adjacent unexposed brain cortex. Volume rendered 3D reconstructions were generated by using AVS Express software. The Nissl (cell damage) and the Timm stainings (mossy fiber sprouting) were used in quantitative histology. The density of mossy fiber sprouting was scored according to Cavazos et al. (1991) [6].

Results

Epileptic rats started to show spontaneous seizures three weeks after KA treatment. Significant enlargement of ventricles and atrophy of several brain structures bilaterally with know vulnerability to STE, including amygdala and entorhinal cortex, were evident in MRI 3 weeks after STE. Both in epileptic and in control animals, enhanced T_1 signal in MEMRI was detected in entorhinal cortex and in perforant pathway in hippocampus. The most pronounced enhancement was detected in CA3 and dentate gyrus subregions matching the mossy fiber pathways. In volume rendered 3D images Mn^{2+} enhancement revealed fine structural features of perforant pathway, such as lamellar organization of the mossy fiber pathway (Fig A and B). Semiautomatic threshold analysis of images showed increased volume of Mn^{2+} enhancement in the hippocampal CA3 subregion and dentate gyrus in the epileptic animals relative to controls (Fig C). The Timm staining demonstrated substantial mossy fiber sprouting (grade 1.0-3.0) both in CA3 and DG in epileptic rats which was correlated ($p<0.05$) with the volume of Mn^{2+} -enhanced pixels (Fig D). In addition to these, strong MEMRI signal was also found in dorsal thalamus in epileptic rats (Fig C).

Conclusions

The present results show good correlation between Mn^{2+} enhancement and mossy fiber sprouting in the CA3 and DG of rat hippocampus during epileptogenesis. Signal increase in MEMRI can be potentially due to two factors as follows: firstly, increased neuronal activity in the hippocampal structures and secondly, axonal sprouting. The latter alternative is favoured, because mossy fiber sprouting has been shown not to correlate with occurrence of seizures [7] Increased MEMRI signal in thalamus may result from non-specific diffusion of $MnCl_2$ from entorhinal to postrhinal cortex and activation of postsubiculum–thalamus pathway in epilepsy.

References

[1] Masukawa, L.M et al., Brain Res 678 (1995) 65-75
[2] Watanabe, T et al, Magn Reson Med 48 (2002) 852-9
[3] Takeda, A et al., Brain Res Bull 45 (1998) 149-52
[4] Pautler, R.G et al., Magn Reson Med 40 (1998) 740-8
[5] Paxinos G., Watson C. in: The Rat Brain in Stereotaxic Coordinates Academic Press (1986)
[6] Cavazos, J.E et al, J Neurosci 11 (1991) 2795-803
[7] Nissinen, J et al., Hippocampus 11 (2001) 299-310

Supported by grants from the Academy of Finland and the Sigrid Juselius Foundation

Fig. A: Volume rendered 3D MEMRI from a control rat. Lamellar structure of mossy fibers in hippocampal CA3 subregion is marked by arrow
Fig. B: Volume rendered 3D MEMRI from an epileptic rat. Enhancement of dorsal thalamus (below the hippocampus) is marked by arrow
Fig. C: Semiautomatic pixel analysis reveals stronger MEMRI signal in the epileptic rat brain: a mild difference from controls in CA1 was seen, but large difference in CA3, dentate gyrus (origination of mossy fibers, where also sprouting is the most intensive) and dorsal thalamus was evident.
Fig. D: An example of correlation ($p\leq0.05$) between mossy fiber sprouting and MEMRI signal 5 days after Mn^{2+} injection. (counted MEMRI labelled pixels on x and Timm score by Cavazos [6] on y axis)