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Effects of Phenytoin and Diazepam on the Seizure Activity in the Cortical Dysplasia Animal Models

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Dysplasia-associated seizure disorders are markedly resistant to pharmacological intervention. Relatively little research has been conducted studying 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 AEDs 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 amplitude and numbers of population spikes, and paired pulse inhibition in response to stimulation of commissural pathway. Two commonly used AEDs were tested: diazepam 5, 2.5 mg/kg; phenytoin 40, 60 mg/kg. Diazepam (DZP) and phenytoin (PHT) reduced the amplitude of population spike in control and MAM-exposed rats. However, the amplitude of population spike was nearly eliminated in control rats as compared to the MAM-exposed rats. Pharmaco-resistance was tested by measuring seizure latencies in awake rats after pilocarpine administration (320 mg/kg, i.p.) with and without pre-treatment with AEDs. Pre-treatment with PHT 60 mg prolonged seizure latency in control rats, but not in MAM-exposed animals. The main findings of this study are that acute seizures initiated in MAM-exposed rats are relatively resistant to standard AEDs assessed in vivo. These data suggest that animal model with cortical dysplasia can be used to screen the effects of potential AEDs.

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Introduction

Development of the mammalian brain occurs through a complex and highly ordered sequence of events. Disturbances of these developmental processes are now recognized as resulting in a wide spectrum of brain malformations (Castro *et al.*, 2002). More than 40,000 infants born each year have seizure disorders associated with cortical malformations (Smyth *et al.*, 2002). Clinically malformations are broadly classified as neuronal migration disorders and are often associated with medically intractable seizures, developmental delay, or neurological deficit. (Barth, 1987; Aicardi, 1994; Palmini, 2000).

Epileptic seizures associated with a brain malformation are frequently severe and resistant to conventional anti-convulsant drugs. Often, surgical removal of abnormally organized tissue is the only effective form of seizure control for these early-onset epilepsies (Calcagnotto *et al.*, 2002).

Traditional AED testing has been used in the acute-seizure models based on chemical and electrical induction of seizures in normal animal. Efficacious AEDs may be ineffective in these models (Stables *et al.*, 2002). Hypothetically, new AEDs that would be effective in pharmacoresistant epilepsy may be discovered by testing them in animal models with cortical malformation, and these new AEDs may be ineffective in the acute-seizure models (Loscher and Honack, 1993; White, 2003). Relatively little research has been conducted studying the effects of AEDs on spontaneous seizures in animals with cortical dysplasia-associated

epilepsy. If epileptogenesis involves new mechanisms not present in the normal brain (e.g., altered receptor subunits or new circuits), then traditional AED testing in acute-seizure models may not identify effective versus ineffective drugs, because they tested on animals whose brains have not undergone the epileptogenic changes (Dudek, 2005).

Brain malformations are found not only in neocortical structures but also in the hippocampal formation (Houser, 1990; Hirabayashi *et al.*, 1993). These hippocampal malformation, which include microdysgenesis, granule cell dispersion, and nodular heterotopia, are especially intriguing given that the hippocampus is implicated in temporal lobe epilepsy (Buckmaster and Schwarzkroin, 1994). To study the functional and molecular properties of dysplastic neurons, a number of animal models have been developed that feature hippocampal dysgenesis. These include rats exposed to irradiation or methylazoxymethanol (MAM) *in utero* (Baraban and 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 have been reported.

In rats, the application of MAM in utero (E15) results in the formation of dysplastic regions in the neocortex and the CA1 region of the hippocampus, as well as heterotopic clusters of neurons in the subcortical white matter (Chen and Hillman, 1986; Ciaroni *et al.*, 1989). Some MAM-treated pups with both neocortical and hippocampal dysgenesis have a lower threshold for seizure activity (Germano and Sperber, 1994). Recording in the slices from MAM-treated rats have been suggested that the dysplastic neocortex and subcortical heterotopia can be induced to exhibit epileptiform bursting, but the electrophysiological characteristics *in vivo* of hippocampal neurons have not been examined in this model. Heterotopic CA1 pyramidal neurons appear to have atypical electrophysiological and morphological characteristics (Chevassus-au-Louis *et al.*, 1998a) and may also form abnormal connections with neocortical regions (Chevassus-au-Louis *et al.*, 1998b).

The present study was focused on rats exposed to MAM *in utero* because they are highly seizure susceptible (de Feo *et al.*, 1995) and share many anatomical similarities with human malformation-associated epilepsies (Spreafico *et al.*, 1998). Although heterotopic neurons in the MAM model lack Kv4.2 A-type potassium channels and exhibit burst firing properties (Sancini *et al.*, 1998), there is no evidence of either spontaneous epileptic seizures *in vivo* or independent burst generation *in vitro*. On going anatomical, molecular, and electrophysiological characterizations of these animals are contributing to improve the understanding whether how seizure activity develops in the dysplastic brain. However, whether any of these animals respond to common AEDs have not been systematically investigated. To address this issue, the MAM-exposed rats were used to evaluate potential anticonvulsant resistance *in vivo* in the present study. In the present study, in vivo pharmacological experiments were performed on MAM-exposed animals to test whether AEDs, phenytoin and diazepam, alter pilocarpine-induced epileptiform activity and seizures.

Materials and Methods

Animals

Pregnant Sprague-Dawley (SD) rats were injected with 25 mg/kg MAM dissolved in 0.9% saline (ip) on embryonic day 15. 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

SD 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 and MAM-exposed rats were injected (i.p.) with a dose of 320 mg/kg of pilocarpine in 0.9% saline. After the administration of pilocarpine, the convulsive behaviors were scored according to the Racine scale: grade 0, in which rats showed no convulsion; grade 1, in which rats showed head bobbing, tremor, backward walking, wet dog shake; grade 2, intermittent forepaw myoclonus, rearing and falling; grade 3, continuous chronic convulsion; grade 4, tonic flexion; and grade 5, respiratory arrest.

In vivo recording: SD rats were anesthetized with urethane (1.3 g/kg), according to procedures reported in Ref (Choi *et al*, 2004). Briefly, the recording electrode was positioned 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. Commissural pathway stimulation was made using single or paired pulses. Paired pulse stimulation was used to assess inhibition in the CA1 network. Pairs of stimuli were delivered at interstimulus intervals of 30 and 70 milli-seconds 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 t-test. The quantitative values are expressed as means± SEM.

Drugs

Pilocarpine hydrochloride and PHT were purchased from

Sigma. Pilocarpine was dissolved in 0.9% saline and PHT was dissolved in DMSO. Together with pilocarpine, PHT was injected i.p. at a volume of 1 ml/kg. DZP was dissolved in a commercial solution (Diazepam-ratiopharm) and injected in a volume of 1 mg/kg, i.p.

Results

Data for this study were obtained from a total of 92 rats (41 MAM-exposed rats and 51 control rats) treated with AEDs. The latencies to the onset time of status epilepticus (SE, Stage 4) were slightly shorter for MAM-exposed rats in comparison with control rats injected with pilocarpine (Stage 4: control = 23±2.82 min; MAM = 16±1.75 min; $P < 0.05$). Although pre-treatment with 60 mg of PHT significantly prolonged the mean latency to the stage 4 seizures (n=6; 51.0±13.0 min; $P<0.05$), it had little effect in the MAM-exposed rats (n=5; 22.0±10.7 min). DZP suppressed the convulsive behavior in control and the MAM-exposed rats (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 (Fig. 2). 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 (6 control rats and 5 MAM-treated rats). In control rats, pilocarpine-induced increase of population spike amplitude was nearly eliminated by pre-administration of AEDs. For the current study, control and MAM-exposed rats were perfused with DZP (2.5 and 5 mg) and PHT (40 and 60 mg). DZP and PHT reduced the amplitude of population spike in control (4 at DZP 2.5 mg, 4 at DZP 5 mg, 6 at PHT 40 mg, 6 at PHT 60 mg) and MAM-exposed rats (5 at DZP 2.5 mg, 5 at DZP 5 mg, 5 at PHT 40 mg, 5 at PHT 60 mg). However, the amplitude of population spike was nearly eliminated in control rats as compared to the MAM-exposed rats (Fig. 2).

Multiple population spikes, indicative of synaptic hyper-excitability, were observed in hippocampal CA1 area

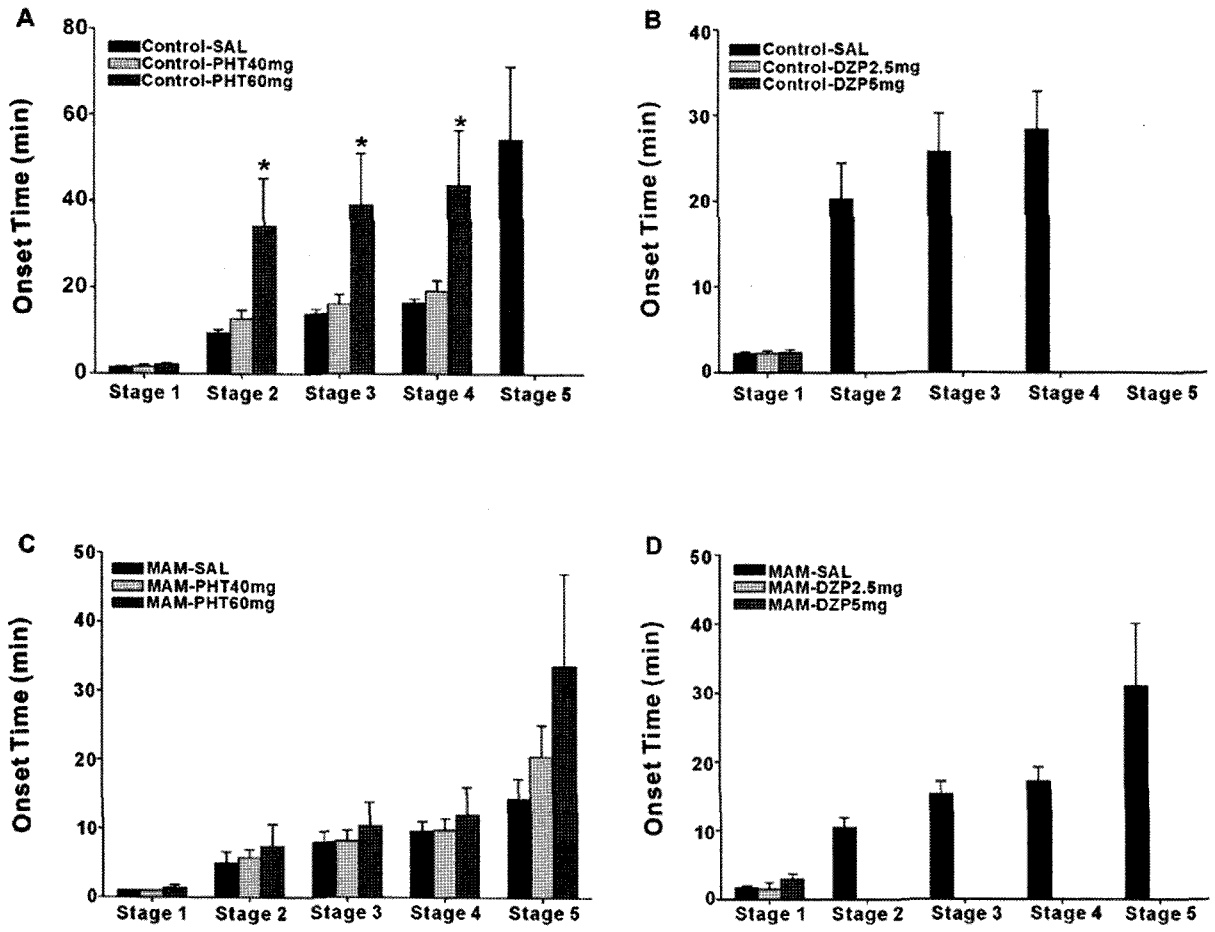

Fig. 1. Effect of AED pre-treatment on the latencies to generalized seizure activity induced by pilocarpine injection. (A) Onset time of each behavioral seizure stage in control rats (9 at PHT 40 mg, 6 at PHT 60 mg) after PHT pre-treatment. (B) After administration of PHT, the convulsive behavior in MAM-exposed rats (5 at PHT 40 mg, 5 at PHT 60 mg). Pilocarpine-induced seizures were graded according to the Racine scale using stage 1-5. (C) Behavioral seizure in control (4 at DZP 2.5 mg, 6 at DZP 5 mg) and MAM-exposed rats (4 at DZP 2.5 mg, 6 at DZP 5 mg) by DZP perfusion. Data are presented as the mean±SEM. * represents $P<0.05$ compared to the saline groups.

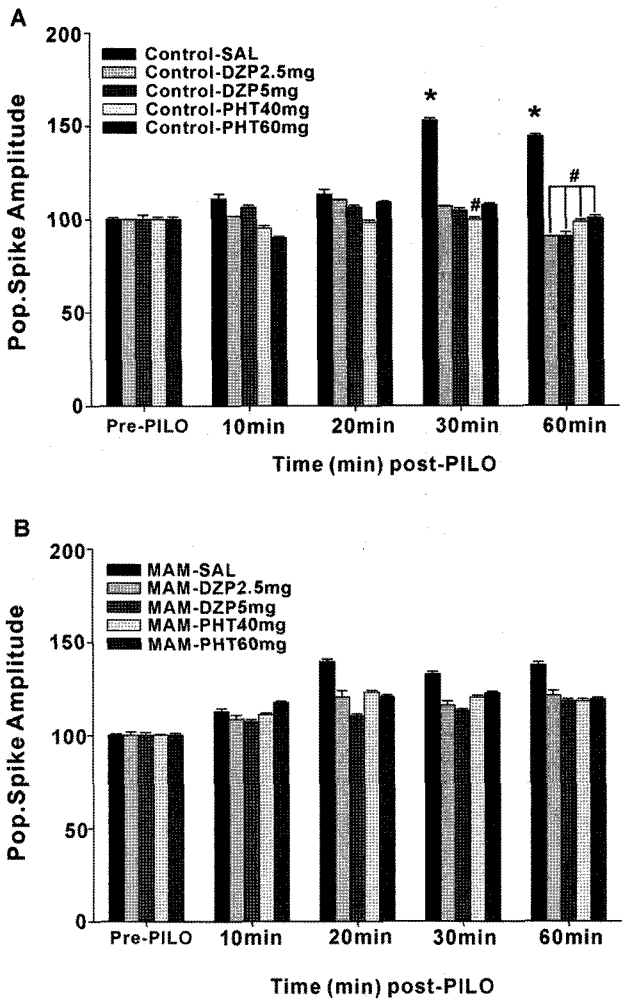

Fig. 2. Time-course of pilocarpine-induced changes in the amplitude of population spike evoked upon commissural stimulation with and without pre-treatment with AEDs. (A) Representative extracellular field recordings in control rats before and after co-perfusion with AEDs. (B) The amplitude of population spike in MAM-exposed rats with and without pre-treatment with AEDs. Normalized amplitude of population spikes as a function of stimulus intensity in control and MAM-treated rats before and after co-perfusion with AEDs. The amplitude of stimulation intensity at 5T was considered to be 100% 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.

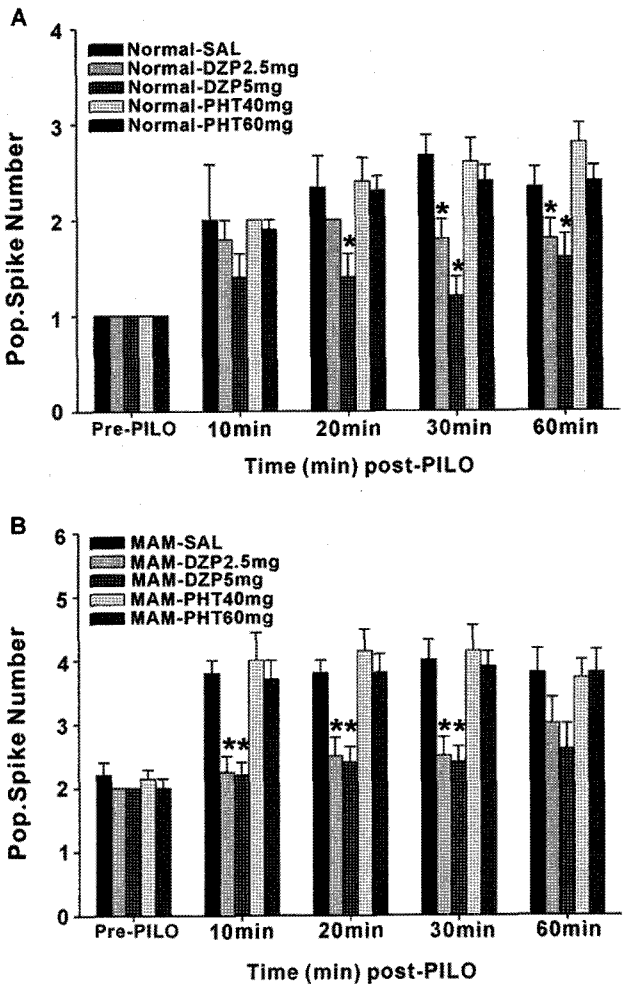

Fig. 3. Effects of AEDs on pilocarpine-induced increase in number of the population spike. (A) Population spike number in control rats before and after co-perfusion with AEDs. (B) The number of population spike in MAM-exposed rats with and without pre-treatment with AEDs. Data are presented as the mean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

perfused with pilocarpine (Fig. 3). The mean number of population spikes after pilocarpine perfusion was higher in MAM-exposed rats (4±0.32 spikes; 5 MAM-treated rats; 30 min after pilocarpine) than in control rats (2.7±0.21 spikes; 3 control rats; 30 min after pilocarpine). DZP 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- and 70-msec interstimulus intervals, paired-pulse responses were analyzed in control rats and MAM-exposed rats (Fig. 4). The control rats displayed less paired-pulse inhibition by pilocarpine administration (0.2853±0.14 ratio, before pilocarpine; 1.035±0.22, 30 min after pilocarpine; 0.9268±0.10, 60 min after pilocarpine; n=4). In control rats, DZP (2.5 and 5 mg) and PHT (60 mg) inhibited the paired-pulse response during pilocarpine perfusion at 30-msec interstimulus intervals. At 70-msec interstimulus intervals, DZP (5 mg) reduced 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 all AEDs.

Spontaneous field potential was observed in hippocampal CA1 area perfused with PHT. The control rats showed spontaneous field potential at 30 minutes after pilocarpine injection with pretreatment of 60 mg PHT (40%, 4/10 rats) and with perfusion of 40 mg PHT (14.3%, 1/7 rats). The MAM-exposed rats showed spontaneous field potential with perfusion of 60 mg PHT (90%, 9/10 rats) and with

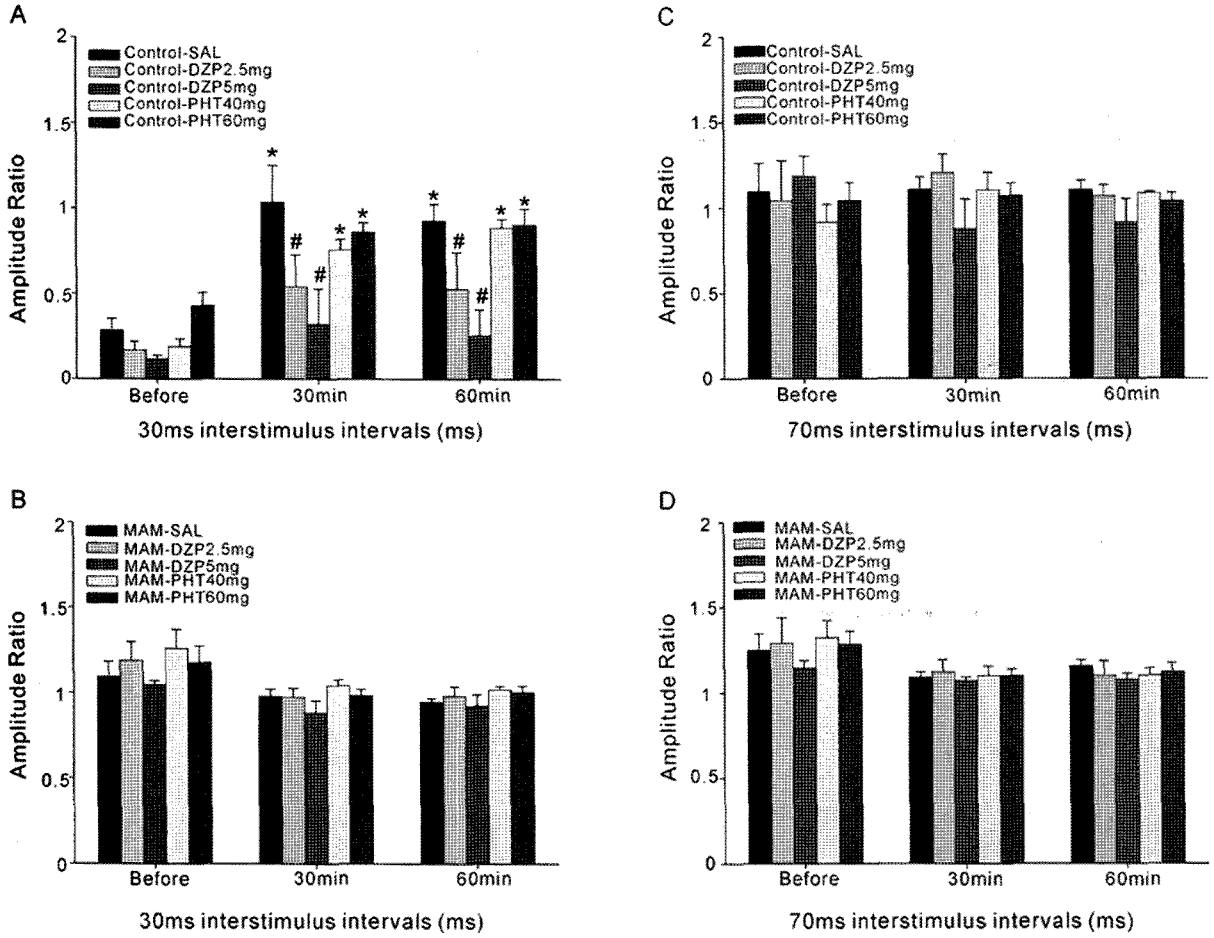

Fig. 4. Effects AED on paired pulse inhibition at 30 and 70 msec interstimulus intervals in CA1. (A) Paired pulse inhibition at 30 msec interstimulus intervals in control rats. (B) Paired pulse inhibition at 30 msec interstimulus intervals in MAM-exposed rats. (C) Field potential responses to 70 msec interstimulus intervals in control rats. (D) Field potential responses to 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.

perfusion of 40 mg PHT (88.9%, 8/9 rats). At 60 minutes after pilocarpine injection, the control rats showed spontaneous field potential with perfusion of 60 mg PHT (30%, 3/10 rats) and with perfusion of 40 mg PHT (28.6%, 2/7 rats). The MAM-exposed rats showed spontaneous field potential with perfusion of 60 mg PHT (60%, 9/10 rats) and with perfusion of 40 mg PHT (88.9%, 8/9 rats). Spontaneous field potential was higher for MAM-exposed rats in comparison with control rats injected with PHT (Fig. 5).

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 maximum stimulation intensity (5 T, 30-msec interstimulus intervals, Fig. 6, 7). At 30 minutes after pilocarpine injection in control animals, single pulse showed pre-treatment with PHT blocked the secondary after-discharges. At 30 minutes after pilocarpine injection, paired pulse showed in 3 out of 10 control animals by pre-treatment with 60 mg PHT and in 2 out of 7 control animals by pre-treatment with 40 mg PHT. In the MAM-exposed rats after pre-administration of PHT, single pulses resulted in secondary after-discharge in at least some of the trials from 3 out of 9 animals by pre-administration of 60 mg PHT and from 5 out of 10 animals by pre-administration of 40 mg PHT (Fig. 6). The control rats showed secondary after discharge at 60 minutes after pilocarpine injection with pretreatment of 60 mg PHT (3/10 rats after single and paired pulse) and with perfusion of 40 mg PHT (2/6 rats after single pulse; 2/7 after paired pulse). The MAM-exposed rats showed secondary after discharge with perfusion of 60 mg PHT (3/9 rats after single pulse; 5/9 rats after paired pulse) and with perfusion of 40 mg PHT (4/8 rats after single pulse; 5/7 rats after paired pulse). These data indicated that afferent activation could result in a long-latency after-discharge in the MAM-exposed rats.

Discussion

An important issue in the development of animal models for cortical dysplasia epilepsy is whether they exhibit behavioral and cellular changes that are similar to those

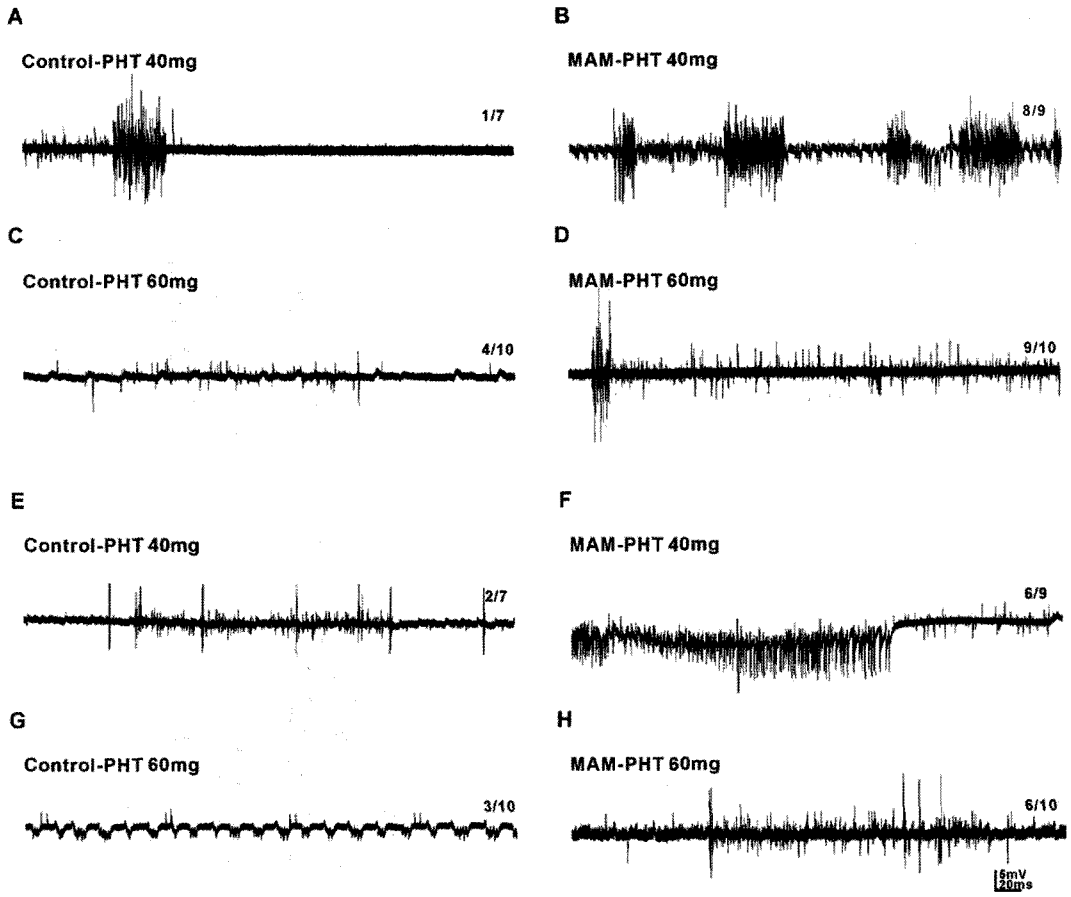

Fig. 5. Spontaneous field potential in control rats (A, C) and in MAM-exposed rats (B, D) at 30 minutes after pilocarpine injection with pre-treatment of PHT. At 60 minutes after pilocarpine injection with perfusion of PHT, spontaneous field potential in control rats (E, G) and in MAM-exposed rats (F, H).

found in cortical dysplasia epilepsy patients. Perhaps the most easily identified behavioral criterion for cortical dysplasia epilepsy development in animal models is the presence of recurrent, spontaneous seizures (Hellier *et al.*, 1998). Neuronal heterotopias are frequently seizure foci in patients suffering from epilepsies associated with a cortical malformation (Palmini *et al.*, 1991). It is now fairly well established that dysplastic brain regions are potential sites of hyperexcitability and seizure genesis. Intra-operative recordings obtained in numerous patients with cortical dysplasia associated epilepsy have confirmed that abnormal electrical activity can be initiated within the dysplasia (Loscher, 2002; Glien *et al.*, 2002; Baba *et al.*, 1983; Babb *et al.*, 1998). The present data demonstrate that the MAM-pilocarpine rats develop spontaneous seizures and undergo mossy fiber sprouting after this treatment. Initial studies from MAM-exposed rats indicated that the threshold for generation of paired pulse inhibition (PPI), amplitude of population spike, and after discharge was significantly lower than in age-matched controls (Prayson and Estes, 1995). Consistent with these findings, we now report that pilocarpine-induced PPI, amplitude of population spike, and after discharge were easier to initiate in the dysplastic brain, possibly related to the intrinsic hyperexcitability of hippocampal heterotopic neurons. Recordings from the heterotopic neurons in the MAM-treated rats suggest that the heterotopic CA1 neurons were in communication with each other as well as with neurons in the overlying neocortex (Baraban *et al.*, 2000; Chevassus-au-Louis *et al.*, 1998a). The present results for the intact animals indicate that the synaptic excitability of the pyramidal neurons in the CA1 of the MAM-treated rats with hippocampal dysplasia and subcortical nodular heterotopia is greater than that of the CA1 pyramidal neurons in the control rats. Further studies will be necessary to ascertain in the connectivity patterns of normotopic CA1 neurons render the neurons susceptible to epileptiform activity. The issue of whether an animal model undergoes cellular changes in the brain to render it more susceptible to spontaneous seizure generation is paramount (Dudek and Spitz, 1997). Several animal models of chronic epilepsy, including the pilocarpine and kainate models, have shown that adult rats develop aberrant mossy fiber sprouting with associated hippocampal cell loss following status epilepticus (Mello *et al.*, 1993; Ben-Ari, 1985). These studies suggested that axon sprouting is correlated with an elevated propensity for increased network excitability.

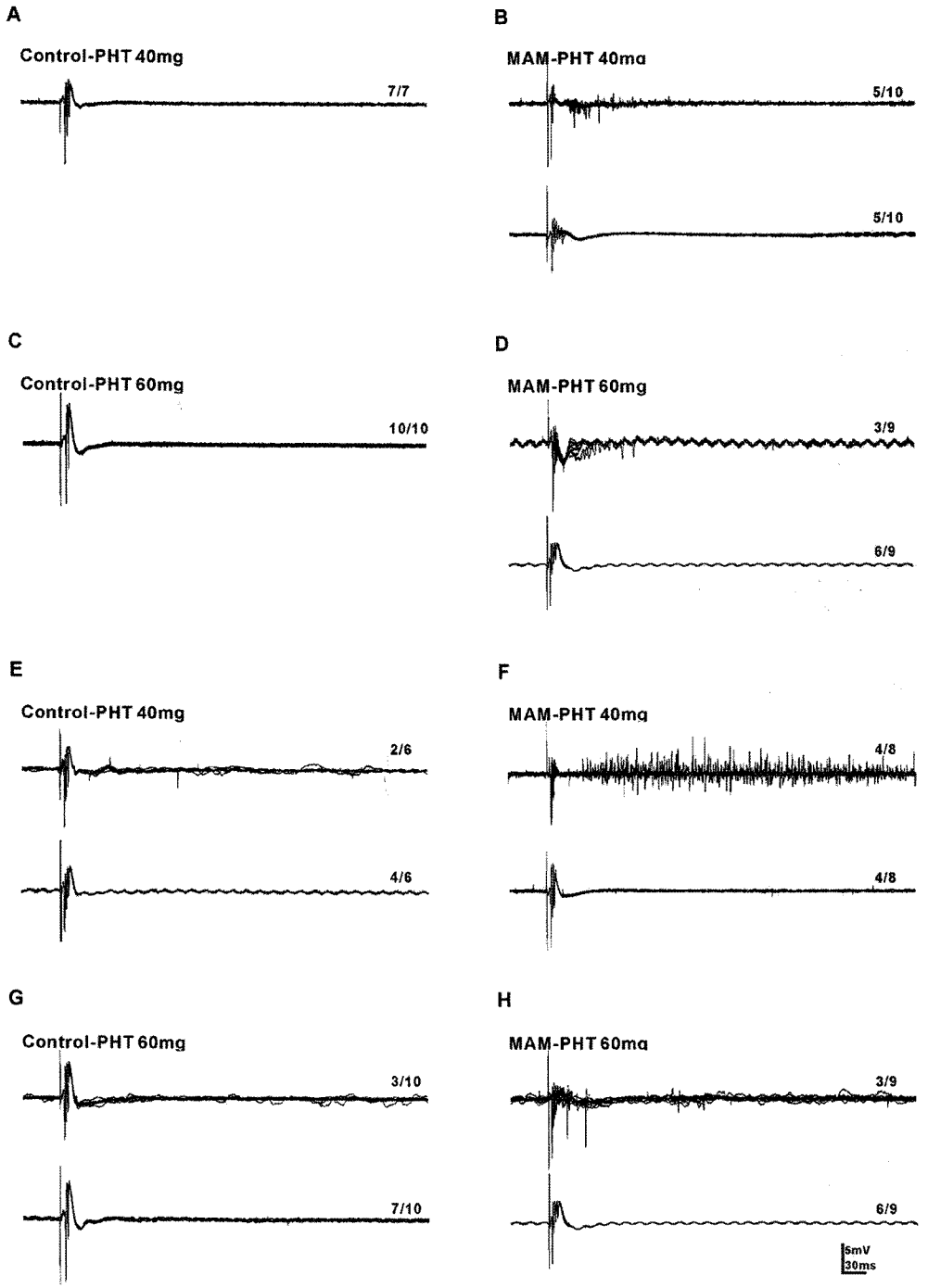

Fig. 6. Field potential afterdischarges in the CA1 region after single pulses with PHT. The response to single pulse stimulation in a control rats (A, C) and in a MAM-exposed rats (B, D) at 30 minutes after pilocarpine injection of pre-treatment of PHT. Population responses in the CA1 pyramidal cell layer of a control rats (E, G) and in a MAM-exposed rat (F, H) at 60 minutes after pilocarpine injection of co-perfusion with PHT. Ten overlapping traces are shown for each data set.

Recurrent spontaneous seizures and mossy fiber sprouting in MAM-pilocarpine rats is replicating similar experiments conducted on rats (Mello *et al.*, 1993) and albino mice (Cavalheiro *et al.*, 1996). Difference between MAM-treated model and other models was the seizure silence period.

The present study demonstrated increased seizure susceptibility of MAM-exposed animals using a variety of techniques including kainate acid (Germano and Sperber, 1997), kindling (Chevassus-au-Louis *et al.*, 1998), hyperthermia (Germano *et al.*, 1996), flurothyl (Baraban and Schwartzkroin, 1995), and bicuculline (de Feo *et al.*, 1995). We observed a tendency toward increased seizure susceptibility between control and MAM-exposed rats following intraperitoneal administration of pilocarpine.

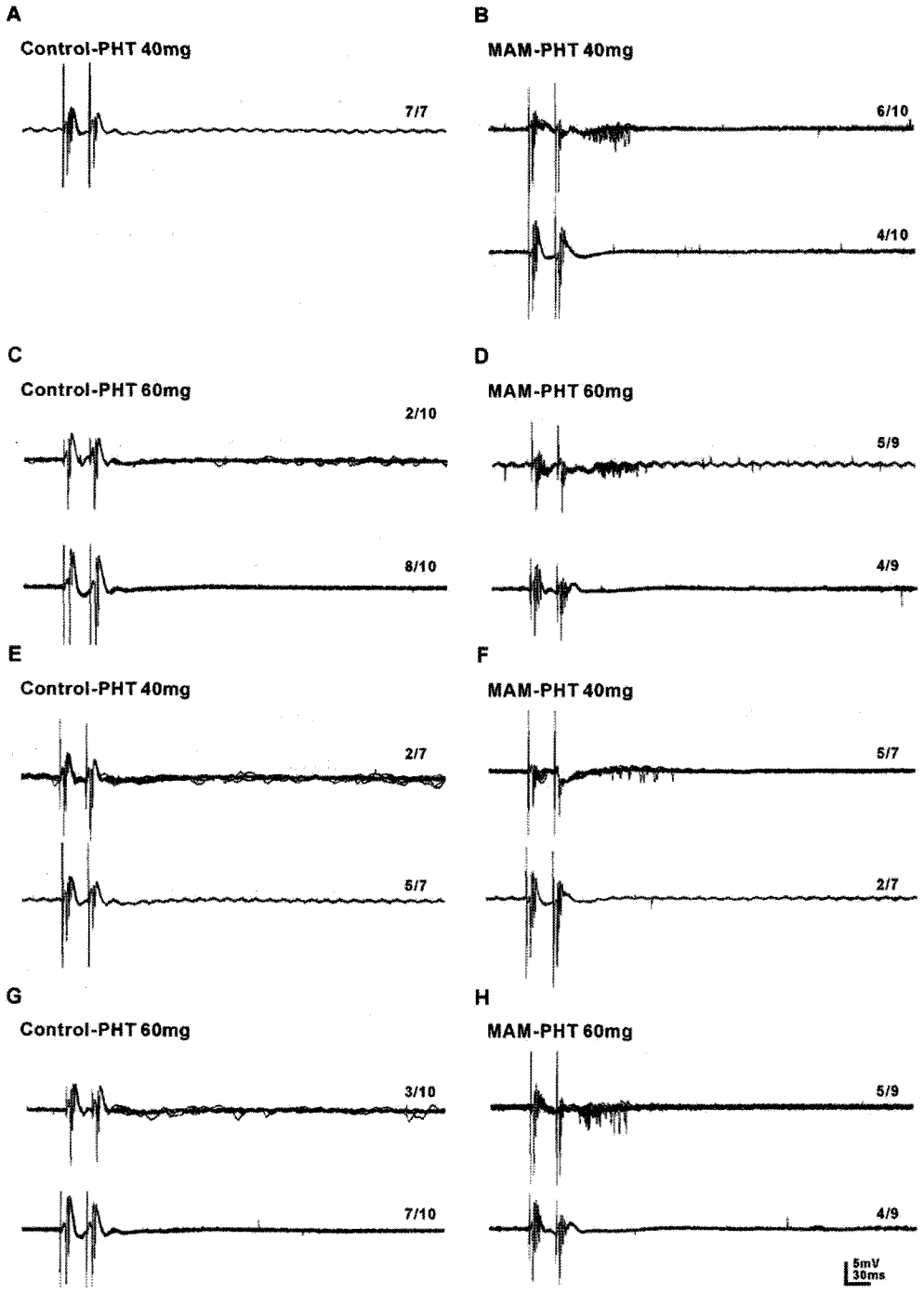

Fig. 7. Field potential afterdischarges in the CA1 region after paired pulses with PHT. The response to paired pulse stimulation in a control rats (A, C) and in a MAM-exposed rats (B, D) at 30 minutes after pilocarpine injection of pre-treatment of PHT. Population responses in the CA1 pyramidal cell layer of a control rats (E, G) and in a MAM-exposed rat (F, H) at 60 minutes after pilocarpine injection of co-perfusion with PHT. Ten overlapping traces are shown for each data set.

Here we show that MAM-treated rats have a striking AEDs pharmaco-resistance when pilocarpine was used to induce seizure. For example, administration of PHT in control animals resulted in prolongation of latency to generalized seizures, but no effect on seizure latency in MAM-exposed rats. These behavioral findings extend and confirm our electrophysiological results while closely mirroring the clinical situation (Hirabayashi *et al.*, 1993; Palmini *et al.*, 1991). Given the MAM-exposed animals mimic salient features of cortical dysplasia assocaited epilepsies and are resistant to standard AEDs, it is likely that further analysis of this model may prove useful in the development of novel treatment options.

A number of standard AEDs have previously been

evaluated in the hippocampal area with pilocarpine-induced seizure activity (Bruckner and Heinemann, 2000; Bruckner *et al.*, 1999; Fueta and Avoli, 1992; Yamaguchi and Rogawski, 1992). Kainic acid, kindling, or a number of other manipulations can also reliably evoke seizure activity, however, to more efficiently compare control and MAM-exposed animals we restricted our analysis of AEDs to the pilocarpine-induced bursting model with well-characterized pharmacological profile (Bruckner and Heinemann, 2000; Fueta and Avoli, 1992).

Phenytoin is the oldest nonsedative antiseizure drug that altered electrically induced seizures in laboratory animals. It alters Na^+, K^+, Ca^{2+} conductances, membrane potentials, and the concentrations of amino acids and neurotransmitters such as norepinephrine, acetylcholine, and GABA (Duncan, 2002). Studies with neurons in cell culture show that phenytoin blocks sustained high-frequency repetitive firing of action potentials. It is a use-dependent effect on Na^+ conductance, arising from preferential binding to the inactivated state of the Na^+ channel. At high concentrations, phenytoin also inhibits the release of serotonin and norepinephrine, promotes the uptake of dopamine, and inhibits monoamine oxidase activity (Hachad *et al.*, 2002). The drug interacts with membrane lipids; this binding might promote the stabilization of the membrane. In addition, phenytoin paradoxically causes excitation in some cerebral neurons. Diazepam given intravenously or rectally is highly effective for stopping continuous seizure activity, especially generalized tonic-clonic status epilepticus (Mohler *et al.*, 2002). Diazepam, one of the benzodiazepine derivatives, binds to molecular component the $GABA_A$ receptor present in neuronal membranes in the central nervous system (Crestani *et al.*, 2001). This receptor, which functions as a chloride ion channel, is activated by the inhibitory neurotransmitter GABA. GABA is the major inhibitory neurotransmitter in central nervous system (Korpi *et al.*, 2002). Electrophysiological studies have shown that benzodiazepines potentiate GABAergic inhibition at all levels of the neuraxis, including the spinal cord, hypothalamus, hippocampus, substantia nigra, cerebellar cortex, and cerebral cortex (Chadha *et al.*, 2000; Kohno *et al.*, 2006; Korpi *et al.*, 2002; Mikkelsen *et al.*, 2005; Xu *et al.*, 2005). Diazepam appear to increase the efficiency of GABAergic synaptic inhibition. Diazepam does not substitute for GABA but appears to enhance GABA effects without directly activating GABA receptors or opening the associated chloride channels (Rudolph *et al.*, 2001). 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, we observed significant effects of AEDs on quantitative assessment of population spike (amplitude and number) and convulsive behavior test that were largely consistent with previous reports. In contrast to control studies, pilocarpine-induced seizure activity in MAM-exposed rats was relatively resistant to AEDs tested (Smyth *et al.*, 2002). PHT 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. DZP evoked a modest suppression of population spike number and convulsive behavior in MAM-exposed animal. Because the mechanism of action for PHT probably involves modulation of Na^+ channel activity, our findings in the MAM model suggest that inhibition of these channels may not be an effective means to reduce hyperexcitability in the dysplastic brain. DZP resulted in a prompt and pronounced fall of extracellular hippocampal glutamate levels. This is consistent with the finding that DZP markedly decreased high K^+ or veratridine-evoked glutamate release in hippocampal area (Smyth *et al.*, 2002). DZP, which works, at least in part, suppressed amplitude in both control and MAM animals.

Pharmaco-resistant seizure disorders, such as those seen in association with cortical dysplasia, present a challenge to the treating physician, the care-givers, and the afflicted patient straddled with intractable epilepsy. Multiple AED regimens are employed in an attempt to ameliorate seizures in these patients, generally with only partial success and often with troublesome and sometimes injurious side effects. Surgical resection of dysplastic brain regions is frequently successful, but entails significant risk. Although a large body of clinical information is available regarding the morphologic and histologic properties of malformed brains, no attempt had been made to systematically investigate pharmaco-resistance in patients with cerebrocortical malformations. While it is not yet clear how a dysplastic brain becomes resistant to AED intervention, it is evident both from clinical and now experimental studies, that a characteristic feature of dysplastic tissue is pharmaco-resistance to available medications. Taken together, the behavioral and electrophysiological data presented here represent systematic evaluation of the effects of standard AEDs in a rodent model of cortical dysplasia associated epilepsy. At present, traditional AED development involves the use of two well-established screening techniques to predict the ability of a compound to reduce or eliminate seizures in some, but not all seizure disorders. MAM-exposed rats have served as a useful model of cortical dysplasia-associated epilepsy, and they reproduce some of the key histopathologic features of dysplasia-associated epilepsy. Nonetheless, it would be prudent to conduct similar studies in other models that have malformation to reduce the possibility that the observed AEDs effects result from some idiosyncrasy of the MAM model. Given that our findings confirm the presence of pharmacological resistance in an animal model designed to mimic cortical dysplasia,

our data support the notion that mechanisms underlying epileptogenesis in dysplasia-associated epilepsy differ from those at work in other forms of epilepsy more responsive to standard AEDs. Furthermore, such findings argue for a model-specific (and disease-specific) approach to the development of new antiepileptic compounds.

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