Abstracts of the Alumni Association Memorial Lectures of the 75th Annual Meeting of the Medical Association of Nippon Medical School

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Abstract of the Alumni Association Medical Research Fund Prize Memorial Lecture (1)

Epileptogenesis Factors with Regard to Oxidative Stress and Theophylline Administration

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Introduction

The role of antioxidative agents (AOAs) in cerebrospinal fluid (CSF) in epileptic patients has been studied. It has been shown that the oxidative stress (redox) is closely related to the pathogenesis during ictogenesis (Brain Dev 28, 2006: 243-6). However, the role of up-regulated AOAs in the central nervous system (CNS) in epileptic patients still remains unknown. It is practically impossible to collect CSF samples before seizure attacks. Without control studies, it is not yet determined whether the up-regulated AOAs is the cause or effect of seizures.

The drug-induced seizure models of epilepsy are frequently used for experiments. Drug-induced seizures have two major problems from the clinical aspects. One is that the drugs which are frequently used in the pediatric field (for example, theophylline, anti-histaminergic agents, etc) may have the seizure provocation effects. The other is that the pathophysiology of the drug-induced model still remains unknown. The relationship between the onset of drug-induced seizures and the serum level of drug and/or the interictal electricencephalogram (EEG)finding in patients is unclear. Furthermore, there are some limitations in research studies using human samples, because of the ethical problems.

Materials and Methods

In this study, a mutant animal model of epilepsy (El mouse) was used. The El mouse is an inbred, epileptic mutant model of secondarily generalized seizures. Several lines of evidence indicate that in El mice, the parietal cortex is the seizure initiation site, and the hippocampus is responsible for the seizure generalization. The developmental formation of the focus complex, which consists mainly of the parietal cortex and the hippocampus, has been hypothesized as the key to epileptogenesis in El mice. Epileptogenesis is established at

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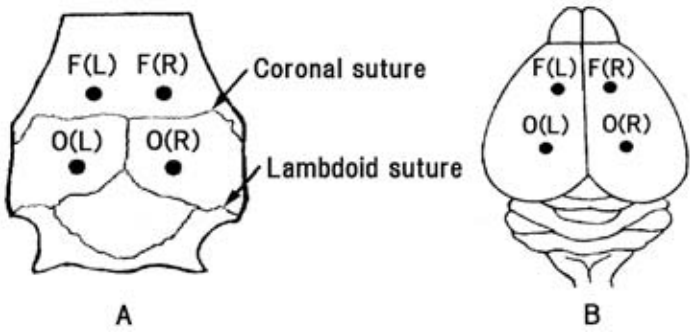

Fig. 1 Locations of the electrodes on the brain.
F: frontal, O: occipital, R: right, L: left.

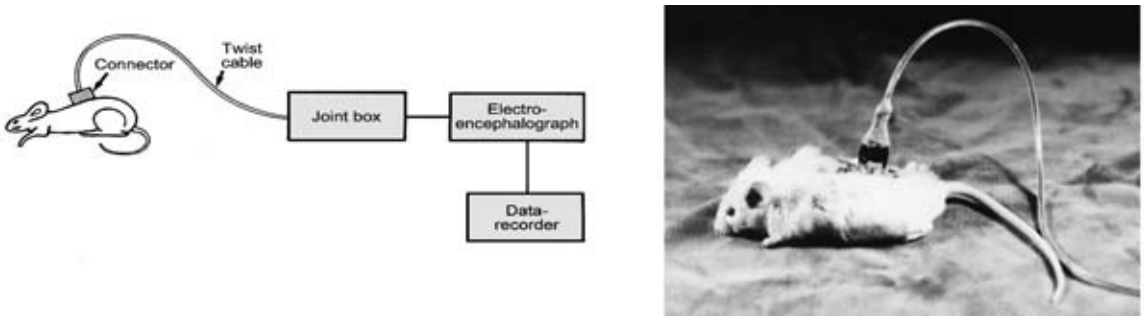

Fig. 2 A mouse implanted with the ensemble of electrode and cassette connector.

around 10 weeks of age during development in El mice and they have never showed seizures before 5 weeks of age. The ddY mice were used as control animals. They were inbred from the mother strain of El.

The redox in the epileptic brain was studied. Six mice were sacrificed by decapitation and the brains were removed and placed on ice. The parietal cortex and the hippocampus were dissected out from the brains and weighed (8～20 mg). To obtain the 10 % brain region homogenates, 20 mM Tris-HCl (pH 8.0) was added. Glutathione activities were determined by the enzymatic cycling method and glutathione peroxidase activities were determined by UV rate method as previously reported, respectively.

EEG recording method for mice was established. It is possible to record EEG stably under uninhibited condition of mice. The electrode materials and other operational procedures are described as follows (see **Fig. 1, 2**). Four burr holes were placed symmetrically anterior to the coronal and lambdoid sutures for electrode placement. The epidural electrodes were silver balls of 1 mm diameter and the dural attachment was 0.2 mm in diameter at the tip. A reference electrode was placed on the nasal bone and a ground electrode was placed subcutaneously near the rump. The apparatus used to record the EEG which was sampled by an A-D converter and was recorded using a digital data recorder.

The pathophysiology of drug-induced seizures was studied. Theophylline was administrated orally to mice after implantation of the electrodes and then EEG was recorded.

Results

Both glutathione and glutathione peroxidase were up-regulated in the brain of El compared with ddY before 10 weeks of age (**Fig. 3, 4**).

The spikes occurred synchronously at all the electrode positions, with differing amplitudes and various

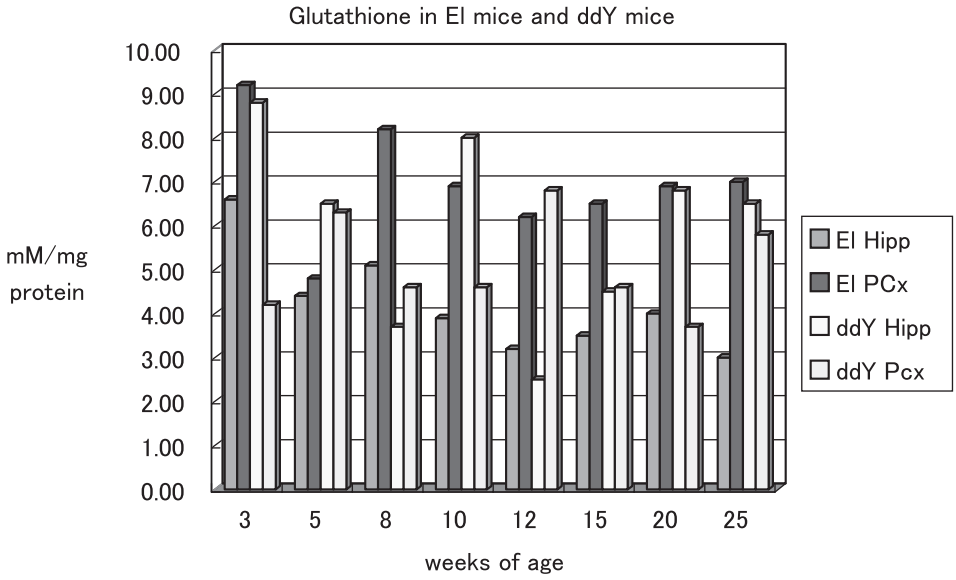

Fig. 3
Hipp: hippocampus, PCx: parietal cortex

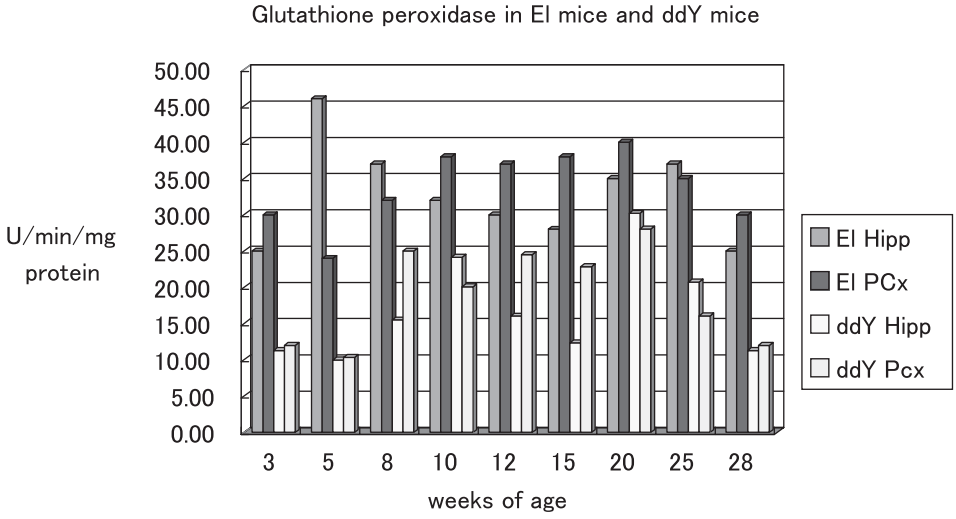

Fig. 4
Hipp: hippocampus, PCx: parietal cortex

fluctuations being evident. All mice survived the surgery and maintained a good condition. A raw EEG signal containing spike discharges (duration: 2 seconds at most) in a sleeping El mouse before oral theophylline administration was recorded **(Fig. 5)**. After theophylline administration, spike discharges were recorded more frequently, and its duration was not changed.

Sleep EEG of ddY mouse was recorded **(Fig. 6)**. It did not contain spike discharges, because naïve ddY mice show no seizures at all. However, theophylline could induce seizures even to ddY mice. A raw EEG signal tended to contain spike discharges (duration: 1 second at most) in a sleeping ddY mouse after oral theophylline administration was recorded (data not shown).

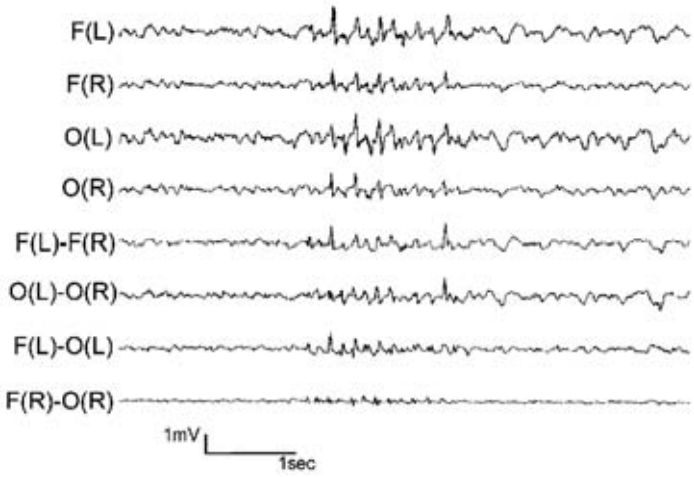

Fig. 5

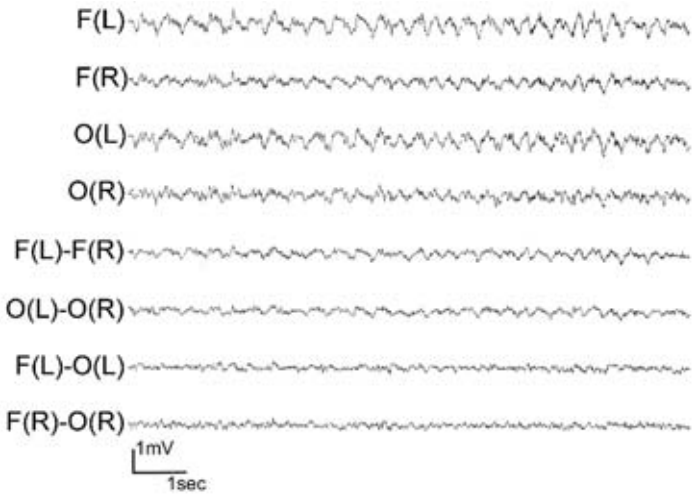

Fig. 6

Conclusions

The oxidative stress is closely related with the establishment of epileptogenesis in El mice. And theophylline might have inductive potency to neural hyperexcitability.