

Seizures Induced by Chronic Pentylenetetrazol Treatment Result in Progressive Neuronal Injuries in Rat Hippocampus: An *In vivo* ^1H MRS Study

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Introduction *In vivo* proton magnetic resonance spectroscopy (^1H -MRS) has been used to measure metabolic changes in the brain of epilepsy patients. Significantly reduced NAA/total creatine (tCr) ratios have been observed in the hippocampus and/or thalamus of patients with temporal lobe epilepsy (TLE), indicative of neuronal dysfunction/loss in these regions [1]. Pentylenetetrazol (PTZ) is a GABA_A receptor antagonist, repeated administration of which in subconvulsive dosages produces chemical kindling in rats [2]. PTZ-induced kindling is a widely used animal model in epilepsy research [3]. Similar to the observations in the patients with TLE, significant neuron loss and gliosis in the hippocampus of PTZ-induced kindled rats have been demonstrated [4]. In this study, we measured the dynamic metabolic changes in the hippocampus of the rats subjected to repeated treatments of PTZ with *in vivo* ^1H MRS, and correlated the spectroscopic findings with the results of behavioral and histological evaluation. The aim is to find out how the neuronal dysfunction/loss observed in the hippocampus of PTZ-induced kindled rats is related to the history of seizures occurring during the course of kindling (i.e., epileptogenesis).

Materials and Methods Sprague-Dawley rats weighing 180-200 g were used, which received i.p. injections of either PTZ (35 mg/kg) or the same amount of saline solution (Group C, n=10) every 48 hrs. A total of 14 doses of PTZ or saline were given. The convulsant responses of the PTZ-treated rats were observed for at least 20 minutes after each injection, and a score for the seizure severity was obtained with 0) no response; 1) facial movements; 2) myoclonic jerks of the forelimb; 3) myoclonic jerks with upright position; 4) clonic seizures, turn over onto side position; 5) generalized clonic-tonic seizures, turn over onto back position [5]. At two weeks after the 28-day treatment, the PTZ-treated rats received another dose of PTZ (35 mg/kg), and the convulsant responses were scored as done previously (i.e., the screening test). The animals had a score of 3 or higher in the screening test and had scores of 4 or higher in three consecutive behavioural tests during the 28-day PTZ treatment were considered being kindled (Group K, n=10). Those animals met neither of the two criteria were considered being unkindled (Group U, n=10). *In vivo* ^1H MRS were performed in chloral hydrate-anesthetized (i.p., 5 ml/kg) rats at 1d after the 7th treatment (2W) and at 7-10d after the 14th treatment (4W) on a Bruker Biospec 4.7 T/30 cm spectrometer. A PRESS sequence was used to acquire spectra from the right hippocampus of each rat with voxel size 2.5 mm×2.5 mm×2.5mm (Fig. 1a), TR 1500 ms, TE 136 ms, spectral width 1500 Hz, 1k data points and 512 averages. The rats were then sacrificed, perfused and fixed with 4% paraformaldehyde. The brains were obtained, embedded with paraffin, cut into 5 μm -thick coronal sections and stained with H&E. The NAA/tCr ratios were calculated with integrated peak intensities and compared among the three groups with one-way ANOVA and *post hoc* Tukey's test. For each PTZ-treated rat, the sum of the seizure scores obtained during the 28-day treatment was calculated. The correlation between the sum of the seizure scores and the hippocampal NAA/tCr ratio measured at 4W was assessed.

Results No matter whether they were found being kindled or not after the 28-day treatment, the PTZ-treated rats did not show any significant changes in the hippocampal NAA/tCr ratios at 2W (Fig. 1c). Both the kindled and unkindled rats showed decreases in the hippocampal NAA/tCr ratios at 4W, compared to the control rats. However, only the changes in the kindled rats were statistically significant (Fig. 1c). In the PTZ-treated rats, the hippocampal NAA/tCr ratio measured at 4W correlated negatively ($p<0.05$) with the sum of the seizure scores obtained during the 28-day treatment (Fig. 2). Both the kindled and unkindled rats showed neuronal injuries in the hippocampus at 4W, as revealed by H&E staining (Fig. 3).

Discussion In a rat model of electrogenic amygdala kindlings, repeated brief seizures were shown to induce progressive neuronal damages in the hippocampal formation and entorhinal cortex, but not in the somatosensory cortex [6]. Clinical studies have also shown that, in the TLE patients, the NAA/tCr ratios in the temporal lobes and the hippocampal volume correlate negatively with the duration of epilepsy, and the patients with frequent generalized tonic-clonic seizures had lower NAA/tCr ratio in the temporal lobes and smaller hippocampal volume than the patients with none or rare generalized tonic-clonic seizures [7]. The results obtained in this study agreed with these previous results. It may be concluded that the neuronal injuries in the hippocampus of the rats subjected to chronic PTZ treatment are likely acquired from repeated seizures during epileptogenesis.

References [1] Hetherington HP et al. *Neurology* 2007;69(24):2256-2265. [2] Hansen SL et al. *Prog Neuropsychopharmacol Biol Psychiatry* 2004;28(1):105-113. [3] Sarkisian MR. *Epilepsy Behav* 2001;2(3):201-216. [5] Racine RJ. *Electroencephalogr Clin Neurophysiol* 1972;32(3):281-294. [6] Cavazos JE, et al. *J Neurosci* 1994;14(5):3106-3121. [7] Tasch E, et al. *Ann Neurol* 1999;45(5):568-576.

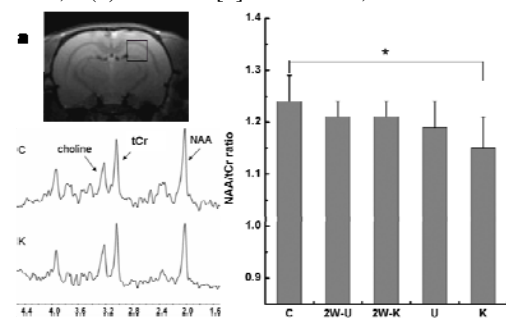


Fig. 1 *In vivo* ^1H MR spectra (b) were acquired from the right hippocampus (a) of the control rats (C), unkindled rats (U) and kindled rats (K). The NAA/tCr ratios (c) in the hippocampus of the PTZ-treated rats did not change significantly after 7 doses (2W). After receiving 14 doses of PTZ, the kindled rats showed significantly lower NAA/tCr ratios compared to the control rats.

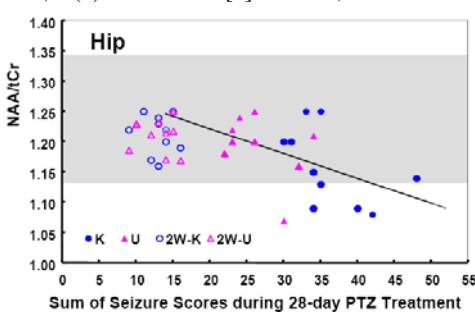


Fig. 2 The hippocampal NAA/tCr ratio in the rats treated with PTZ correlates negatively ($p<0.05$) to the sum of the seizure scores obtained during the 28-day treatment and. K and U: kindled and unkindled rats after 14 doses of PTZ, respectively; 2W-K and 2W-U: kindled and unkindled rats after 7 doses of PTZ, respectively.

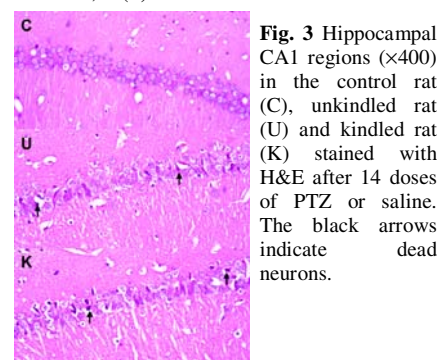


Fig. 3 Hippocampal CA1 regions ($\times 400$) in the control rat (C), unkindled rat (U) and kindled rat (K) stained with H&E after 14 doses of PTZ or saline. The black arrows indicate dead neurons.

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