MRI Changes in CBF, Diffusion and T2 after seizures induced by GluR5 agonist infusion in rat before 180 mins.

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Introduction

Epilepsy, a serious neurological disorder, affects 1% of the world; at least 1/3 of the affected people have inadequate seizure control. To develop new therapeutic approaches, animal models are necessary to understand underlying mechanisms, regional anatomic vulnerability, and response to therapy. Altered excitation and inhibition may lead to functional and structural derangements associated with epileptogenesis. Glutamate receptors are particularly implicated in this process. MRI can elucidate lesion evolution associated with seizures. We used ATPA ((RS-2-amino-3-(3-hydroxy-5-tert-butylisoxazole-4-yl) propanoic acid), a specific KAr GluR5subtype agonist, Kainic acid (KA), activating several receptor subtypes, and AMPA (alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid), agonist for AMPA gluR1-4subtype, to produce seizures after intra amygdalar (*i.a.*) injection in rats, and measured Cerebral Blood Flow (CBF) with Arterial Spin labeling (ASL) MRI. For comparison, KA was infused intravenously (*i.v.*). After *i.a.* and *i.v.* infusions, T_2 and diffusion image maps were generated to quantify changes in brain regions after seizures lasting 60 minutes.

Methods

Rats anesthetized with ketamine / xylazine had MR-compatible cannulae placed stereotaxically in right basolateral amygdala. After several days rest, they were intubated and anesthetized with isoflurane. Lines were placed in the femoral artery to monitor blood pressure, and femoral vein for drug and fluid administration. Body core temperature was maintained at 37 degrees with a heated circulating water pad. Blood gas was analyzed at frequent intervals. MR imaging was performed on a 7T Bruker Avance scanner. The heads were stereotaxically fixed and rats placed in a 72 mm radio frequency coil; for *i.v.* scans this coil acted as transmitter for a 25 mm receive only surface coil. Pilot images were obtained to locate the cannula/amygdala site. Five 1 mm thick axial images centered on the cannula/amygdala delineated morphological details (FOV = 3.2 cm, matrix 128x128). CBF was measured using ASL on a 2 mm axial slice containing the cannula. A labeling pulse of 2 ms with power of 81 mG/cm was used at 2 cm caudal to the site of cannula to label arterial spins. (parameters: FOV = 3.2 cm, matrix 64x64, total time per scan = 4.5 mins). Baseline images (control) were acquired before infusion of drug or saline. 5 microliters of 10 nanomolar ATPA, AMPA or KA (n = 4 rats) was injected over five minutes. Two rats had saline alone. ASL images were acquired continuously for an hour from the start of the infusion. Approximately 2 hours after infusion, two more sets of spin labeled images were acquired. Eighty minutes post infusion, multi echo T_2 weighted (TE=10 ms, 16 echoes, TR= 3 s, Matrix 128x128) and diffusion weighted (in three orthogonal directions) sequences (b = 4, from 0 to 3000 g/cm, TE= 20 ms, Δ=32 ms, TR= 2.5 s, matrix 64x64) with same geometry (FOV=3.2 cm) were performed. T_2 and ADC maps were generated. The signal difference between control and labeled images was used as a measure of CBF. CBF, T_2 and ADC were evaluated for regions of interest (ROI) placed in left and right amygdalae, hippocampus, sensory and motor cortices of both hemispheres to investigate any widespread brain activation.

Results

Animals' vital signs were stable during pre and post agent administration. Approximately 16 minutes after infusion, CBF begun to increase. Changes due to KA were greater than ATPA and were shorter temporally (Fig-1A-B). KA, AMPA, and ATPA increased CBF in all regions to different extents. Saline had no effect; thus interference due to agent accumulation before dispersion, and local edema proximal to the cannula site, is negligible. The right amygdala cannula site showed maximum variation gradually generalizing in intra-amygdala rats. In comparison, *i.v.* KA animals showed more rapid global increases. All three agents increased T_2 in left and right hippocampus (Fig-2A). KA and AMPA led to larger changes than AMPA. KA also reduced average ADC in hippocampus (Fig-2B). Also, there was a significant drug effect on average ADC value in right amygdala ($F = 7.9$; $p<0.01$). However, average ADC was not significantly affected in most other regions.

Discussion

Our results show that focal specific amygdala glutamate receptor stimulation leads to widespread brain activation. Systemic administration also led to global activation, more prominent in neocortex. Seizures induced by glutamatergic agonist amygdalar infusion lead to decreased diffusion in the epileptogenic region, and increased T_2 signal in more widespread limbic cortex. T_2 and ADC changes may have been limited to limbic, rather than affecting more widespread cortical regions due to the relatively short seizure duration. MRI evidence of potential cytotoxic injury (reduced diffusion and increased T_2 may be related to cytotoxic edema) can occur after one hour of seizure activity.

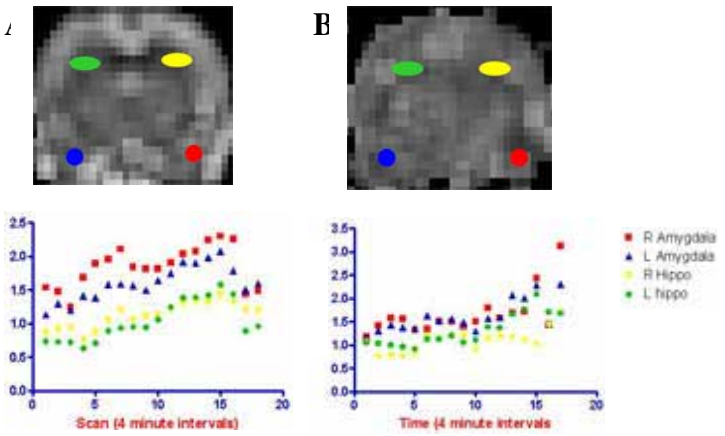

Fig-1: Temporal variation of Normalized Blood flow of four selected regions in rat brain during infusion of **A**) KA **B**) ATPA (data points at 4 min intervals)

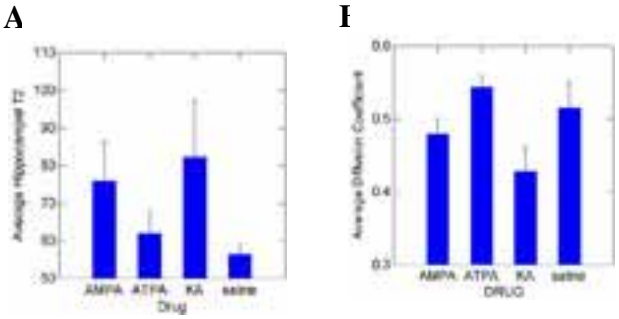

Fig-2: Average T_2 (**A**) and ADC (**B**) for the hippocampal regions as a function of drug.