Effects of antiepileptic drugs on induced epileptiform activity in a rat model of dysplasia

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Abstract

Seizure activity associated with cortical dysplasia (CD) is often resistant to standard pharmacologic treatments. Although several animal models exhibit CD, virtually nothing is known about antiepileptic drug (AED) responses in these animals. Here 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. 4-aminopyridine (100 μM), a K⁺ channel blocker, was used to induce interictal epileptiform bursting in acute hippocampal slices from MAM-exposed and age-matched vehicle-injected control animals. Extracellular field recordings were used to monitor seizure activity in vitro. Five commonly used AEDs were tested: phenobarbital, 25–400 μM; carbamazepine, 25–200 μM; valproate (VPA), 0.19–4 mM; ethosuximide (ESM), 0.5–8 mM; and lamotrigine (LTG), 49–390 μM. 4-AP-induced bursting occurred with shorter latencies in slices from MAM-exposed rats in comparison with slices from controls, confirming the intrinsic hyperexcitability of dysplastic tissue. Each AED tested demonstrated significant burst suppression in control slices, but interictal epileptiform bursting in MAM-exposed slices was resistant to these treatments. Even at the highest concentrations, VPA, ESM and LTG had no effect on burst amplitude in slices from MAM-exposed rats. Pharmacoresistance was further tested by measuring seizure latencies in awake, freely-moving rats after kainate administration (15 mg/kg, i.p.) with and without pre-treatment with VPA (400 mg/kg i.p.). Pre-treatment with VPA prolonged seizure latency in control rats, but had no effect in MAM-exposed animals. These results suggest MAM-exposed rats exhibit a dramatically reduced sensitivity to commonly prescribed AEDs. © 2002 Elsevier Science B.V. All rights reserved.

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1. Introduction

Cortical dysplasia (CD) is increasingly implicated as a major contributing factor in many types of epileptic disorders (Aicardi, 1994; Becker, 1991; Gambardella et al., 1997). Histopathological hall-
marks of CD include disrupted cytoarchitecture, disorganized lamination, clusters of displaced cells, and abnormal dendritic arborization (Crino and Eberwine, 1997). Clinical studies have directly linked epileptogenesis with human dysplastic tissue (Mattia et al., 1995; Morioka et al., 1999; Palmini et al., 1995) and surgical resection often provides the only effective form of seizure control for patients with CD-associated epilepsy (Guerrini et al., 1992; Hirabayashi et al., 1993; Jay and Becker, 1995; Palmini et al., 1991b; Raymond et al., 1995). Although many forms of epilepsy are successfully managed with antiepileptic drug (AED) therapy, dysplasia-associated seizure disorders are markedly resistant to pharmacologic intervention (Guerrini et al., 1992; Hirabayashi et al., 1993; Palmini et al., 1991a; Taylor et al., 1971).

Presently available animal models of CD with specific clinical pathologies include, but are not limited to, TISH rats (Lee et al., 1997) (double cortex), Ihara rats (Amano et al., 1996) (microdysgenesis), Lis1 knockout mice (Hirotsune et al., 1998) (Type 1 lissencephaly), p35 knockout mice (Wenzel et al., 2001) (granule cell dispersion), freeze-lesion rats (Jacobs et al., 1996, 1999) (polymicrogyria) and rats exposed to methylazoxymethanol acetate (MAM) in utero (Baraban and Schwartzkroin, 1995) (nodular heterotopia). Invariably, CD in each of these rodent models is associated with some form of hyperexcitability and either spontaneous epileptic behavior or enhanced seizure susceptibility (for review, see Chevassus-au-Louis et al. 1999). On-going anatomical, molecular, and electrophysiological characterization of these animals is contributing to improved understanding of how seizure activity develops in the dysplastic brain. However, whether any of these animals respond to common AEDs has not been systematically investigated. To address this issue, we have been studying MAM-exposed rats to evaluate potential anticonvulsant resistance both in vitro and in vivo.

Previous work in our laboratory has demonstrated that MAM-exposed rats exhibit distinct nodular heterotopia, hyperexcitability within dysplastic brain regions, altered synaptic connectivity and abnormal potassium channel expression/function (Baraban and Schwartzkroin, 1995; Baraban et al., 2000; Castro et al., 2001). In the present study, in vitro pharmacological experiments were performed on brain slices from MAM-exposed animals to test whether AEDs alter 4-aminopyridine (4-AP)-induced epileptiform activity. A potent K\(^+\) channel blocker, 4-AP is a powerful convulsant drug that readily induces the appearance of spontaneously occurring epileptiform activity in vitro and has been used extensively for analysis of basic epileptogenic mechanisms (Barbarosie and Avoli, 1997; Perreault and Avoli, 1991, 1992; Rutecki et al., 1987) and AED drug screening (Fueta and Avoli, 1992). In vivo studies were performed to examine AED effectiveness against acute kainate-induced seizures. Here we report that commonly available AEDs have little effect on induced interictal epileptiform bursting in hippocampal slices from MAM-exposed rats and do not prolong seizure latencies in MAM-rats exposed to kainic acid.

2. Methods

2.1. Methylazoxymethanol acetate injection

Control and dysplastic rats were generated by injecting timed-pregnant Sprague–Dawley mothers (Charles River Laboratories, Hollister CA) with MAM (25 mg/kg i.p., Midwest Research Institute, Kansas City, MO) or vehicle alone (10% DMSO in 0.3 ml 0.9% saline i.p.) on embryonic day 15. Representative cresyl violet stained hippocampal sections (coronal; 30 \(\mu\)m) of a rat exposed to MAM in utero are shown in Fig. 1. A total of 53 animals were used for in vitro studies and 28 for in vivo experiments, including both control and MAM-exposed animals. Animal care and handling was performed according to NIH guidelines approved by the UCSF Committee on Animal Care.

2.2. In vitro studies

Recordings were performed in acute hippocampal slices (horizontal; 450 \(\mu\)m) obtained from adult vehicle- or MAM-exposed rats (P40–P50). Horizontal slices were prepared as described previously (Baraban and Schwartzkroin, 1995).
Hippocampi were not dissected out, and all slices included entorhinal cortex and other overlying cortical structures. After cutting, slices remained submerged in a holding chamber containing oxygenated recording medium (nACSF) consisting of (in mM): 124 NaCl, 3 KCl, 1.25 NaH2PO4, 2
MgSO$_4$, 26 NaHCO$_3$, 2 CaCl$_2$ and 10 dextrose. A slice was then transferred to a gas-interface recording chamber (Harvard Apparatus, South Natick, MA) and perfused with oxygenated nACSF at flow rate of $\sim 2.5$ ml/min (temperature $= 33.5 \pm 1$ °C). Borosilicate glass electrodes were pulled, filled with 2 M NaCl ($2$–$8$ MΩ) and placed in the CA1 region of stratum pyramidale and/or within neuronal heterotopias under visual microscopic control. A mono-polar stimulating electrode was placed in stratum radiatum (for simplicity, the recording configuration schematic focuses on EC–hippocampal structures; Fig. 1G). Voltage was recorded with a Neurodata IR-283 amplifier and monitored on a PC running pClamp software (Axon Instruments, Foster City, CA). Spontaneous field activity and evoked population spikes were stored to hard disk for later blinded analysis. Intercitial epileptiform burst activity was initiated with perfusion of nACSF containing 4-AP (100 μM), a potassium channel blocker known to cause seizures in humans and spontaneous epileptiform activity in hippocampal slice preparations; (Spyker et al., 1980; Voskyul and Albus, 1985). The 4-AP in vitro seizure model is based on blockade of A-type potassium channels leading to the appearance of giant excitatory postsynaptic potentials (EPSPs) generated by the prolonged firing of pyramidal neurons in CA3 burst-generating regions of hippocampus (Perreault and Avoli, 1991). Although we recently demonstrated a lack of A-type potassium channels on hippocampal heterotopic neurons (Castro et al., 2001), epileptiform bursting initiating in CA3 can be reliably induced using 4-AP in intact hippocampal–entorhinal cortex slices from MAM-exposed rats (Baraban et al., 2000). Burst frequency was determined by counting the number of interictal epileptiform events (bursts; 100–250 ms in duration) per second during a 3-min epoch before and after 60 min of AED co-perfusion, and was expressed as Hz. Burst amplitude (1.5–6 mV) was determined by measuring the average peak-to-peak interval for 10 consecutive representative bursts during the same epoch (Fig. 1H). Evoked synaptic responses were analyzed by averaging the number of population spikes obtained on 10 consecutive sweeps recorded after stratum radiatum stimulation (0.1–3 mA pulses; 100 μs pulse-width). A downward voltage deflection $\geq 0.5$ mV superimposed on the population EPSP was defined as a ‘population spike’; the number of population spikes was computed for each slice during perfusion with normal ACSF (baseline), ACSF plus 4-AP and ACSF plus 4-AP and AED. For each slice experiment, population spike amplitude was monitored every 15 min and data were discarded if the population spike was significantly decreased or lost. All in vitro data were analyzed for statistical difference using a Student’s $t$ test (significance taken as $P < 0.05$).

2.3. In vivo studies

Control and MAM-exposed animals were administered 15 mg/kg of kainic acid (Opika-1™, Ocean Produce International, Canada), a concentration that reliably produces acute seizure activity (Velisek et al., 1992). Behavioral seizure activity was scored on a 6-stage scale, as described previously (Germano and Sperber, 1997). Animals were pre-treated with valproate (VPA; 400 mg/kg i.p. dissolved in 0.9% NaCl; 30 min before kainate injection), as described previously (Klotz and Antonin, 1977; Otsuki et al., 1998; Walker et al., 2000; Wilder, 1992). Latencies to the first sign of hyperexcitability (Stage I/II) and to the first generalized tonic–clonic seizure (Stage V/VI) were recorded. Data were plotted graphically as ‘survival’ curves (Kaplan and Meier, 1958), and differences in mean latencies were ranked and analyzed using a non-parametric Kruskal–Wallis One-Way ANOVA (significance taken as $P < 0.05$).

2.4. Histology

Acute brain slices from in vitro studies on MAM-exposed animals were saved and post-fixed in fresh 4% paraformaldehyde for 48–72 h at 4 °C. Animals used for in vivo studies were deeply anesthetized and decapitated 2 h after kainate injection. Brains were rapidly removed and fixed in fresh 4% paraformaldehyde for 72 h and then cryoprotected in 20% sucrose-PBS for another 72 h at 4 °C. Brains were then blocked and glued to
the stage of a cryostat where 30 μm coronal sections were cut through the hippocampus. All tissue slices/sections were mounted on glass slides, stained with cresyl violet, cover-slipped and examined on an upright light microscope. Data from tissue slices/sections from MAM-exposed animals without microscopic evidence of dysplasia (defined as at least one section containing a nodular heterotopia with greater than 30 displaced cells; Fig. 1A) were excluded from the analysis.

2.5. Drugs

All drugs were freshly prepared each day with the exception of 4-AP (Sigma, Milwaukee, WI), which was prepared from a concentrated stock solution made weekly. 4-AP concentrate (0.94% dissolved in nACSF) was added to the nACSF to achieve a final concentration of 100 μM. Each AED tested was then added to 4-AP-containing nACSF. Valproic acid (VPA; Sigma), ethosuximide (ESM; Sigma), and phenobarbital (PB; Sigma) readily dissolved in 4-AP/nACSF. Carbamazepine (CBZ; Sigma) was dissolved in DMSO (Sigma) before adding it to 4-AP/nACSF (0.1% DMSO final concentration), and lamotrigine (LTG; GlaxoWellcome, Research Triangle Park, NC) was prepared by dissolving crushed 25 mg tablets in 4-AP/nACSF.

3. Results

A total of 10 litters each of MAM-exposed and vehicle-injected pups were generated, ranging in size from 6 to 13 pups. One or more distinct clusters of displaced neurons (e.g. nodular heterotopia) were observed in stratum radiatum or interrupting CA1–CA2 stratum pyramidale (Fig. 1A–F) in the hippocampus of all MAM-exposed animals included for analysis. Excluded animals (n = 17) came entirely from 2 MAM-exposed litters lacking significant microscopic abnormalities on post hoc analysis.

3.1. AED effect on 4-AP-induced epileptiform activity

The latency to initiation of 4-AP-induced interictal epileptiform activity was significantly shorter in slices from MAM-exposed rats (n = 75; μ = 6.4 ± 3.4 min) in comparison with slices from age-matched controls (n = 97; μ = 18.8 ± 8.3 min; Student’s t test P < 0.0001). There were no differences in burst morphology, amplitude, or frequency for these interictal epileptiform events when comparing slices from vehicle- and MAM-exposed animals (Amplitude: control μ = 1.6 ± 0.8 mV; MAM μ = 1.5 ± 0.8 mV and Frequency: control μ = 1.6 ± 0.6 Hz; MAM μ = 1.5 ± 0.5 Hz). Furthermore, interictal epileptiform bursts were stable in our gas-interface recording chamber for up to 4 h in the presence of continuous 4-AP perfusion for both control and MAM-exposed slices (data not shown).

In control slices, AEDs suppressed burst frequency, burst amplitude, or both, with complete cessation of interictal epileptiform burst activity occasionally observed at lower AED concentrations and always observed at higher concentrations. In slices from MAM-exposed rats, there was a consistent resistance to burst suppression (frequency and/or amplitude) with all AEDs tested, and complete burst suppression was never observed even at the highest AED concentrations (Figs. 2 and 3). Specific responses to individual AEDs are described in the following sections.

3.2. Valproate

At low concentrations, VPA is thought to exert its anticonvulsant actions via inhibition of Na^+ channels (Schauf, 1987) and enhancement of brain GABA levels (Macdonald, 1989). At higher concentrations, VPA also reduces excitability by increasing a K^+ conductance (Porter and Meldrum, 1995). For the current study, a total of 20 control slices were perfused with VPA/4-AP/nACSF at 5 concentrations (0.19, 0.50, 1, 2 and 4 mM). 15 MAM-exposed slices were perfused with VPA/4-AP/nACSF at 4 concentrations (0.50, 1, 2 and 4 mM). Representative extracellular recordings before, and approximately 60 min
following VPA perfusion, are shown in Fig. 2A. VPA reduced burst amplitude in slices obtained from both control and MAM-exposed animals (Fig. 2B). However, the maximum amount of burst suppression observed in slices from MAM-exposed animals was ~20% (at the highest drug concentration) whereas 60–70% inhibition was observed in slices from control animals. Although low concentrations of VPA did not consistently reduce burst frequency in control slices, inhibition was consistently observed at concentrations between 1 and 4 mM (Fig. 2C).

3.3. Ethosuximide

ESM is the primary treatment for generalized absence-type seizures and is thought to exert anticonvulsant activity by reducing low-threshold

Fig. 2. (A) Representative extracellular field recordings of 4-AP-induced interictal epileptiform bursting in slices from control and MAM-exposed rats before (above) and after (below) co-perfusion with VPA. (B) Concentration–response curve for burst amplitude suppression at escalating doses of VPA in slices from control (circles) and MAM-exposed (triangles) rats. (C) Concentration–response curve for burst frequency suppression at escalating doses of VPA in slices from control (circles) and MAM-exposed (triangles) rats. Data represent mean ± S.E.M.; *significance taken as P < 0.05. Each point represents 3–6 slices.

Fig. 3. Dose–response curves for burst amplitude (left) and frequency (right) suppression at escalating doses of the 4 remaining AEDs in control (circles) and MAM-exposed (triangles). (A) ESM dose–response curves. (B) CBZ dose–response curves. (C) LTG dose–response curves. (D) PB dose–response curves. Data represent mean ± S.E.M.; *significance taken as P < 0.05. Each point represents 3–6 slices.
(T-type) Ca\textsuperscript{2+} currents (Coulter et al., 1989), and at higher concentrations ESM depresses cerebral metabolic rates by inhibiting a Na\textsuperscript{+}/K\textsuperscript{+} ATPase (Gilbert and Wyllie, 1976; Porter and Meldrum, 1995). For the current study, a total of 30 slices (17 control and 13 MAM) were perfused with ESM/4-AP/nACSF at 4 concentrations (1, 2, 4 and 8 mM). In slices from control rats, high concentrations of ESM (4–8 mM) often resulted in complete or near-complete suppression of interictal epileptiform bursting in the 4-AP model (Fig. 3A). ESM produced consistent decreases in both amplitude and frequency of bursts observed in control tissue but had minimal or no effect on burst amplitude/frequency in slices from MAM-exposed animals at all concentrations tested.

3.4. Carbamazepine

CBZ is a tricyclic compound closely related to imipramine and other antidepressants. Reported anticonvulsant mechanisms for this drug include blockade of membrane-bound Na\textsuperscript{+} channels (Willow et al., 1984) and presynaptic inhibition of synaptic transmission (DeLorenzo, 1984; Porter and Meldrum, 1995). A total of 37 slices (17 control and 20 MAM) were perfused with CBZ/4-AP/nACSF at 4 concentrations (25, 50, 100 and 200 \mu M). In slices from control rats, CBZ exposure primarily altered burst amplitude with modest effects on frequency (Fig. 3B). Note that CBZ significantly suppressed burst amplitude to 20–60% of baseline at each concentration tested. In contrast, CBZ failed to suppress burst amplitude or frequency, even at the highest concentration, in slices from MAM-exposed rats.

3.5. Lamotrigine

At present, the mechanism(s) of action of LTG are poorly understood, but it has been shown to prolong inactivation of Na\textsuperscript{+} channels (Battino et al., 1993; Messenheimer, 1995; Porter and Meldrum, 1995). For the current study, a total of 26 slices (12 control and 14 MAM) were perfused with LTG/4-AP/nACSF at 4 concentrations (49, 98, 195 and 390 \mu M). Increasing concentrations of LTG produced comparable decreases in burst frequency in slices from either control or MAM-exposed animals (Fig. 3C). Note that LTG significantly decreased burst amplitude in control tissue, but burst amplitude remained stable (or increased slightly) during LTG perfusion in slices from MAM-exposed rats.

3.6. Phenobarbital

PB, one of the oldest AEDs presently available, as a barbiturate acts to enhance GABA receptor-mediated inhibition and attenuate excitatory transmission. It has been shown to selectively suppress abnormal neuronal discharges, and also has effects on both Na\textsuperscript{+} and Ca\textsuperscript{2+} conductances (Ferrendelli and Daniels-McQueen, 1982; Pincus and Hsiao, 1981; Porter and Meldrum, 1995; Zbicz and Wilson, 1981). For the current study, 30 slices (18 control and 12 MAM) were perfused with PB/4-AP/nACSF at 4 concentrations (25, 100, 200 and 400 \mu M). PB significantly depressed burst amplitude in slices from control animals at all concentrations tested (Fig. 3D). In contrast, PB produced a reduction in burst amplitude in slices from MAM-exposed animals at 2 of the 4 doses tested. PB produced a small decrease in burst frequency at higher concentrations that was not significantly different between control and MAM-exposed tissue.

3.7. AED effect on evoked synaptic responses

Multiple population spikes, indicative of synaptic hyperexcitability, were consistently observed in hippocampal slices perfused with 4-AP ACSF (Fig. 4A). Their occurrence coincided with the onset of interictal epileptiform burst discharges during virtually all slice experiments (data not shown). The mean number of population spikes evoked after 4-AP perfusion was similar for slices from control (2.4 ± 1.4 spikes, range 1–5) and MAM-exposed rats (2.5 ± 1.6 spikes, range 1–7). All AEDs tested reduced the number of evoked population spikes observed during 4-AP perfusion, and the degree of suppression was similar for slices from control and MAM-exposed rats (40–60% inhibition; Fig. 4B). This is in contrast with the effect of AEDs on spontaneous bursting, which
were significantly different in slices from control and MAM-treated rats. In addition, these changes were independent of the effect of a particular AED on spontaneous burst amplitude or frequency, i.e. higher dose ESM and VPA had the least effect on multiple population spikes but at the same time strongly suppressed spontaneous burst activity.

3.8. AED effect on behavioral seizures

Based on our in vitro data demonstrating a significant lack of AED efficacy with VPA in slices from MAM-exposed rats, we tested this drug in vivo. The latencies to the first incidence of a generalized seizure (Stage V/VI) were slightly shorter (though not statistically significant) for MAM-exposed rats in comparison with control animals injected with kainate (Stage V/VI: control = 68 ± 15 min; MAM = 61 ± 20 min). Although pre-treatment with VPA significantly prolonged the mean latency to first Stage V/VI seizure in control animals (n = 8; 105 ± 15 min; P < 0.006, Kruskal–Wallis one-way analysis of variance on ranks), it had little effect in MAM-exposed animals (n = 6; 72 ± 14 min). This ‘shift to the right’ in the Kaplan–Meier plot of the percentage of control animals exhibiting Stage V/VI seizures (Fig. 5) is consistent with previous reports (Turski et al., 1990; Velisek et al., 1992) and suggests that VPA is an effective suppressor of kainate-induced seizures in these animals. In contrast, VPA pre-treatment, consistent with our in vitro data (Fig. 2), is not an effective AED treatment for animals with MAM-induced CD.

4. Discussion

More than 40,000 infants born each year have seizure disorders associated with cortical malformations. Of these, a substantial number are...
dysplasia-associated seizure disorders, which often have an early onset, are refractory to pharmacologic intervention and can result in severe neurodevelopmental delay (Guerrini et al., 1992; Hirabayashi et al., 1993; Palmini et al., 1991a; Taylor et al., 1971). Here we report on AED sensitivity following induction of acute seizure activity in one such model e.g. rats exposed to MAM in utero. The main findings of this study are that acute seizures initiated in MAM-exposed rats are relatively resistant to standard AEDs assessed both in vitro and in vivo.

4.1. Hyperexcitability in the MAM model

It is now fairly well established that dysplastic brain regions are potential sites of hyperexcitability and seizure genesis. In humans, dysplastic sites are often hypometabolic when evaluated with positron emission tomography; consistent with the hypothesis that seizure activity is generated in these regions (Chugani, 1992; Chugani et al., 1990). Intra-operative recordings obtained in numerous patients with CD-associated epilepsy have confirmed that abnormal electrical activity can be initiated within the dysplasia (Kutsy, 1999; Morioka et al., 1999; Rosenow et al., 1998). Initial studies using brain slices from MAM-exposed rats indicated that the threshold for generation of epileptiform activity was significantly lower than in non-dysplastic slices from age-matched controls (Baraban and Schwartzkroin, 1995). In particular, raising the extracellular potassium concentration from 3 to 6 mM induced spontaneous and evoked epileptiform-like activity in the majority of slices from MAM animals but in very few control slices. Consistent with these findings, we now report that 4-AP-induced spontaneous interictal epileptiform bursting was ‘easier’ to initiate in the dysplastic brain, possibly related to the intrinsic hyperexcitability of hippocampal heterotopic neurons (Castro et al., 2001). Although burst morphology, amplitude, and frequency were indistinguishable between slices from control and MAM-exposed rats, the latency to burst onset was significantly shorter (6 vs. 19 min) in slices from MAM-exposed rats perfused with the same concentration of 4-AP.

4.2. AED resistance in the MAM model: in vitro

A number of standard AEDs have previously been evaluated in the hippocampal or combined entorhinal cortex– hippocampal slice preparation with 4-AP-induced epileptiform activity (Bruckner and Heinemann, 2000; Bruckner et al., 1999; Fueta and Avoli, 1992; Yamaguchi and Rogawski, 1992). Bicuculline, high potassium, or a number of other in vitro manipulations can also reliably evoke epileptiform activity, however, to more efficiently compare control and MAM-exposed animals we restricted our analysis of AEDs to the 4-AP-induced bursting model with its well-characterized pharmacological profile (Bruckner and Heinemann, 2000; Fueta and Avoli, 1992). In this study, the hyperexcitability of hippocampal slices induced by 4-AP was measured in two ways: quantitative assessment of spontaneous bursts (amplitude and frequency) and analysis of multiple population spikes evoked by electrical stimulation. In control studies, we observed significant effects of AEDs on spontaneous epileptiform bursts that were largely consistent with previous reports. Interestingly, interictal epileptiform burst amplitude, and not burst frequency or evoked population spike response, was the most reliable measure of AED efficacy in the 4-AP model.

In contrast to control studies, 4-AP-induced epileptiform activity in brain slices from MAM-exposed rats was relatively resistant to all five AEDs tested. Even at high drug concentrations, VPA, ESM and LTG had little or no effect on burst amplitude in dysplastic tissue. CBZ and PB evoked a modest suppression of burst amplitude in slices from MAM-exposed rats but required significantly higher concentrations to achieve a level of suppression comparable to that observed in control tissue (Fig. 3B and D). Similar, but less dramatic effects were seen when we analyzed interictal epileptiform burst frequency. VPA and ESM had essentially no effect on burst frequency at escalating concentrations in slices from MAM-exposed rats, and CBZ had a higher ECS0 for frequency suppression in dysplastic tissue. Because the mechanism of action for VPA, ESM, CBZ and phenytoin probably involve modulation of Na+ or Ca2+ 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. Interestingly, LTG, which works, at least in part, by stabilizing the Na$^+$ channel’s inactivated state, suppressed burst frequency in a concentration-dependent fashion in both control and MAM animals. These latter findings are consistent with the improved efficacy of LTG in medically refractory and CD-associated epilepsy syndromes that has been observed clinically (Jawad et al., 1989; Messenheimer, 1995; Vossler et al., 1999; Wallace, 1994) and suggests that drugs which enhance Na$^+$ channel function may be an effective form of treatment for CD-associated epilepsies.

4.3. AED resistance in the MAM model: in vivo

Previous reports demonstrated increased seizure susceptibility of MAM-exposed animals using a variety of techniques including kainic acid (Germano and Sperber, 1997), kindling (Chevassus-au-Louis et al., 1998), hyperthermia (Germano et al., 1996), flurothyl (Baraban and Schwartzkroin, 1996), and bicuculline (de Feo et al., 1995). Although we observed a trend toward increased seizure susceptibility between control and MAM-exposed animals following intraperitoneal administration of kainate, the difference was not statistically significant, in contrast to the findings of Germano and Sperber (1997). This may be related to the younger age of animals studied here (P40–50), variability associated with i.p. injections, the small sample size, or the use of synthetically derived, and potentially less potent, kainic acid (Opika-1™).

Nonetheless, MAM-exposed animals showed a striking AED pharmacoresistance when kainate was used to induce seizures. For example, administration of VPA in control animals resulted in an 80% prolongation of latency to generalized seizures, but had no effect on seizure latency in MAM-exposed rats. These in vivo findings extend and confirm our in vitro results while closely mirroring the clinical situation (Hirabayashi et al., 1993; Palmini et al., 1991a). Given that MAM-exposed animals mimic salient features of CD-associated 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.

4.4. Clinical relevance of the described studies

Pharmacoresistant seizure disorders, such as those seen in association with CD, present a challenge to the treating physician, the caregivers, 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 (now more readily identifiable with high-resolution MRI) is frequently successful, but entails significant risk (Palmini et al., 1991b, 1995). Although a large body of clinical information is available regarding the morphologic and histologic properties of malformed brains (Babb et al., 1998; Battaglia et al., 1996; Hannan et al., 1999; Prayson and Estes, 1995), no attempt had been made to systematically investigate pharmacoresistance 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 pharmacoresistance to available medications. Taken together, the in vitro and in vivo data presented here represents the first systematic evaluation of the effects of standard AEDs in a rodent model of CD-associated epilepsy. We propose that future examination of potential mechanisms of pharmacoresistance in the dysplastic brain (either in MAM-exposed rats or other animal models of CD) will lead to a more complete understanding of how CD-associated epilepsies may be treated.

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: the maximal electroshock test is the classical animal model of a generalized tonic–clonic convulsion and is used to identify compounds that can prevent the spread of seizures. It
is reasonably predictive of a compound’s effectiveness against partial and generalized tonic–clonic seizures. The subcutaneous administration of pentylenetetrazol test is used to identify compounds that can raise seizure threshold and predicts effectiveness against absence seizures (White et al., 1995). This process does not include testing of animal models that mimic specific clinical syndromes i.e. animal models of CD (Chevassus-au-Louis et al., 1999). Given that our findings confirm the presence of broad pharmacological resistance in an animal model designed to mimic CD, 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.

5. Conclusions

Here we describe AED actions in rats exposed to MAM in utero. Our data demonstrate a direct correlation between in vitro electrophysiological recordings and the in vivo response to AEDs. The consistent finding of drug resistance using a panel of standard AEDs in the acute hippocampal slice preparation, and one AED in a common behavioral seizure model, mimics the clinical situation reported in dysplasia-associated epilepsy. As such, our data support the continued use of animal models in the rational design and testing of novel anticonvulsant treatments.

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