Diffusion in the rat cerebral cortex during pilocarpine-induced status epilepticus

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Introduction: The diffusion of neuroactive substances through the extracellular space (ECS) of the central nervous system is the underlying mechanism of extrasynaptic transmission [1], an important mode of communication between nerve cells. The objective of the present work was to determine the diffusion parameters in the brain cortex of adult rats after pilocarpine-induced seizures - an experimental model widely used to study the pathophysiology of epilepsy. We employed the real-time iontophoretic tetramethylammonium (TMA) method to measure the ECS volume fraction (α = ECS volume/total tissue volume) and tortuosity (λ), which is related to the apparent diffusion coefficient of TMA in the extracellular space ($ADC_{TMA}=D/\lambda^2$, where D is the free diffusion coefficient). Although this method provides detailed information about diffusion in the brain, it cannot be used in humans. Diffusion-weighted (DW) MRI, which is widely available for clinical use, was used to determine the apparent diffusion coefficient of water (ADC_W) in the tissue, and the results were compared to the TMA measurements.

Subjects and methods: Experiments were performed *in vivo* on 3-month-old male rats (Wistar strain). The rats were anesthetized by urethane (1200mg/kg), artificially ventilated and administered pilocarpine (300 mg/kg, i.p.). To potentiate the effects of pilocarpine, lithium chloride (127 mg/kg, i.p.) was given to the animals 14-18 h before the experiment. TMA and DW-MRI measurements were made up to four hours after the administration of pilocarpine. The TMA method monitors the diffusion of the small TMA cation, which diffuses predominantly in the ECS, after its iontophoretic application. The local concentration of TMA^+ was measured in the primary somatosensory cortex using ion-selective microelectrodes [2]. The DW-MRI method measures the apparent diffusion coefficient of water (ADC_W) in both the extracellular and intracellular compartments. Using a stimulated echo sequence, we acquired 4 axial slices with the following parameters: FOV 3.2 cm, TR 1.2 s, TE 47 ms, 1.0 mm slice thickness, 0.5 mm gap, 256 x 128 image matrix, slice diffusion gradient direction and 4 b-values (75- 1731 s/mm^2). The resulting ADC_W maps were evaluated in the primary somatosensory cortical region, the area corresponding to the site of the TMA measurements. In addition, the extracellular potassium concentration $[K^+]_e$ was recorded using ion-selective microelectrodes.

Results: The mean values of α and λ before the application of pilocarpine were α=0.19 ± 0.004 and λ =1.58 ± 0.01, (n=7, mean ± SEM). Following pilocarpine application, there were no significant changes in tortuosity λ. The volume fraction α started to decrease several minutes after the application of pilocarpine, reaching a minimum (0.134 ± 0.012) 80 - 100 minutes later, which was significantly different ($p<0.001$) from control values. At 120 minutes, α started to increase and reached 0.176 ± 0.009 four hours after the application of pilocarpine. ADC_W was significantly ($p<0.05$) decreased 80 minutes after pilocarpine application (563 ± 18 $\mu m^2 s^{-1}$, n=5) compared to controls (655 ± 11 $\mu m^2 s^{-1}$, n=5); by the end of the experiments, ADC_W had returned to control values. These changes were accompanied by an increase in extracellular potassium, which reflects increased neuronal activity. Detailed data of the ECS volume fraction, tortuosity, $[K^+]_e$ and ADC_W after the application of pilocarpine are summarized in Figure 1.

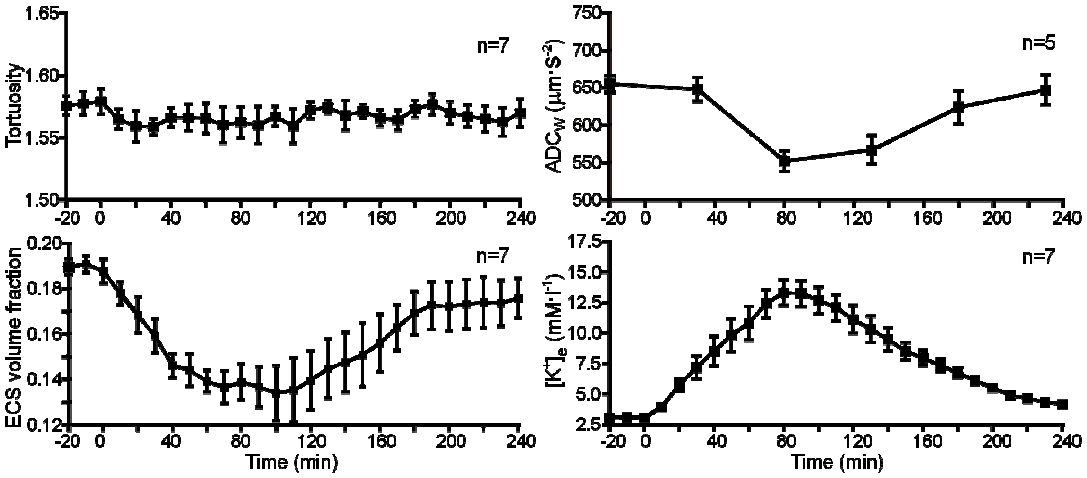

Fig. 1: The graphs show the time course of changes in extracellular volume fraction α, tortuosity λ, extracellular potassium concentration $[K^+]_e$ and the apparent diffusion coefficient of water (ADC_W) in the primary somatosensory cortex. Note the similar time course of changes in α, ADC_W and $[K^+]_e$. Data are expressed as mean ± S.E.M.

Discussion and conclusion: Our data show that pathologically increased neuronal activity during pilocarpine-induced seizures causes cell swelling followed by a long-lasting reduction in the ECS volume fraction, but not significant changes in tortuosity (which reflects the geometry of the ECS and diffusivity in the ECS). The reduction in ECS volume correlated with a decrease in the apparent diffusion coefficient of water, in agreement with our previous results [3]. These diffusion changes can influence synaptic as well as extrasynaptic transmission and neuron-glia interactions and can affect the development of seizures. Moreover, the ECS volume decrease can contribute to the accumulation of metabolites to toxic levels, which can lead to damage of the central nervous system.

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