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Rapid loss of efficacy to the antiseizure drugs lamotrigine and carbamazepine: a novel experimental model of pharmacoresistant epilepsy

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Abstract

Purpose—Kindling is a well established model of secondarily generalized partial seizures that is widely employed in the search for novel antiseizure drugs. During the kindling and post-kindling acquisition phase, an active process of neuronal remodeling occurs. We tested the hypothesis that exposure to the voltage-gated sodium channel blockers, lamotrigine (LTG) and carbamazepine (CBZ) during the period of active remodeling will lead to a diminished therapeutic effect.

Methods—Two days after the last kindling stimulation, fully kindled rats were randomized to receive either 0.5% methyl cellulose (MC), LTG (30 mg/kg), or CBZ (40 mg/kg). The effect of LTG and CBZ on behavioral seizure severity and electrographic afterdischarge duration (ADD) was recorded. One week after this treatment, rats in both groups were re-challenged with LTG 30 or CBZ 40 mg/kg and their seizure score and ADD recorded. *In vitro* efficacy of LTG on neuronal action potentials was also evaluated using whole cell current clamp recording in hippocampal brain slices obtained from rats treated similarly to the *in vivo* experiments.

Key Findings—When acutely administered 48 hrs after the last kindling stimulation, LTG and CBZ blocked the expression of behavioral seizures and reduced the ADD. In contrast, a second challenge dose of LTG or CBZ administered after a 7 day “no drug, no stimulation” period, did not result in reduction of either the seizure score or the ADD. Interestingly, the potassium channel opener, ezogabine also known as retigabine (EZG; 40 mg/kg), blocked the expression of behavioral seizures at both time points evaluated (i.e., 2 days and 9 days after last stimulation). *In vivo* resistance to LTG was associated with a similar reduction in LTG’s ability to limit action potential firing in CA1 neurons. LTG (50 μ M) significantly decreased the number of action potentials generated by a depolarizing current pulse in neurons recorded from slices obtained from kindled control and LTG-sensitive rats, but not in slices obtained from LTG-resistant rats.

Significance—Collectively, results obtained from both *in vivo* and *in vitro* studies demonstrate that even a single exposure to the sodium channel blockers, LTG or CBZ during the post kindling remodeling phase leads to an altered pharmacological response to these two ASDs, but not to EZG. The LTG and CBZ-resistant amygdala kindled rats may serve as a useful model of therapy-resistant epilepsy.

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We confirm that we have read the Journal’s policy on ethical publication and assure that this report is consistent with those guidelines.

Keywords

Pharmacoresistance; kindling; lamotrigine; carbamazepine; seizure animal model

INTRODUCTION

Pharmacoresistant epilepsy represents a major clinical problem in a substantial proportion (30%) of patients with epilepsy (Kwan, et al. 2008). Recent clinical and experimental studies have suggested that pharmacoresistance could result from an insufficient antiseizure drug (ASD) concentration in the brain due to over-expression of drug transporter proteins and/or functional changes in the drug targets such as voltage-gated sodium channels. (Loscher 2007), Remy and Beck 2006). Animal models of pharmacoresistance can provide an important tool to help understand the molecular basis underlying therapy resistance to ASDs.

Kindling is a well established model of acquired focal epilepsy and synaptic plasticity in the nervous system (Akbar, et al. 1996). Studies using the kindling model have confirmed that the brain actively remodels itself in response to excessive neural activation, such as seen during seizures (Geinisman, et al. 1988). During kindling acquisition and the post-kindling phase (once kindling has been established), an active process of synaptic plasticity and remodeling occurs which may contribute to a long-lasting alterations in seizure susceptibility. Contributing factors to remodeling likely also involve a variety of molecular and cellular changes in ion channel receptors and neurotransmitters (Blumenfeld, et al. 2009, Brooks-Kayal, et al. 1999, Ellerkmann, et al. 2003, Morimoto, et al. 2004). One of the changes that occur during the remodeling that follows pilocarpine-induced status epilepticus and amygdala kindling is an alteration in expression and functional properties of voltage-gated sodium channels (VGSCs) (Blumenfeld, et al. 2009, Ellerkmann, et al. 2003). Recently Blumenfeld et al, have found that once established, the long term facilitation observed in kindling was associated with increased expression in Nav1.6 subunit of VGSC in CA3 hippocampal neurons (Blumenfeld, et al. 2009). In contrast, decreased expression of Nav1.6 in med^{tg} heterozygote mice resulted in an increased resistance to the initiation and development of kindling (Burgess, et al. 1995). These changes are of clinical relevance because VGSCs are one of the primary targets for many of the conventional first line ASDs including (CBZ), phenytoin (PHT), oxcarbazepine (OXC), and LTG (Catterall 1999), Ragsdale and Avoli 1998). Changes in expression of VGSCs may lead to a loss of therapeutic efficacy of ASDs with sodium channel blocking properties (Remy, et al. 2003b, Vreugdenhil and Wadman 1999). Indeed, in CBZ-resistant epilepsy patients with refractory epilepsy, the use-dependent block of VGSCs by CBZ is substantially diminished (Remy, et al. 2003a). In addition, impaired modulation of sodium currents in CA1 neurons by CBZ has also been reported in epileptic rats (Remy, et al 2003b). Therefore, a model of epilepsy wherein there is resistance to commonly prescribed ASDs would be helpful in identifying innovative therapies that may be helpful for the treatment of pharmacoresistant epilepsy. In the present investigation, we tested the hypothesis that *kindled rats exposed to sodium channel blocking ASDs during the post-kindling remodeling phase leads to the development of a pharmacoresistant state*. Herein, we describe a novel state of pharmacoresistance that develops rapidly following single exposure to the VGSC blockers, LTG and CBZ. This novel model of LTG- and CBZ-resistance may prove useful in the identification of therapeutic approaches for the treatment of pharmacoresistant epilepsy and furthering our understanding of the mechanisms underlying pharmacoresistance.

METHODS

Animals

Adult male Sprague-Dawley rats (225–250 g; Charles River, Wilmington, MA) were group housed in a temperature- and humidity-controlled facility and maintained on a constant 12 hr light/dark cycle with access to standard laboratory chow and water *ad libitum*. Animal care and use was in accordance with the guidelines set by National Institute of Health and the University of Utah Institutional Animal Care and Use Committee (IACUC) committee in an AAALAC approved facility.

Surgical placement of kindling electrode

Rats were anesthetized with Ketamine/Xylazine (120 mg/kg/12 mg/kg; i.p). Under aseptic conditions, a bipolar electrode (Plastic One, Roanoke, VA, USA) was implanted stereotaxically into the right basolateral amygdala (AP –2.2, ML –4.7, and DV –8.7), with reference to bregma (Paxinos 1998). These electrodes consisted of two twisted, Teflon-coated stainless steel wires. The bipolar electrodes implanted into the basolateral amygdala were used to record the after discharge (AD) evoked by the kindling stimulus. The electrode assembly was anchored to the skull and fixed by dental acrylic cement. Following surgery, animals received a single injection of Penicillin G (60,000; s.c; MWI, Meridian, ID, USA) and were allowed to recover for one week post-operatively.

Amygdala kindling and drug treatment protocol

On the day of the experiment rats were placed in a recording chamber to acclimatize for 30 min. A 10 sec baseline EEG recording was obtained using a Biopack MP100 system. Rats were then stimulated once daily using a supra-threshold stimulus (200 μ Amp for 50 Hz, 2 sec duration) and observed for the presence or absence of behavioral seizures. Seizure severity was assessed using the Racine scale: 0, no response; stage 1, grooming, hyperactivity; stage 2, head nodding; tremor; stage 3, unilateral forelimb clonus; stage 4, clonus with rearing; stage 5, generalized tonic-clonic seizure with loss of righting reflex (Racine 1972). The EEG following stimulation was recorded for a period of 180 sec and the AD duration (ADD) was determined. Rats were considered to be fully kindled when they showed consecutive stage 4 or 5 seizures for 3–4 days. Only fully kindled animals were advanced for subsequent pharmacological treatment. For the LTG experiments, a total of 97 rats were kindled in 7 separate experiments; 6 rats lost their electrodes. The remainder of the fully kindled rats (n = 91) were randomized into two groups. One group received LTG 30 mg/kg (n = 56) and the other 0.5% MC (n = 35) two days following the last kindling session (day 2). For the CBZ studies, forty three (n = 43) animals were implanted and kindled in 3 separate experiments. We excluded four rats from the study due to loss of their electrodes. The remaining fully kindled rats (n = 39) were randomized into two groups. One group received; CBZ 40mg/kg (n = 19) and the other 0.5% MC (n = 20) two days after their last kindling stimulation. CBZ and LTG were both administered 60 min prior to stimulation. The times employed in the present study correspond to the time to peak pharmacodynamic effect for each of the three drugs evaluated. This approach is considered appropriate for pharmacological testing. The dose of LTG and CBZ used in the present investigation are also consistent with efficacious doses of these two drugs in standard kindling models and other rodent seizure tests (Krupp, et al. 2000). The seizure score and ADD were recorded. After a one-week stimulus- and drug-free period (day 9), rats received a single dose of either the same ASD or MC prior to being stimulated. The seizure score and ADD were recorded. In a separate experiment, 8 fully kindled rats received a single intraperitoneal dose of EZG (40mg/kg) 10 min prior to the kindling stimulation on day 2 and day 9 after their last kindling stimulation.

The drugs were kindly provided by Glaxo-Smith Kline, Brentford, UK (LTG) and Valeant Pharmaceuticals, Costa Mesa, CA (EZG). CBZ was purchased from Sigma (St. Louis, MO). Drugs were suspended in 0.5% MC and were administered in a volume that did not exceed 1 ml/100 g body weight.

Brain slice preparation and electrophysiology

Hippocampal brain slices were prepared from naïve, kindled and LTG-treated kindled rats, as described previously (Otto, et al. 2006). Coronal sections (400 μ m) were cut using a vibratome (Vibratome, St. Louis, MO) and transferred to a holding chamber containing oxygenated Ringer solution similar to the sucrose Ringer, but with 126 mM NaCl in place of sucrose (pH 7.30–7.32; 290–300 mOsm). Slices were incubated at room temperature for approximately one hour before recording.

Whole-cell patch clamp recordings were obtained from hippocampal CA1 pyramidal neurons using a MultiClamp 700A amplifier (Molecular devices, Sunnyvale, CA). Patch clamp electrodes of resistance 2.5–3 M Ω were filled with an internal solution containing (in mM): 130 K gluconate, 10 KCl, 10 HEPES, 1 EGTA, and 0.2 CaCl₂ (pH 7.28–7.30 290 mOsm). The external Ringer solution was supplemented with picrotoxin (50 μ M), APV (50 μ M), and CNQX (10 μ M) to block GABAergic, NMDA and non-NMDA receptor-mediated receptors, respectively. Recordings were made in current clamp mode using Clampex 8.0 and data stored for offline analysis using Clampfit (Molecular Devices, Sunnyvale, CA) and Mini Analysis (Synaptosoft Inc., Fort Lee, NJ). The cells were held at –65 mV and current was injected in a series of steps ranging from –80 to + 240 pA of 1.25 sec duration. Corrections for liquid junction potentials were not made. The trains of action potentials generated were recorded under control conditions and in the presence of 50 μ M LTG (20 min). A steady state pharmacological effect was observed in most experiments by 15–20 min, and hence the effects of LTG on action potential firing were compared at 20 min and expressed as % of control for each cell. The ability of CA1 neurons to accommodate action potential frequency was determined by plotting interspike interval number *vs.* normalized spike frequency. Each cell served as its own control, and the frequency of each subsequent interspike interval was normalized to that of the first interspike interval.

Statistical analysis

Seizure score data are presented as mean, except the ADD which is expressed as mean $\pm$ SD. Significant differences in behavioral seizure scores between treatment groups were determined by the non-parametric Mann-Whitney U test. Statistical difference in percent incidence of generalized seizures was determined by Fischer's exact test. Statistical differences between the treated groups in *in vitro* slice recordings were determined by ANOVA. A $p < 0.05$ was considered significant.

RESULTS

Rapid development of pharmacoresistance to LTG and CBZ in kindled rats

Earlier studies have reported that administration of LTG reduces the severity of amygdala-kindled seizures and increases the afterdischarge and seizure thresholds in fully kindled rats (Postma, et al. 2000). In the present investigation, MC administered two days after the establishment of kindling (MC control group; day 2) did not affect either the behavioral seizures or the ADD of animals exposed to the same stimulation employed to establish a stable kindled seizure (Fig 1D, E, F; 3D, E, F). In contrast, LTG administration two days following the establishment of kindling successfully blocked the stimulation-induced behavioral seizures in fully kindled rats. For example, LTG (30 mg/kg) significantly reduced the seizure score from 5 to 1.7 (Fig 1B). At this dose only 21 % of the animals (12/56)

displayed generalized behavioral seizures (Fig 1A). This dose of LTG also significantly reduced the ADD from 82 ± 44 sec to 26 ± 24 sec (Fig 1C). When a second dose of LTG (30 mg/kg) was administered 7 days later (day 9), the rats that were previously found to be sensitive to the anticonvulsant effects of LTG were now resistant to the same dose of LTG (seizure score, 4.6; ADD, 90 ± 43 sec). LTG resistance was observed in 91 % of animals (51/56). In contrast, when LTG (30 mg/kg) was administered to the MC control group (on day 9), the seizure severity score (1.9) and ADD (34 ± 34 sec) (Fig 1 E, F) were reduced. These animals were considered to be *LTG-sensitive*. In our pilot studies, rats that received 30 mg/kg LTG without a stimulus on day 2, did not develop pharmacoresistance with a 2nd challenge dose of LTG at day 9 (data not discussed).

To determine if the pharmacoresistance that developed after the initial LTG treatment was specific to only LTG, a group of kindled animals were treated using the same kindling and dosing paradigm as above with the sodium channel blocker CBZ. CBZ treatment also resulted in the rapid development of pharmacoresistance in kindled animals. CBZ (40 mg/kg) administration initially conferred complete protection against behavioral and electrographic seizures in fully kindled rats when administered 48 hours (day 2) after the last kindling session. Indeed, CBZ (40 mg/kg) treatment significantly reduced the seizure score from 5 to 1.1 (Fig 3B) and reduced the ADD from 50 ± 22 sec to 12 ± 18 sec (Fig 3C). However, as was observed with LTG treatment, rats exposed to a second dose of CBZ 7 days after the first treatment (day 9), displayed complete resistance to the anti-seizure effects of CBZ (40 mg/kg). Following the second dose of CBZ, the observed seizure score (4.3) and ADD (59 ± 23 sec) were not statistically different from the vehicle control group (5) and (50 ± 22 sec), respectively (Fig 3 B, C). Further, 89 % of the animals (17/19) displayed generalized behavioral seizures when they received a second injection of CBZ one week after the initial treatment, as compared to 5 % of the animals (1/19) that were protected at day 2. (Fig 3 A)

Ezogabine (EZG) treatment did not result in pharmacoresistance

In a separate study, EZG completely blocked the expression of behavioral seizures in fully kindled rats at 2 days and 9 days after the last stimulus (Fig. 2C). The present results suggest that the resistance that develops in this model is specific to the ASD administered. For the EZG experiment, we used a maximal tolerable dose (i.e., 40 mg/kg). EZG has been found to be active (ED_{50} range = 4.0–18.6 mg/kg, i.p.) against electrically induced seizures and chemical seizures (Rostock, et al. 1996).

LTG is less effective in reducing action potential firing in CA1 pyramidal neurons in LTG-resistant kindled rats

To better understand the mechanism underlying the development of pharmacoresistance at the neuronal level, we examined the effects of LTG on action potential firing of CA1 pyramidal neurons of the hippocampus in acute brain slices prepared from naïve, kindled control, LTG-sensitive and LTG-resistant group of rats. Rats from the naïve group did not have an implanted electrode and did not receive stimulation. Rats in the kindled group were kindled according to the protocol described in the methods but never exposed to LTG. Kindled animals treated with LTG in the paradigm described earlier, were grouped into LTG-sensitive and LTG-resistant rats. Brain slices were prepared one day after the last stimulation (and drug treatment; day 9) by an investigator blinded to the treatment group and action potentials in CA1 pyramidal cells were recorded using the whole cell patch clamp technique in current clamp mode. The resting membrane potential of the neurons were not significantly different among groups (-62.2 ± 0.89 mV in naïve rats, as compared to -64.5 ± 2.06 mV in kindled control and -64.13 ± 1.13 mV in LTG-treated rats). Likewise, the firing threshold of CA1 neurons were not different in slices prepared from LTG-treated, kindled

control and naïve rats (-56.13 ± 0.6 , -58 ± 0.82 and -55.82 ± 0.68 mV respectively). The input resistance of the neurons from these groups of rats did not show any significant difference ($p > 0.05$, Student's *t*-test). The input resistance in LTG-treated, kindled control and naïve rats were 88.25 ± 5.98 , 98.90 ± 13.63 , and 102.56 ± 7.37 M Ω , respectively. Injection of a depolarizing current pulse (80 pA; 1.25 sec duration) elicited a train of action potentials (Fig 4A) in CA1 neurons recorded in slices from all groups of rats. Application of LTG (50 μ M) reduced the number of action potentials elicited in CA1 neurons (40–45%) in brain slices prepared from naïve, kindled and LTG-sensitive rats (Fig 4A, B; $p < 0.05$, one way ANOVA). In contrast, the same concentration of LTG was less effective in reducing action potential firing ($21.8 \pm 4.5\%$) in brain slices prepared from LTG-resistant rats (Fig 4). Since naïve, kindled and LTG-sensitive rats exhibited a similar degree of response to LTG application (Fig. 4A, B), only untreated kindled rats (kindled controls) were used as controls for further *in vitro* analysis.

As shown in Fig 4C, LTG was found to inhibit action potential firing in CA1 neurons in a concentration-dependent manner. However, when compared to slices obtained from kindled control animals, there was a decrease in the efficacy of LTG in slices obtained from LTG-resistant rats. In the presence of LTG (100 μ M), the firing frequency of CA1 neurons obtained from LTG-resistant rats was $46 \pm 10\%$ of control, as compared to $24 \pm 5.4\%$ in kindled control rats.

Effect of LTG on spike frequency adaptation of CA1 neurons

Depolarizing current injections of long duration induces repetitive firing of action potentials in CA1 neurons. These spike trains also exhibit spike frequency adaptation. Since frequency adaptation is an important mechanism in controlling network excitability, the effect of LTG in modulating spike frequency adaptation was examined in slices obtained from kindled and LTG-resistant rats. LTG (50 μ M), did not affect the ability of CA1 neurons to adapt to spike frequency in either kindled control or LTG-resistant rats (Fig 5A, B). However, LTG (100 μ M) increased spike frequency adaptation in neurons from kindled rats, but remained ineffective in slices obtained from LTG-resistant rats (Fig 5C, D).

DISCUSSION

The present study demonstrates that LTG, when administered to amygdala kindled rats two days after the last kindling stimulation, leads to the development of profound LTG resistance. A unique feature of this model is that near complete resistance develops following a single exposure to LTG and a single stimulus. In order to verify if observed LTG resistance is a permanent phenomenon, we administered a 3rd dose of LTG, a week after the last dose in LTG-resistant rats. Interestingly, rats remained insensitive to a challenge dose of LTG. These LTG-resistant rats were non responsive to even higher doses of LTG (up to 45 mg/kg) (Preliminary findings). Interestingly, resistance developed in rats exposed to another sodium channel blocker; i.e., CBZ. A single dose of CBZ when administered 2 days after the last kindling session significantly blocked the expression of behavioral seizures, and the same animals developed resistance to the challenge dose of CBZ administered seven days later. Although not tested in this model, we recently reported an interesting cross-resistance between LTG and CBZ in another model of pharmacoresistant epilepsy; i.e., animals resistant to LTG were also found to be resistant to a therapeutic dose of CBZ (Srivastava and White 2013). However, the same experimental protocol using the M-current activator EZG did not result in the development of pharmacoresistance. It is not clear what the clinical impact of these findings might be and further studies are clearly needed before any firm conclusion can be made.

To test the hypothesis that pharmacoresistant animals have an altered neuronal response to LTG, electrophysiology experiments were performed using brain slices obtained from naïve, kindled control, LTG-sensitive and LTG-resistant animals. LTG inhibits repetitive neuronal firing *via* blockade of voltage-dependent sodium channels (Cheung, et al. 1992). In the present study, LTG produced a concentration-dependent reduction in action potential firing of CA1 neurons. LTG preferentially blocked action potentials generated towards the end of the depolarizing pulse; an effect consistent with an activity dependent block of sodium currents (Calabresi, et al. 2003). The extent of inhibition in slices obtained from naïve, kindled and LTG-sensitive animals was similar; evidence that kindling itself did not change the firing pattern of CA1 neurons (Fig 4B). However, in slices obtained from LTG-resistant animals, the ability of LTG to block evoked action potentials was attenuated when compared to the LTG-sensitive rats. The loss of *in vivo* efficacy of LTG is considerably more marked than the loss of pharmacological sensitivity in brain slices. This may be due to reduction in sustained repetitive firing of neurons by LTG, without actually interfering with normal action potential firing and synaptic transmission.

The precise mechanism underlying the reduced efficacy of LTG in resistant animals is not currently known. One testable hypothesis is that LTG-resistance is due to altered expression of sodium channel subunits. This hypothesis is supported in part by the results from previous human and experimental epilepsy studies, wherein numerous changes in sodium channel subunit expression have been reported (Aronica, et al. 2001, Bartolomei, et al. 1997, Whitaker, et al. 2001). In this respect, decreased expression of accessory Na⁺ channel β 1 and β 2 subunits following experimentally induced status epilepticus appears to be a consistent finding (Gastaldi, et al. 1998). Indeed, Blumenfeld *et al.*, has shown that the kindling process *per se* was associated with an increased expression of the Nav1.6 subunit of sodium channels and increased persistent sodium currents in hippocampal CA3 neurons (Blumenfeld, et al. 2009). Kindling is an ongoing activity-dependent plasticity phenomenon. Exposure to sodium channel blockers while plastic changes continue may lead to an even more robust dysregulation in the expression of sodium channel subunits as compared to the kindling process in the absence of sodium channel blockers. Although we have not yet tested this hypothesis, it remains plausible that altered expression of sodium channel subunits may participate in the mechanism underlying LTG resistance. However, there is currently no evidence that different subunits of sodium channel will have different pharmacological sensitivities to LTG. This is further supported by our preliminary observation that the LTG-resistant rats were non-responsive to even a 3rd dose of LTG (a week after the last dose), indicating the pharmacoresistance observed in this model is robust and lasting.

The pharmacoresistant kindled rat model described in this study is unique among the previously described models of ASD resistance (Loscher, 1993). The finding that resistance develops to a single dose of sodium channel blocking ASDs (LTG, CBZ) had led to the characterization of a unique platform to evaluate the molecular mechanisms underlying pharmacoresistance. Although the molecular basis underlying the observed pharmacoresistance in this model has yet to be defined, the finding that drug exposure during the active synaptic remodeling phase may lead to the development of drug resistance was supported by *in vitro* electrophysiological findings. Lastly, the rapidity to which resistance to LTG or CBZ develops is an important attribute of this unique model that can be utilized to help define the cellular and molecular mechanisms of pharmacoresistance to sodium channel blocking drugs. The present model could become an important tool for evaluating pharmacoresistant seizures and aid in the search for novel ASDs that are useful in the treatment of pharmacoresistant epilepsy.

Conclusion

Our present results support the hypothesis that treatment during the post-kindling remodeling phase with sodium channel blockers may contribute to pharmacoresistance in a well-established animal model of epilepsy. Further investigations are required before any substantial conclusions can be made regarding the clinical implications of this observation.

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Figure 1.

Two groups of fully kindled rats (MC, n = 35; LTG group, n = 56) were randomized to receive 0.5% MC (i.p.) or a single dose of LTG (30 mg/kg, i.p.) two days following the last kindling session. For seizure score, scatter plots are used to illustrate the effect of LTG in both protected and non protected rats at day 2 and day 9. A significant reduction in the percent incidence of generalized seizures, seizure severity and mean ADD was seen in the LTG-treated rats, but not in the MC group. Seven days after a "LTG- and stimulation-free" period a single challenge dose of 30 mg/kg LTG given to both groups of rats significantly reduced the mean seizure score ($p < 0.001$ Mann-Whitney U test) and ADD ($p < 0.05$ Student's t-test) in the MC group, but not in LTG group.

Figure 2.

A: Representative EEG traces from LTG-sensitive (**A**) and LTG-resistant (**B**) rats are shown. Note that whereas LTG significantly reduced the ADD at Day 2 (**B**), the ADD at Day 9 was quite prolonged. In contrast, LTG administration on Day 9 to rats in the MC group was effective in blocking the ADD. **C:** EZG (40 mg/kg), completely blocked the expression of behavioral seizures in fully kindled rats ($n=8$) at both treatment times (i.e., 2 days and 9 days after their last kindling session; $p < 0.05$, Fischer's exact test). MC treatment was not repeated in this experiment, as MC *per se* does not affect the seizure score or mean ADD.

Figure 3.

Thirty nine (n=39) fully kindled animals were randomized into two groups, Control (MC, n = 20) and CBZ group (n = 19). Control group received 0.5% MC (i.p.), while CBZ-treatment group received a single dose of CBZ (40 mg/kg, i.p.), two days following the last kindling session. For seizure score, scatter plots are used to illustrate the effect of CBZ on seizure severity in both protected and non protected rats at day 2 and day 9. A reduction in the incidence of generalized seizures, seizure severity, and mean ADD was seen in the CBZ-treated rats, but not in the MC group. Seven days after a "CBZ- and stimulation-free" period (day 9), a single challenge dose of CBZ (40 mg/kg) in both groups significantly reduced the mean seizure score and mean ADD in the MC group, but not in the CBZ group.

Figure 4.

A, B: LTG (50 μ M) attenuated action potential generation in CA1 hippocampal neurons. The number of cells in each group is indicated by “n”, obtained from 4–12 rats (N) in each group. A significant reduction in action potential firing was observed in brain slices obtained from naïve (N= 10), kindled control (N= 4), and LTG-sensitive rats (N= 7) ($p < 0.05$ one-way ANOVA), as compared to LTG-resistant rats (N= 12). **C:** Concentration-response of LTG (10–100 μ M) on action potentials elicited in CA1 neurons of slices obtained from kindled control and LTG-resistant rats. The number of action potentials in slices obtained

from LTG resistant rats were significantly greater than those seen in kindled control rats ($p < 0.05$, two-way ANOVA) following bath administration of 50 and 100 μM LTG.

Figure 5.

Spike frequency adaptation (SFA) in CA1 hippocampal neurons in slices from kindled control and LTG-resistant rats. The interspike interval (ISI) number was plotted against normalized ISI frequency. Each cell served as its own control. Frequency of each subsequent ISI was normalized to the first ISI. There was no significant difference in spike frequency adaptation in the presence of LTG (50 μ M) in slices from either kindled controls or LTG-resistant animals (**A**, **B**). However, spike frequency adaptation (SFA) was increased in the presence of LTG (100 μ M) in kindled control rats, shifting the curve to the left (**C**). No effect on SFA was seen in slices obtained from LTG-resistant rats (**D**).