Korean J Physiol Pharmacol
Vol 10: 235–242, October, 2006

Effects of Ethosuximide on the Pilocarpine Induced Seizure in Rat Model of Neuronal Migration Disorder

Byung-Kon Kim[1], In-Sun Choi[1], Jin-Hwa Cho[1], Il-Sung Jang[1], Maan-Gee Lee[2] and Byung-Ju Choi[1]

Departments of [1]Dental Pharmacology and [2]Pharmacology, College of Medicine, Kyungpook National University, Daegu 702-412, Kore

Cortical malformation-associated epileptic seizures are resistant to conventional anticonvulsant drugs. Relatively little research has been conducted on the effects of antiepileptic drugs (AEDs) on seizure activity in a rat model of dysplasia. We have used rats exposed to methylazoxymethanol acetate (MAM) in utero, an animal model featuring nodular heterotopia, to investigate the effects of ethosuximide (ETX) in the dysplastic brain. Pilocarpine was used to induce acute seizure in MAM-exposed and age-matched vehicle-injected control animals. Field potential recordings were used to monitor the amplitude and number of population spikes, and paired pulse inhibition in response to stimulation of the commissural pathway. Pharmaco-resistance was tested by measuring seizure latencies after pilocarpine administration (320 mg/kg, i.p.) with and without pre-treatment with ETX. Pre-treatment with 300 mg of ETX significantly prolonged the latency to the status epilepticus (SE) in both control and MAM-treated groups. Pre-treatment with ETX 100mg and ETX 200 mg had little effect in MAM-exposed rats. However, ETX 200 mg prolonged the latency to the SE in control groups. Spontaneous field potential and secondary after-discharges were higher for MAM-treated rat in comparison with control rats injects with ETX. The main findings of this study are that acute seizures initiated in MAM-exposed rats are relatively resistant to standard ETX assessed in vivo. These data suggest that ETX do not prolong seizure latencies in MAM-rats exposed to pilocarpine.

Key Words: Ethosuximide, Dysplasia, Methylazoxymethanol acetate, Epilepsy, Pilocarpine

INTRODUCTION

Disturbances of brain developmental processs result in a cortical malformations (Castro et al, 2002). Infants with seizure disorders associated with cortical malformations (Smyth et al, 2002) are increasing. Clinically neuronal migration disorders are classified as malformations and are often associated with medically intractable seizures. (Barth, 1987; Aicardi, 1994; Palmini A, 2000)

Cortical malformation-associated epileptic seizures are resistant to conventional antiepileptic drugs. These early-onset epilepsies are effectively controlled by surgical removal of abnormal brain tissue (Calcagnotto et al, 2002).

There are several kinds of animal models for investigating mechanisms of epileptogenesis associated with heterotopic malformation (Baraban & Schwartzkroin, 1995). Testing of efficacy of antiepiletic drug (AED) include acute seizure models based on chemical and electrical induction of seizures in normal animal. Sometimes efficacy of AEDs may be ineffective in these models (Stables et al, 2002). Animal models with cortical malformation may be necessary for discovering new AEDs that would be effective in pharmacoresistant epilepsy, and since these new AEDs may be ineffective in the acute-seizure models (Loscher & Honack, 1993; White, 2003). If epileptogenesis involves new mechanisms not present in the normal brain, then traditional AED testing in acute-seizure models may not identify effective versus ineffective drugs, because they being tested on animals whose brains have not undergone the epileptogenic changes (Dudek, 2005). The effects of AEDs on spontaneous seizures in animals with cortical dysplasia-associated epilespy have been conducted relatively little.

Researcher has found brain malformations in neocortical structures and in the hippocampal formation (Houser, 1990; Hirabayashi et al, 1993). A number of animal models have been developed that feature hippocampal dysgenesis to identify the electrophysiological and morphological properties of dysplastic neurons. These include rats exposed to irradiation or methylazoxymethanol (MAM) *in utero* (E15) (Baraban & Schwartzkroin, 1995), *Lis 1* and *p35* knock-out mice (Fleck et al, 2000; Wenzel et al, 2001) and Ihara rats (Amano et al, 1996). In each of these animal models, spontaneous seizures or an increased susceptibility to convulsant agents has been reported.

The formation of dysplastic regions and heterotopic clusters of neurons were found after the application of MAM *in utero* (E15) in the neocortex and the CA1 region of the hippocampus, as well as in the subcortical white matter (Chen & Hillman, 1986; Ciaroni et al, 1989). The threshold for seizure activity is low in some MAM-treated pups with both neocortical and hippocampal dysgenesis (Germano &

Corresponding to: Byung-Ju Choi, Department of Dental Pharmacology, College of Dentistry, Kyungpook National University, 101, Dongin-dong 2-ga, Jung-gu, Daegu 702-412, Korea. (Tel) 82-53-660-6880, (Fax) 82-53-424-5130, (E-mail) bjchoi@knu.ac.kr

Sperber, 1994). Atypical electrophysiological and morphological characteristics were found in heterotopic CA1 pyramidal neurons (Chevassus-au-Louis et al, 1998a) and abnormal connections with neocortical regions were formed in these area (Chevassus-au-Louis et al, 1998b).

The present study used a MAM-treated rat that shares many anatomical similarities with human malformation-associated epilepsies (Spreafico et al, 1998) and highly seizure susceptible (de Feo et al, 1995, Sancini et al, 1998). To address whether any of these animals respond to ethosuximide, the present study used MAM-exposed rats to evaluate potential anticonvulsant resistance and pharmacological experiments were performed on MAM-exposed animals to test whether ethosuximide alter pilocarpine-induced epileptiform activity and seizures.

METHODS

Animals

Female rats (Sprague-Dawley) with known insemination times were obtained. Pregnant rats were injected with 25 mg/kg MAM dissolved in 0.9% saline. Intraperitoneal injections were made on 15-day old embryos. Experimental procedures were performed in accordance with the animal care guidelines of NIH and the Korean Academy of Medical Sciences. All animals were maintained in a 12-hour light-dark cycle and were provided with food and water ad libitum.

Pilocarpine treatment

Sprague-Dawley rats (250~300 g, n=92) were injected intraperitoneally with atropine at a dose of 1 mg/kg before the injection of pilocarpine to reduce the peripheral effects of pilocarpine. Control rats and MAM-exposed rats were injected (i.p.) with a dose of 320 mg/kg of pilocarpine in 0.9% NaCl. After the administration of pilocarpine, the convulsive behavior was scored according to the Racine scale (Racine, 1972): stage 0, in which rats showed no convulsion; stage 1, in which rats showed head bobbing, tremor, backward walking, wet dog shake; stage 2, intermittent forepaw myoclonus, rearing and falling; stage 3, continuous chronic convulsion; stage 4, tonic flexion; and stage 5, respiratory arrest.

In vivo recording

Sprague-Dawley rats were anesthetized with urethane (1.3 g/kg) and fixed in a stereotaxic apparatus with a small-animal thermoregulatory device. The scalp was reflected and two small bone windows (1×2 mm) were formed for recording and stimulation. The recording electrode was located in the hippocampus (AP: −3.8 mm from bregma; L: 2.5 mm). A concentric bipolar stimulating electrode was inserted into the contralateral fimbria-fornix (AP: −1.3; L: 1.0; V: 4.8 mm) to stimulate commissural inputs in the CA1 area. Ten field potentials were recorded with electrodes made from glass micropipettes blunted to an outer diameter of ~ 15 μm and filled with 0.9% NaCl. The population spike amplitude was calculated by averaging the sum of the value measured from the first peak positivity to peak negativity of the spike, and the same peak negativity to the last peak positivity of the spike. The stimulus intensity was standardized by the stimulus threshold for a population spike T, which was determined by delivering stimuli (frequency 0.1 Hz, duration 100 μs) of increasing intensity until a small-amplitude population spike was evoked consistently. Commissural pathway stimulation was made using single or paired pulses.

Paired pulse stimulation was used to assess inhibition in the CA1 network. Recording sites were verified to use a standard method of dye placement or traced the recording electrode tract penetrating into hippocampus, with *post hoc* tissue processing and mapping. Pairs of stimuli were delivered at interstimulus intervals of 30 msec, 50 msec, 70 msec, and 100 msec, 250 msec that generated inhibition of the second population spike of the pair. Amplitude ratios of the population spike were calculated by dividing the amplitude of the second response of the pair by that of the first response. Therefore, amplitude ratios <1 indicated paired pulse inhibition, and amplitude ratios >1 indicated facilitation. The significance of differences between the pre-drug and *post-drug* recordings was evaluated using one-way analysis of variance (ANOVA) followed by LSD *post hoc* analysis. Comparisons between the groups were made with an unpaired Student's t-test. The quantitative values are

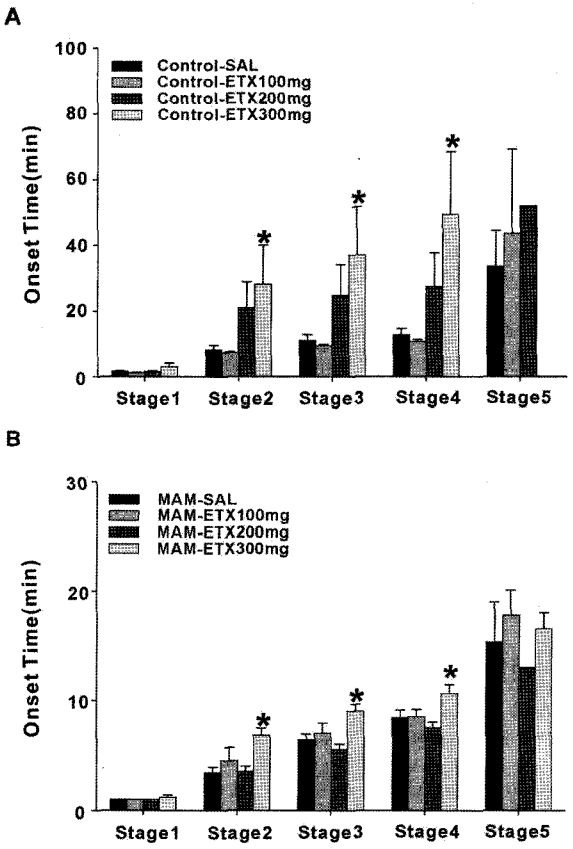

Fig. 1. Effect of ethosuximide (ETX) pre-treatment on the latencies to generalized seizure activity induced by pilocarpine injection (n=43 control rats; n=34 MAM-treated rats). (A) Onset time of each behavioral seizure stage in control rats after ETX pre-treatment. (B) In MAM-exposed rats, the convulsive behavior after administration of ETX. Pilocarpine-induced seizures were graded according to the Racine scale using stage 1~5. Data represented as the mean± SEM. *represents $p<0.05$ compared to the saline group (SAL).

expressed as means±SEM.

Drugs

Pilocarpine hydrochloride and ethosuximide (ETX) were purchased from Sigma. Pilocarpine was dissolved in 0.9% saline and ETX was dissolved in 0.1% DMSO. Together with pilocarpine, ETX was injected i.p. at a volume of 1 ml/kg.

RESULTS

Data for this study were obtained from a total of 77 rats (34 MAM-exposed rats and 43 control rats). The latencies to the onset time of SE (stage 4) were slightly shorter for MAM-exposed rats in comparison with control rats injected with pilocarpine (stage 4: control = 12.7±1.96 min; MAM = 8.4±0.68 min). Seizure latencies with ETX prolonged the mean latency to the stage 4 seizures (control-ETX 100-mg = 10.7±0.60 min; control-ETX 200-mg=27.5±10.08 min). Especially pre-treatment with 300-mg of ETX significantly prolonged the latency to the SE (control-ETX 300-mg=49.4± 18.88 min; $p<0.05$). Pre-treatment with ETX 100-mg and ETX 200-mg had little effect in MAM-exposed rats (MAM-ETX 100-mg=8.5±0.65 min; MAM-ETX 200-mg=7.5± 0.50 min). However ETX 300-mg significantly prolonged the latency to the SE (MAM-ETX 300-mg=10.6±0.81 min; $p<0.05$, Fig. 1).

Pilocarpine increased the amplitude of population spike, evoked in CA1 area by the stimulation of the commissural pathway, in a time-dependent manner. The population spike in the MAM-exposed rats reached the maximum amplitude at 20 min after pilocarpine administration whereas it took 30 min in control rats. In control rats, pilocarpine-induced increase in population spike amplitude was significantly eliminated by pre-administration of ETX (Fig. 2).

Multiple population spikes, indicative of synaptic hyper-

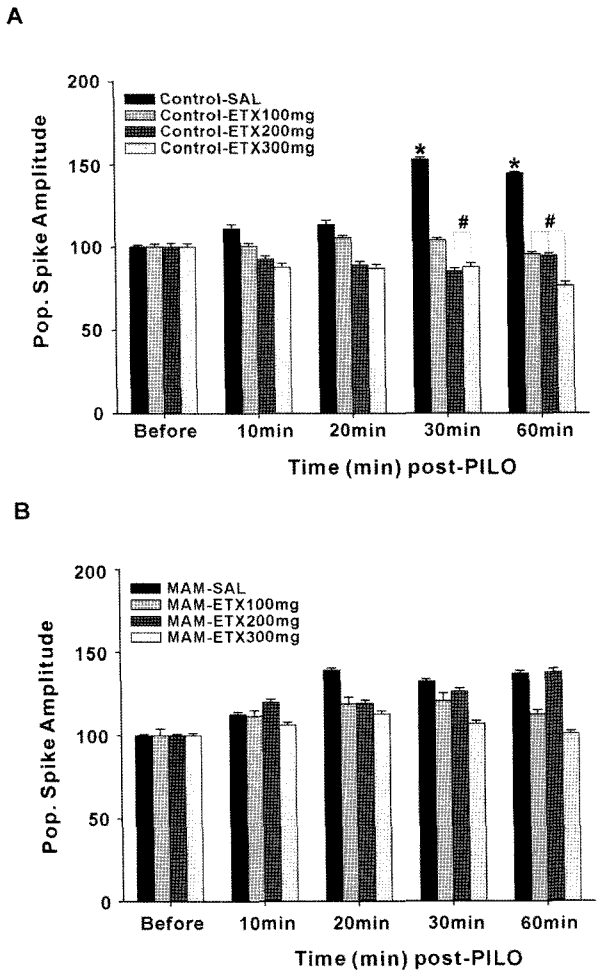

Fig. 2. Time-course of pilocarpine-induced changes in the amplitude of population spike evoked upon commissural stimulation with and without pre-treatment with ETX. (A) The amplitude of population spikes in control rats before and after co-perfusion with ETX. (B) The amplitude of population spike in MAM-exposed rats with and without pre-treatment with ETX. Data represented as the mean±SEM. *represents $p<0.05$ compared to the pre-drug. #represents $p<0.05$ compared to the saline group (SAL).

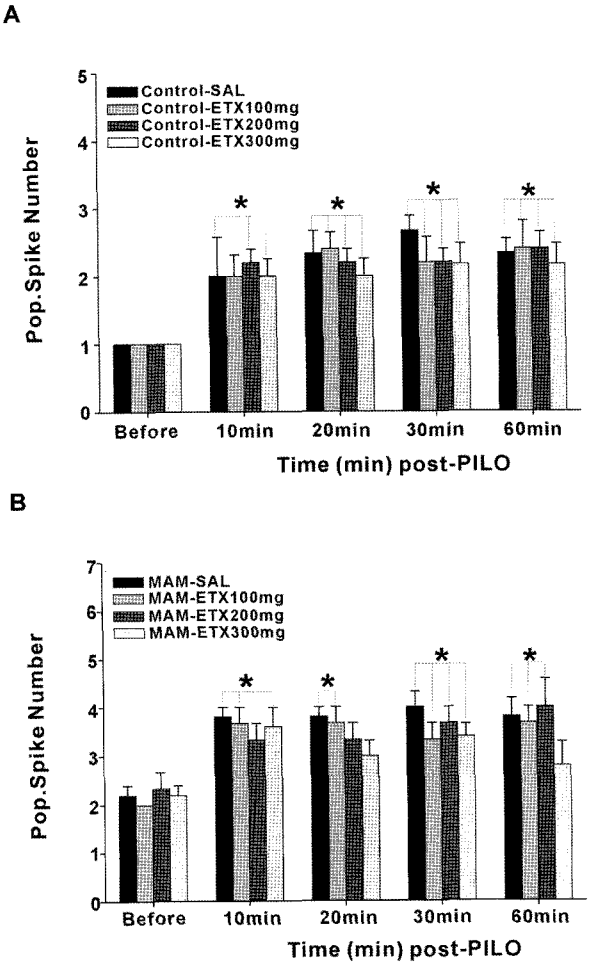

Fig. 3. Effects of ETX on pilocarpine-induced increase in number of the population spike. (A) Population spike number in control rats before and after co-perfusion with ETX. (B) The number of population spike in MAM-exposed rats with and without pre-treatment with ETX. Data are presented as the mean±SEM. *represents $p<0.05$ compared to the pre-drug.

excitability, were observed in hippocampal CA1 area perfused with pilocarpine (Fig. 3). The mean number of population spikes after pilocarpine perfusion was higher in MAM-exposed rats (4±0.32 spikes, 30 min after pilocarpine) than in control rats (2.7±0.21 spikes, 30 min after pilocarpine). ETX reduced the number of evoked population spikes observed during pilocarpine perfusion, and the degree of suppression was similar to control and MAM-exposed rats.

At the 30, 70-msec interstimulus intervals, paired-pulse responses were analyzed in control rats and MAM-exposed rats (Fig. 4, 5). The control rats displayed less paired-pulse inhibition than MAM-treated rats by pilocarpine administration. In control rats, ETX had changed paired-pulse responses but had little inhibited the paired-pulse response during pilocarpine perfusion. Compared with the control rats, MAM-exposed rats showed about the same response as before pilocarpine administration and perfusion of ETX.

Spontaneous field potential was observed in hippocampal CA1 area perfused with ETX (Fig. 6, 7). The control rats showed spontaneous field potential at 30 minutes after pilocarpine injection with pretreatment of 100-mg ETX (1/5 rats), with perfusion of 200-mg ETX (1/5 rats) and with administration of 300-mg ETX (2/5 rats). The MAM-exposed rats showed spontaneous field potential with perfusion of 100-mg ETX (0/3 rats), with perfusion of 200-mg ETX (3/3 rats) and with administration of 300-mg ETX (3/5 rats). Spontaneous field potential was higher for MAM-exposed rats in comparison with control rats injected with ETX. The similar patterns were shown at 60 minutes after pilocarpine injection (control-ETX 100-mg: 2/5 rats; control-ETX 200-mg: 1/5 rats; control-ETX 300-mg: 2/5 rats; MAM-ETX 100-mg: 0/3 rats; MAM-ETX 200-mg: 3/3 rats; MAM-ETX 300-mg: 5/5 rats).

To determine if the synaptic activation of the CA1 population could result in epileptiform after-discharge, we examined the response to single and paired pulses at a maximum stimulation intensity (i.e. 5T or 3T). Pre-treatment with ETX blocked the secondary after-discharges at 30 minutes after pilocarpine injection in control animals. In the MAM-treated rats, ETX did not blocked after-discharges (Fig. 8, 9). These data indicated that afferent activation could result in a long-latency after-discharge in the

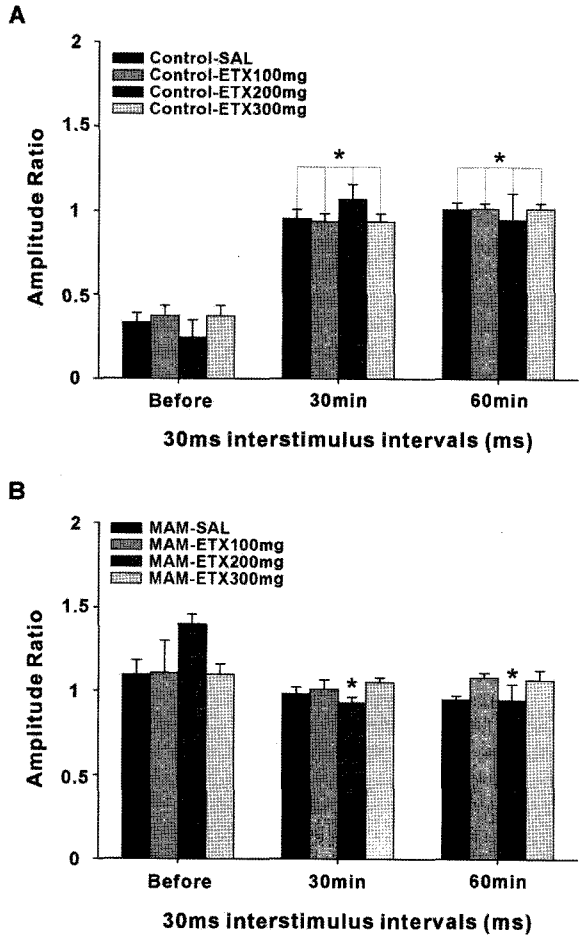

Fig. 4. Effects ETX on paired pulse inhibition at 30 msec interstimulus intervals in CA1. (A) Paired pulse inhibition at 30 msec interstimulus intervals in control rats. (B) Paired pulse inhibition at 30 msec interstimulus intervals in MAM-exposed rats. Data are presented as the mean±SEM. *represents $p<0.05$ compared to the pre-drug.

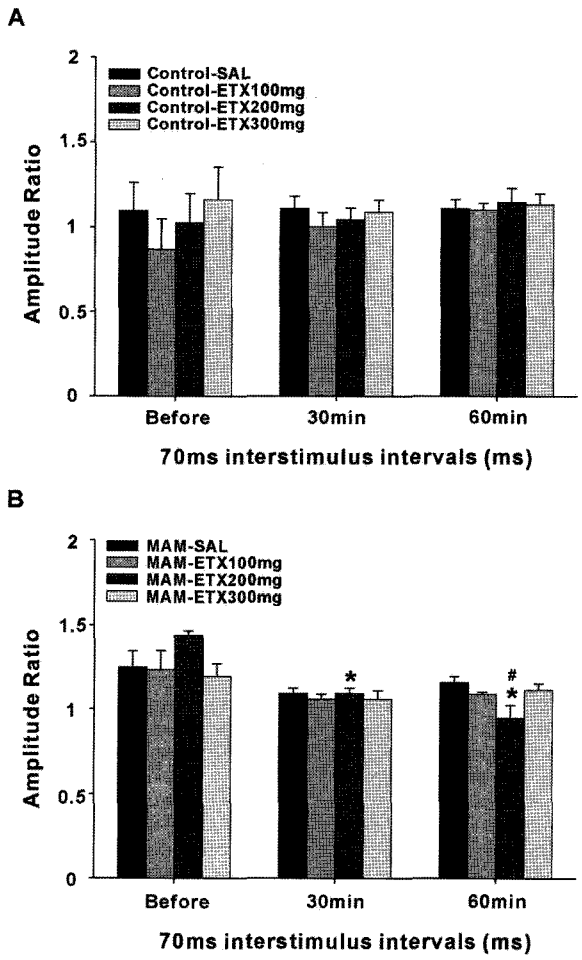

Fig. 5. Effects ETX on paired pulse inhibition at 70 msec interstimulus intervals in CA1. (A) Paired pulse inhibition at 70 msec interstimulus intervals in control rats. (B) Paired pulse inhibition at 70 msec interstimulus intervals in MAM-exposed rats. Data are presented as the mean±SEM. *represents $p<0.05$ compared to the pre-drug. #represents $p<0.05$ compared to the saline group (SAL).

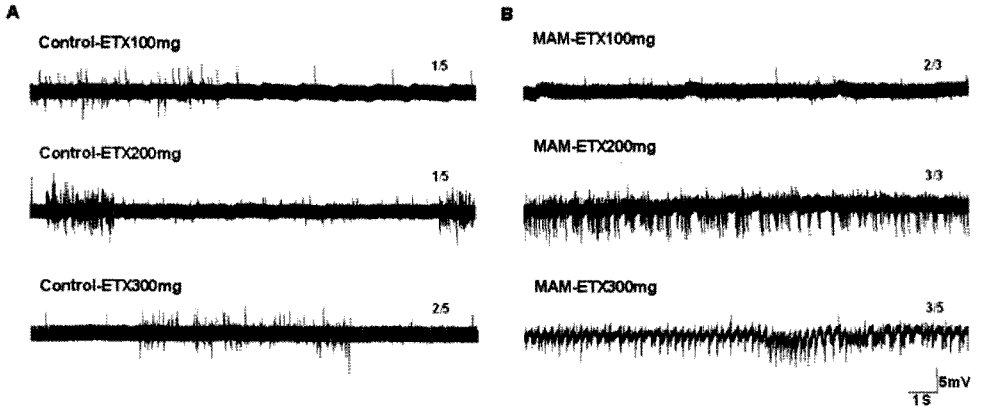

Fig. 6. Effects of ETX on spontaneous field potential 30 minutes after pilocarpine treatment between control (A) and MAM-treated rats in CA1 pyramidal layer of hippocampus (B).

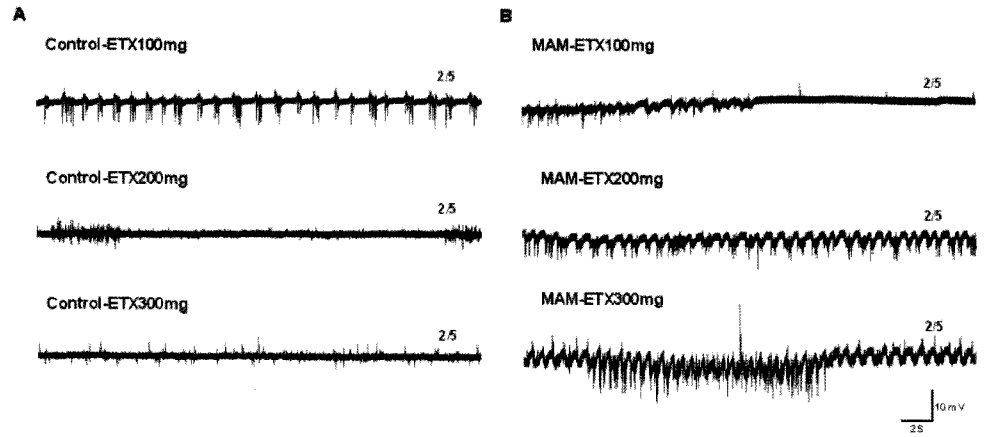

Fig. 7. Effects of ETX on spontaneous field potential 60 minutes after pilocarpine treatment between control (A) and MAM-treated rats in CA1 pyramidal layer of hippocampus (B).

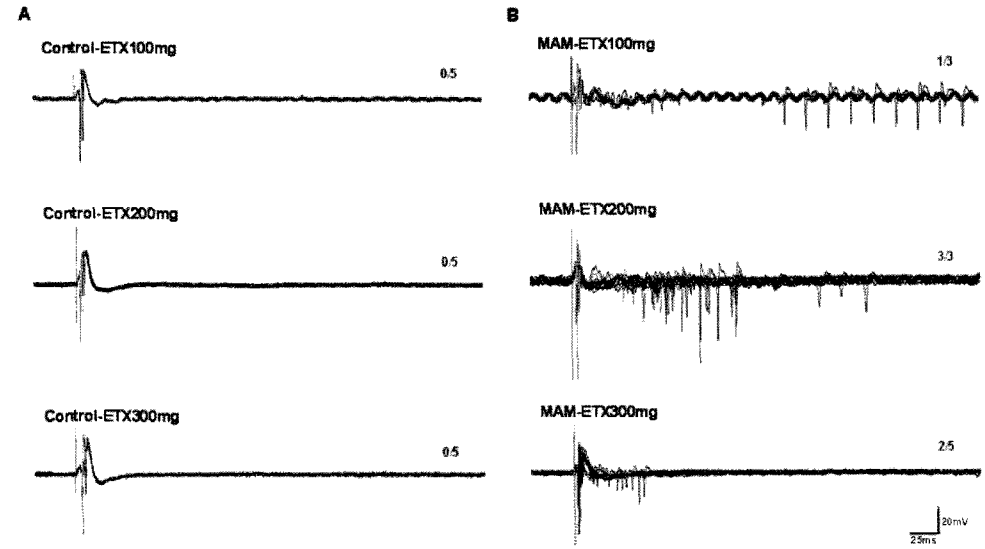

Fig. 8. Field potential after-discharges in the CA1 region after single pulses (5T) with ETX. The response to paired pulse stimulation in control rats with (A) ETX administration. Population responses in the CA1 pyramidal cell layer of MAM-treated rat (B) co-perfusion with ETX. Ten overlapping traces are shown for each data set.

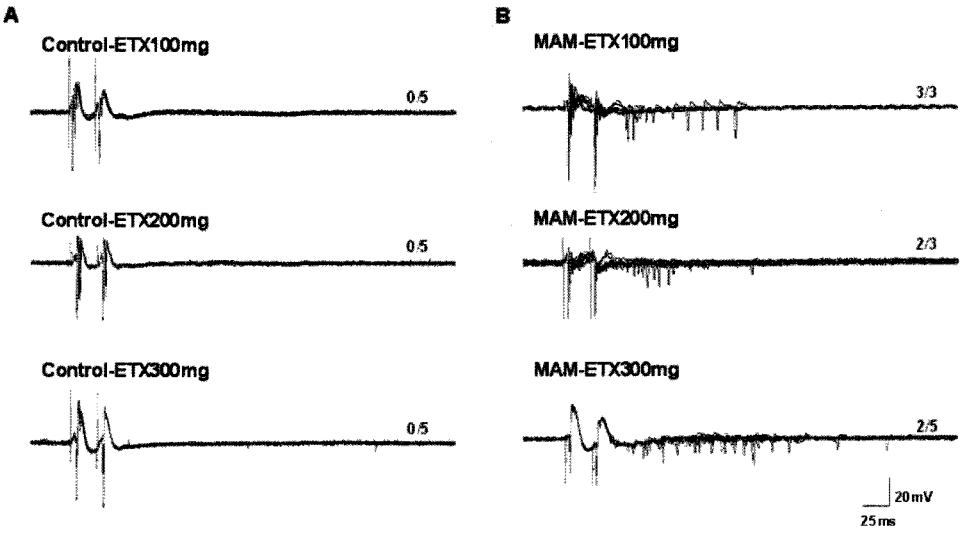

Fig. 9. Field potential after-discharges in the CA1 region after paired pulses (30-msec interstimulus intervals) with ETX. The response to paired pulse stimulation in control rats with (A) ETX administration. Population responses in the CA1 pyramidal cell layer of a MAM-treated rat (B) co-perfusion with ETX. Ten overlapping traces are shown for each data set.

MAM-treated rats but not in the control animals.

DISCUSSION

The exhibition of behavioral and neuropharmacological changes that are similar to those found in cortical dysplasia epilepsy patients is an important issue in the development of animal models for cortical dysplasia epilepsy. The occurrences of recurrent, spontaneous seizures are the most easily identified behavioral criterion for malformation-associated epilepsy development in animal models (Hellier et al, 1998). Epilepsy patients with a cortical malformation have seizure foci in neuronal heterotopias (Palmini et al, 1991). It is established that heterotopic brain regions are potential sites of seizure genesis. Numerous patients with cortical dysplasia associated epilepsy have been recorded to confirm that dysplasia can be the initial focus of abnormal electrical activity (Baba et al, 1983; Babb et al, 1998; Glien et al, 2002; Loscher, 2002). The present data demonstrate that the MAM-pilocarpine rats develop spontaneous seizures after this treatment. Some studies from MAM-exposed rats indicated that the threshold for generation of paired pulse inhibition (PPI), amplitude of population spike, and afterdischarge were significantly lower than in age-matched controls (Prayson & Estes, 1995). Our results that pilocarpine-induced PPI, amplitude population spike, and after-discharge were easier to initiate in the dysplastic brain are consistent with these findings, possibly related to the intrinsic hyperexcitability of heterotopic neurons in neocortex. Electrophysiological data from heterotopic neurons in the MAM-treated rats suggests that the heterotopic CA1 neurons were connected with each other as well as with neurons in the overlying neocortex (Chevassus-au-Louis et al, 1998a; Baraban et al, 2000). The present results indicate that the neurons in the CA1 of the MAM-treated rats with dysplasia and nodular heterotopia are more excitable than that of the CA1 pyramidal neurons in the normal rats. To ascertain the connectivity patterns of normotopic CA1 neurons, further studies will be necessary. It is not confirmed yet whether an animal model undergoes cellular changes in the brain to render it more susceptible to spontaneous seizure generation (Dudek & Spitz, 1997). The pilocarpine and kainate models have shown that aberrant mossy fiber sprouting with associated hippocampal cell loss following status epilepticus was formed (Ben-Ari, 1985; Mello et al, 1993). These studies suggested that network hyperexcitability is correlated with axonal spouting. Similar experiments conducted on rats (Mello et al, 1993) and albino mice (Cavalheiro et al, 1996) had demonstrated the recurrent spontaneous seizures and mossy fiber sprouting. The increased susceptibility to seizure in MAM-exposed animals using a variety of techniques including kainate acid (Germano & Sperber, 1997), kindling (Chevassus-au-Louis et al, 1998b), hyperthermia (Germano et al, 1996), flurothyl (Baraban & Schwartzkroin, 1995), and bicuculline (de Feo et al, 1995) was demonstrated. Present study demonstrated a tendency toward increased seizure susceptibility in control and MAM-exposed rats following intraperitoneal administration of pilocarpine. MAM-treated rats have a pharmaco-resistance when pilocarpine was used to induce seizure. These behavioral findings confirm our electrophysiological results while closely similar with the clinical findings (Palmini et al, 1991; Hirabayashi et al, 1993). If the MAM-exposed animals mimic features of cortical dysplasia-associated epilepsies and are resistant to standard AEDs, further analysis of this model may be useful in the development of novel treatment.

The effects of standard AEDs on pilocarpine-induced seizure activity have been evaluated (Fueta & Avoli, 1992; Yamaguchi & Rogawski, 1992; Bruckner et al, 1999; Bruckner & Heinemann, 2000). Although kainate acid, kindling, or a number of other manipulations can also reliably evoke seizure activity, we used the pilocarpine-induced bursting model of ethosuximide to more efficiently compare control and MAM-exposed animals (Fueta & Avoli, 1992; Bruckner

& Heinemann, 2000).

Ethosuximide has considerable efficacy against pentylenetetrazol seizures and was introduced as a pure petit mal drug. Ethosuximide has an important effect on Ca^{2+} currents, reducing the low-threshold (T-type) current. This effect is seen at therapeutically relevant concentrations in thalamic neurons. Ethosuximide also inhibits Na^{+}/K^{+} ATPase, depresses the cerebral metabolic rate, and inhibits GABA aminotransferase.

In this study, the hyperexcitability of hippocampal CA1 induced by pilocarpine was measured in several ways: quantitative assessment of population spike (amplitude and number) and paired pulse inhibition, analysis of afterdischarge evoked by electrical stimulation, convulsive behavior test, and spontaneous field potential. In control studies, significant effects of ethosuximide on quantitative assessment of population spike (amplitude and number) and convulsive behavior test that were largely consistent with previous reports (Choi, 2005). In contrast to control studies, pilocarpine-induced seizure activity in MAM-exposed rats was relatively resistant to ethosuximide tested. Ethosuximide had little or no effect on quantitative assessment of population spike (amplitude and number) and paired pulse inhibition, analysis of afterdischarge, convulsive behavior test and spontaneous field potential in dysplastic brain. Ethosuximide evoked a modest suppression of population spike number and convulsive behavior in MAM-exposed animal. Because the mechanism of action for ethosuximide probably involve modulation of Ca^{2+} channel activity, our findings in the MAM model suggests that inhibition of these channels may not be an effective means to reduce hyperexcitability in the dysplastic brain.

Cortical dysplasia with pharmaco-resistant seizure disorders makes it intractable epilepsy. Multiple trials using new drugs to treat convulsion in epilepsy patients made partial success and often with troublesome and sometimes injurious side effects. Sometimes surgical resection of dysplastic brain regions is frequently successful. Little attempt had been made to investigate pharmaco-resistance in patients with dysplasia, even though a large body of clinical information is available regarding morphological properties of malformed brains. The pathophysiological mechanism by which a dysplastic brain becomes resistant to AED intervention is not fully understood, it is evident both from clinical and now experimental studies, that a characteristic feature of dysplastic tissue is pharmaco-resistance to available medications. In present study, systematic evaluation of the effects of ethosuximide in malformed epilepsy model had done. MAM-exposed rats have served as a useful model of cortical dysplasia-associated epilepsy. Our data demonstrate a correlation between in vivo electrophysiological recordings and behavior response to ETX. If present findings confirm the pharmacological resistance in an animal model designed to mimic malformation-induced epilepsy, our experiment support that pathological basis underlying epileptogenesis in malformation-associated epilepsy differ from those at work in other forms of epilepsy more responsive to standard AEDs.

REFERENCES

Aicardi J. The place of neuronal migration abnormalities in child neurology. *Can J Neurol Sci* 21: 185–193, Review, 1994

Amano S, Ihara N, Umeura S. Development of novel rat mutant with spontaneous limbic-like seizures. *Am J Pathol* 149: 329–336, 1996

Baba A, Okumura S, Mizuo H, Iwata H, The inhibition of diazepam and gamma-aminobutyric acid of depolarization-induced release of [14C] cysteine sulfinate and [3H] glutamate in rat hippocampal slices. *J Neurochem* 40: 280–284, 1983

Babb TL, Ying Z, Hadam J, Penrod C. Glutamate receptor mechanisms in human epileptic dysplastic cortex. *Epilepsy Res* 32: 24–33, 1998

Baraban SC, Schwartzkroin PA. Electrophysiology of CA1 pyramidal neurons in an animal model of neuronal migration disorders: prenatal methylazoxymethanol treatment. *Epilepsy Res* 22: 145–156, 1995

Baraban SC, Wenzel HJ, Hochman DW, Schwartzkroin PA. Characterization of heterotopic cell clusters in the hippocampus of rats exposed to methylaxzoxymethanol in utero. *Epilepsy Res* 39: 87–102, 2000

Barth PG. Disorders of cerebral migration. *Can J Neurol Sci* 14: 1–16, 1987

Ben-Ari Y. Limbic seizure and brain damage produced by kainic acid: mechanisms and relevance to human temporal lobe epilepsy. *Neuroscience* 14: 375–403, 1985

Bruckner C, Heinemann U. Effects of standard anticonvulsant drugs on different patterns of epileptiform discharges induced by 4-aminopyridine in combined entorhinal cortex-hippocampal slices. *Brain Res* 859: 15–20, 2000

Bruckner C, Stenkamp K, Meierkord H, Heinemann U. Epileptiform discharges induced by combined application of bicuculline and 4-aminopyridine are resistant to standard anticonvulsants in slices of rats. *Neurosci Lett* 268: 163–165, 1999

Calcagnotto ME, Maredes MF, Baraban SC. Heterotopic neurons with altered inhibitory synaptic function in an animal model of malformation-associated epilepsy. *J Neurosci* 22: 7596–7605, 2002

Castro PA, Pleasure SJ, Baraban SC. Hippocampal heterotopia with molecular and electrophysiological properties of neocortical neurons. *Neuroscience* 114: 961–972, 2002

Cavalheiro EA, Santos NF, Priel MR. The pilocarpine model of epilepsy in mice. *Epilepsia* 37: 1015–1019, 1996

Chen HX, Roper SN. Reduction of spontaneous inhibitory synaptic activity in experimental heterotopic gray matter. *J Neurophysiol* 89: 150–158, 2003

Chen S, Hillman DE. Selective ablation of neurons by methylazoxymethanol during pre- and postnatal brain development. *Exp Neurol* 94: 103–119, 1986

Chevassus-au-Louis N, Congar P, Represa A, Ben-Ari Y, Gaiarsa JL. Neuronal migration disorders: heterotopic neocortical neurons in CA1 provide a bridge between the hippocampus and the neocortex. *Proc Natl Acad Sci* 95: 10263–10268, 1998a

Chevassus-au-Louis N, Ben-Ari Y, Vergnes M. Decreased seizure threshold and more rapid rate of kindling in rats with cortical malformation induced by prenatal treatment with methylazoxymethanol. *Brain Res* 812: 252–255, 1998b

Choi IS, Cho JH, Lee MG, Choi BJ. Pilocarpine-induced seizure susceptibility in rats following prenatal methylazoxymethanol treatment. *Biol Pharm Bull* 28: 1408–1413, 2005

Ciaroni S, Cecchini T, Gazzanelli G, Grande PD. Methylazoxymethanol (MAMac) effects on ontogenesis of the mouse neocortex. *J Hirnforsch* 30: 699–705, 1989

de Feo MR, Mecarelli O, Ricci GF. Seizure susceptibility in immature rats with micrencephaly induced by prenatal exposure to methylazoxymethanol acetate. *Pharmacol Res* 31: 109–114, 1995

Dudek FE, Spitz M. Hypothetical mechanisms for the cellular and neurophysiologic basis of secondary epileptogenesis: proposed role of synaptic reorganization. *J Clin Neurophysiol* 14: 90–101, Review 1997

Dudek FE. Use of chronic epilepsy models in antiepileptic drug discovery: the effect of topiramate on spontaneous motor seizures in rats with kainate-induced epilepsy. *Epilepsia* 46: 8–14, 2005

Duncan JS. The promise of new antiepileptic drugs. *Br J Clin Pharmacol* 53: 123–131, 2002

Fleck MW, Hirotsune S, Gambello MJ, Phillips-Tansey E, Suares G, Mervis RF, Wynshaw-Boris A, McBain CJ. Hippocampal abnormalities and enhanced excitability in a murine model of human lissencephaly. *J Neurosci* 20: 2439–2450, 2000

Fueta Y, Avoli M. Effects of antiepileptic drugs on 4-aminopyridine-induced epileptiform activitity in young and adult rat hippocampus. *Epilepsy Res* 12: 207–215, 1992

Germano IM, Sperber EF, The effects of neuronal migration disorders on seizure susceptibility. *Epilepsia* 35(Suppl.8): 137, 1994

Germano IM, Sperber EF. Increased seizure susceptibility in adult rats with neuronal migration disorders. *Brain Res* 28; 219–222, 1997

Germano IM, Zhang YF, Sperber EF, Moshe SL. Neuronal migration disorders increase susceptibility to hyperthermia-induced seizures in developing rats. *Epilepsia* 37: 902–910, 1996

Glien M, Brandt C, Potschka H, Loscher W. Effects of the novel antiepileptic drug levetiracetam on spontaneous recurrent seizures in the rat pilocarpine model of temporal lobe epilepsy. *Epilepsia* 43: 350–357, 2002

Hellier JL, Patrylo PR, Buckmaster PS, Dudek FE. Recurrent spontaneous motor seizures after repeated low-dose systemic treatment with kainate: assessment of a rat model of temporal lobe epilepsy. *Epilepsy Res* 31: 73–84, 1998

Hirabayashi S, Binnie CD, Janota I, Polkey CE. Surgical treatment of epilepsy due to cortical dysplasia: clinical and EEG findings. *J Neurol Neurosurg Psychiatry* 56: 765–770, 1993

Houser CR. Granule cell dispersion in the dentate gyrus of human with temporal lobe epilepsy. *Brain Res* 535: 195–204, 1990

Loscher W. Animal models of epilepsy for the development of antiepileptogenic and disease-modifying drugs. A comparison of the pharmacology of kindling and post-status epilepticus models of temporal lobe epilepsy. *Epilepsy Res* 50: 105–123, 2002

Loscher W, Honack D. Profile of UCB L059, A novel anticonvulsant drug, in models of partial and generalized epilepsy in mice and rats. *Eur J Pharmacol* 232: 147–158, 1993

Mello LM, Cavalhiero EA, Tan AM. Circuit mechanisms of seizures in the pilocarpine model of chronic epilepsy: cell loss and mossy fiber sprouting. *Epilepsia* 34: 985–995, 1993

Palmini A, Andermann F, Olivier A, Tampieri D, Robitaille Y. Focal neuronal migration disorders and intractable partial epilepsy: results of surgical treatment. *Ann Neurol* 30: 750–757, 1991

Palmini A. Disorders of cortical development. *Curr Opin Neurol* 13: 183–192, 2000

Prayson RA, Estes ML. Cortical dysplasiz: a histopathologic study of 52 cases of partial lobectomy in patients with epilepsy. *Hum Pathol* 26: 493–500, 1995

Racine RJ. Modification of seizure activity by electrical stimulation, II: motor seizure. *Electroencephalogr Clin Neurophysiol* 32: 281–294, 1972

Sancini G, Franceschetti S, Battaglia G, Colacitti C, Di Luca M, Spreafico R, Avanzini G. Dysplastic neocortex and subcortical heterotopias in methylazoxymethanol-treated rats: an intracellular study of identified pyramidal neurones. *Neurosci Lett* 246: 181–185, 1998

Smyth MD, Barbaro NM, Baraban SC. Effects of antiepileptic drugs on induced epileptiform activity in a rat model of dysplasia. *Epilepsy Res* 50: 251–264, 2002

Spreafico R, Pasquier B, Minotti L, Garbelli R, Kahane P, Grand S, Benabid A, Tassi L, Avanzini G, Battaglia G, Munari C. Immunocytochemical investigation on dysplastic human tissue from epileptic patients. *Epilepsy Res* 32: 32–48, 1998

Stables JP, Bertram EH, White HS, Coulter DA, Dichter MA, Jacobs MP, Loscher W, Lowenstein DH, Moshe SL, Noebels JL, Davis M. Models for epilepsy and epileptogenesis: report from the NIH workshop, Bethesda, Maryland. *Epilepsia* 43: 1410–1420, 2002

Wenzel HJ, Robbins CA, Tsai LH, Schwartzkroin PA. Abnormal morphological and functional organization of the hippocampus in a p35 mutant model of cortical dysplasia associated with spontaneous seizures. *Neurosci Feb* 21: 983–998, 2001

White HS. Preclinical development of antiepileptic drugs: past, present, and future directions. *Epilepsia* 44(suppl 7): 2–8, 2003

Yamaguchi S, Rogawski MA. Effects of anticonvulsant drugs on 4-aminopyridine-induced seizures in mice. *Epilepsy Res* 11: 9–16, 1992

Zhu WJ, Roper SN. Reduced inhibition in an animal model of cortical dysplasia. *J Neurosci* 20(23): 8925–8931, 2000