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Mode of seizure inhibition by sodium channel blockers, an SV2A ligand, and an AMPA receptor antagonist in a rat amygdala kindling model

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ABSTRACT

Purpose: A number of antiepileptic drugs (AEDs) with a variety of modes of action, are effective in treating focal seizures. Several AEDs, such as perampanel (PER), levetiracetam (LEV), lacosamide (LCM), lamotrigine (LTG), and carbamazepine (CBZ), have been shown to elevate the seizure threshold in kindling models. These AEDs are clinically effective, but differences exist in the anti-seizure profiles of drugs with similar modes of action. Therefore, we hypothesized that there are differences in how these AEDs affect seizures. Here, we evaluated the effects of AEDs on various seizure parameters in a rat amygdala kindling model upon stimulation at the after-discharge threshold (ADT) and at three-times the ADT (3xADT) to characterize the differences in the effects of these AEDs.

Methods: PER, LEV, LCM, LTG, CBZ, or vehicle was administered intraperitoneally to fully kindled rats. Changes in Racine seizure score, after-discharge duration (ADD), and latency to Racine score 4 generalized seizure (S_4L) were measured to assess differences in the modes of seizure inhibition among the AEDs. Stimulation at 3xADT was used to eliminate the influence of any AED-induced elevation of the seizure threshold on these parameters.

Results: PER, LEV, LCM, LTG, and CBZ significantly reduced the seizure score from Racine score 5 after stimulation at the ADT; this effect was lost with LEV and LTG after stimulation at 3xADT. PER and LEV significantly shortened the ADD when the seizure focus was stimulated at the ADT, whereas LCM, LTG, and CBZ did not. LEV, LCM, LTG, and CBZ failed to shorten the ADD upon stimulation at 3xADT. PER dose-dependently and significantly increased S_4L, even at doses that were ineffective for seizure score reduction, after stimulation at both the ADT and 3xADT. LEV and LTG significantly increased S_4L after stimulation at the ADT, whereas LCM and CBZ did not significantly increase S_4L at any of the doses tested.

Conclusions: The sodium channel blockers (LCM, LTG, and CBZ) appeared to act by elevation of the seizure threshold via reduction of neuronal excitability, whereas the AMPA receptor antagonist (PER) and the SV2A ligand (LEV), as well as LTG, exerted their effects through the weakening of synaptic transmission in neuronal networks at the seizure focus. Maintenance of the effect of PER even at 3xADT suggests direct and strong modulation of excitatory synaptic transmission by PER, both at the focus and along the seizure propagation route. These findings may provide further rationale for usage of AEDs beyond their respective modes of action.

1. Introduction

More than 20 antiepileptic drugs (AEDs) have been shown to be clinically effective. The molecular targets of AEDs are most often ion channels, neurotransmitters, or synaptic proteins. Common molecular targets include sodium channels, the synaptic vesicle protein (SV2A), gamma-aminobutyric acid (GABA)-related molecules, calcium channels, and glutamate receptors. The majority of AEDs act on the sodium channel (Rogawski et al., 2016) and are effective for focal seizures (NICE clinical guideline, last updated in 2018; https://www.nice.org.uk/guidance/cg137); however, differences in the level of efficacy demonstrated by sodium channel-blocking AEDs against generalized seizures have been reported (Brodie, 2017). For example, lamotrigine (LTG) demonstrates efficacy in absence seizures (Mikati and

Abbreviations: ADD, after-discharge duration; ADT, after-discharge threshold; 3xADT, three-times the after-discharge threshold; AED, antiepileptic drug; AMPA, α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid; ANOVA, analysis of variance; CBZ, carbamazepine; EEG, electroencephalogram; LCM, lacosamide; LEV, levetiracetam; LTG, lamotrigine; PER, perampanel; S_4L, score 4 latency; VPA, valproate

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Holmes, 1997; Hainsworth et al., 2003), whereas others have no effect on seizures of this type. It is therefore important to characterize the modes of seizure inhibition of different AEDs, in addition to their molecular targets.

Currently, there is no established methodology for characterizing modes of seizure inhibition, and to our knowledge there has been no systematic study comparing seizure suppression by different AEDs. This may be due to the range of established animal models used to estimate the clinical spectrum of AEDs. Drug development is facilitated by the use of these animal models, which translate to specific clinical seizure types (Löscher, 2011). However, after an AED is launched, it is initially evaluated as an adjunctive therapy. Rational combination therapy is now a major focus of adjunctive therapy, but there is a paucity of experimental and clinical evidence in support of this (Abou-Khalil, 2017). Multiple non-clinical studies have evaluated pharmacodynamic interactions using various animal models, but these studies have only targeted interactions in terms of anti-seizure effects and adverse reactions (Lee and Dworetzky, 2010; Wu et al., 2014; Sarhan et al., 2015; Russmann et al., 2016). Pharmacodynamic interaction studies from the perspective of mode of inhibition could provide a clearer understanding of rational drug combination.

The kindling model is a well-established animal model of focal to bilateral tonic-clonic seizures (McNamara, 1984). It has been used for examining the elevation of seizure thresholds, but it also allows assessment of additional parameters such as the after-discharge duration (ADD), latency to generalized seizure, and Racine seizure score (Löscher et al., 1986; Hewapathirane and Burnham, 2005; Beheshti Nasr et al., 2013; Cheng et al., 2015). The experimental conditions, including the strength of stimulation, can be modified (Morimoto et al., 1997; Otsuki et al., 1998). Assessment of the effects of AEDs, using various experimental approaches, can enable identification of the mode of action of each AED. Morimoto et al. (1997) evaluated LTG under varying stimulus intensity, and found that the efficacy of LTG decreased with increased intensity, leading the authors to conclude that LTG acts through elevation of the seizure threshold. Wu et al. (2014) also evaluated AEDs under increased stimulus intensity, and reported that only perampanel (PER) maintained its efficacy under these conditions. These results suggest that each AED may have a specific mode of seizure inhibition. Therefore, in this study, we used a rat amygdala kindling model to evaluate the efficacy of five AEDs typically used in the treatment of focal seizures upon various seizure parameters at different stimulus intensities, in order to elucidate the modes of seizure inhibition of these drugs.

2. Materials and methods

2.1. Animals

Male Wistar Kyoto rats (Charles River Laboratories Japan, Inc., Kanagawa, Japan), weighing 450–550 g, were used for all experiments. Animals were individually housed in cages in a controlled environment (12-h dark/light cycle [lights on between 07:00 and 19:00]) and had free access to food (Oriental MF; Oriental Yeast Co., Tokyo, Japan) and water. All procedures were performed in an animal facility accredited by the Center for Accreditation of Laboratory Animal Care and Use, Japan Health Sciences Foundation. All protocols were approved by the Institutional Animal Care and Use Committee and implemented according to the Eisai Animal Experimentation Regulations (Eisai Co., Ltd., Tokyo, Japan).

2.2. Stereotaxic surgery

Animals were acclimatized to the conditions described above for at least 1 week before surgery. On the day of surgery, rats were anesthetized with pentobarbital at 65 mg/kg (Somnopentyl; Kyoritsu Seiyaku Co., Ltd, Tokyo, Japan) administered intraperitoneally. A tripolar electrode (TN201 − 059; Unique Medical Co., Ltd, Tokyo, Japan) was implanted into the basolateral amygdala (anterior–posterior: –2.5 mm; lateral: –4.8 mm; depth: –7.5 mm) in accordance with the coordinates of Paxinos and Watson (2007). A reference electrode was placed on the contralateral cortex. Electrodes were fixed to the skull with acrylic dental cement. After electrode implantation, rats were returned to their cages and allowed to recover.

2.3. Amygdala kindling

After at least 1 week of recovery, the after-discharge threshold (ADT) was determined for each rat. To achieve this, the amygdala was stimulated with an electronic stimulator (SEN-7203, Nihon Kohden, Tokyo, Japan); stimulation consisted of 1-ms monophasic square-wave pulses at 50 Hz for 1 s. Stimulation was initiated at 0.04 mA and was then increased by 25% every 30 s until the ADT was elicited. The ADT was defined as the point at which an abnormal electroencephalogram (EEG) and a behavioral seizure of at least Racine score 1 were observed (Racine, 1972). Racine seizure scores were classified as follows: (1) mouth and facial movements; (2) head nodding; (3) unilateral forelimb clonus; (4) rearing and bilateral forelimb clonus; and (5) rearing and falling. A Racine score ≥ 4 is indicative of a generalized seizure. The rats then received daily stimulation at their ADT until they experienced three consecutive seizures of Racine score 5. Stimulation at the ADT was used to assess baseline Racine seizure score, score 4 latency (S_4L; the length of time from the point of stimulation to the point of appearance of seizures of Racine score ≥ 4), and the ADD (Beheshti Nasr et al., 2013; Cheng et al., 2015). In addition to stimulation at the ADT, stimulation at three-times the ADT (3xADT) was performed (Morimoto et al., 1997; Otsuki et al., 1998).

2.4. Drugs

The α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA) receptor antagonist PER (Eisai Co. Ltd, Tokyo, Japan), the SV2A ligand levetiracetam (LEV; Tokyo Chemical Industry Co., Ltd, Tokyo, Japan), and the sodium channel blockers lacosamide (LCM; Ark Pharm, Inc., Arlington Heights, IL, USA), lamotrigine (LTG; A K Scientific Inc., Union City, CA, USA), and carbamazepine (CBZ; Wako Pure Chemical Industries, Ltd., Tokyo, Japan) were dissolved in a 1:1:1 mixture of water, dimethyl sulfoxide, and polyethylene glycol 200 (hereafter referred to as vehicle). Drugs were administered intraperitoneally either 30 min (PER, LEV, LCM, CBZ) or 60 min (LTG) before ADT evaluation.

2.5. Dose selection

Doses of PER, LEV, LTG, and CBZ were selected in accordance with those used in a previous study (Wu et al., 2014), whereby the ADT of each animal treated with all five AEDs individually increased by more than 20% at the highest two doses administered, and the increase in ADT was significant compared with the vehicle group for at least one dose of each AED (data of LCM were the same as the present study).

2.6. Statistical analysis

Seizure behavior scores are presented in a range from 0 (no seizure behavior) to 5 (full motor seizure), and were analyzed using the Kruskal-Wallis test followed by Dunn's test. S_4L and ADD are presented as percentages of baseline (pre-drug) values, and were analyzed using one-way analysis of variance (ANOVA) followed by Dunnett's multiple-comparison test, comparing the vehicle group with each AED group given different doses. Animals in which seizures were suppressed to Racine score < 4 were excluded from the S_4L analysis, because precise durations of latency were not obtainable in these animals. GraphPad Prism software (Version. 7.02, GraphPad Software, Inc., San Diego, CA, USA) was used for all statistical analyses.

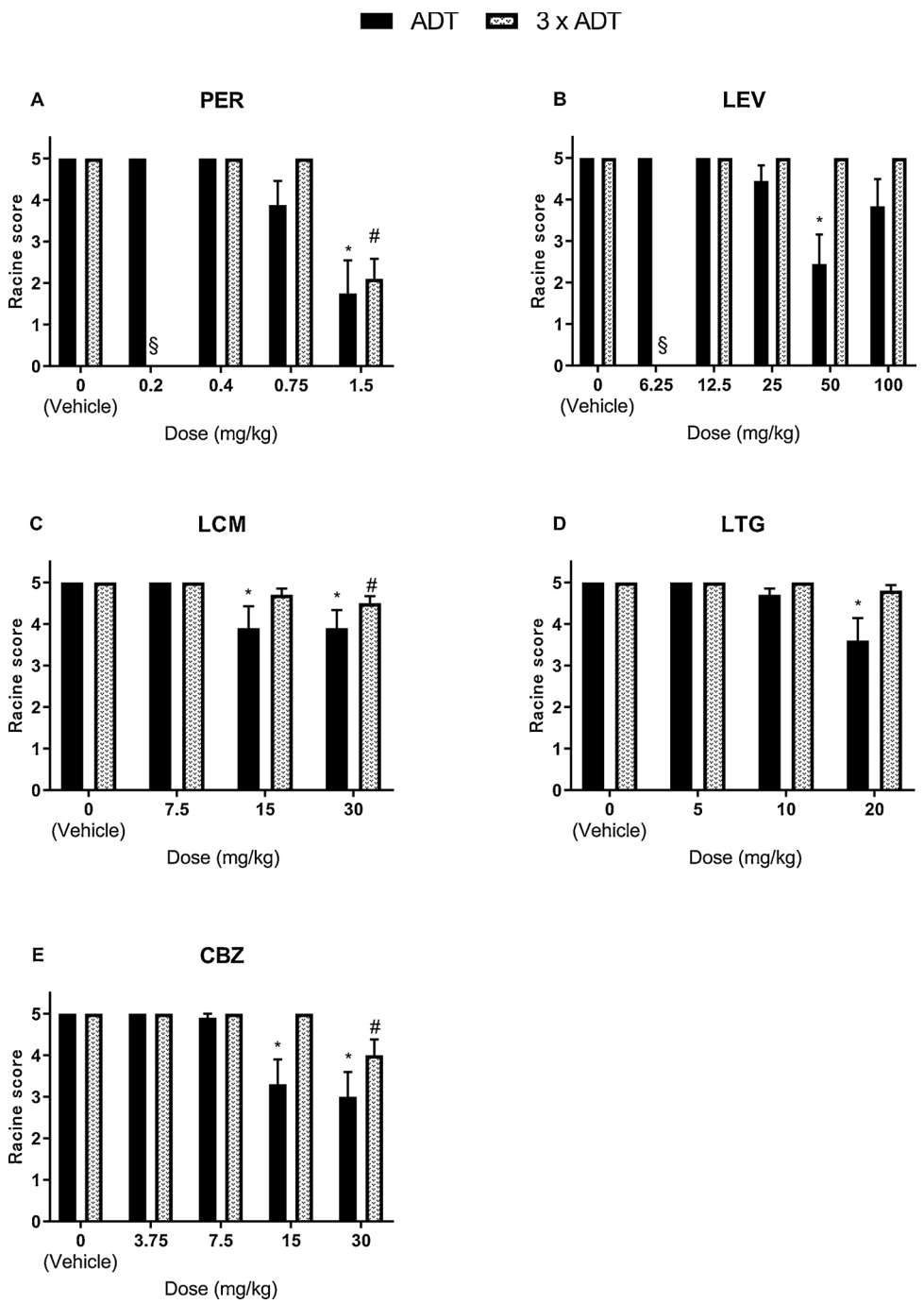

Fig. 1. Effects of (A) PER, (B) LEV, (C) LCM, (D) LTG, and (E) CBZ on Racine seizure score with stimulation at the ADT and at 3xADT.
*$P < 0.05$ compared with vehicle (stimulation at the ADT); #$P < 0.05$ compared with vehicle (stimulation at 3xADT); Kruskal-Wallis test followed by Dunn's test.
§Not tested.

3. Results

3.1. Effects of AEDs on behavioral seizure scores

Upon stimulation at the ADT, the mean Racine scores were 3.9 at 0.75 mg/kg and 1.8 at 1.5 mg/kg in the PER treatment group (Fig. 1A); 2.4 at 50 mg/kg and 3.8 at 100 mg/kg in the LEV treatment group (Fig. 1B); 3.9 at both 15 and 30 mg/kg in the LCM treatment group (Fig. 1C); 3.6 at 20 mg/kg in the LTG treatment group (Fig. 1D); and 3.3 at 15 mg/kg and 3.0 at 30 mg/kg in the CBZ treatment group (Fig. 1E). Doses of 1.5 mg/kg PER, 50 mg/kg LEV, both 15 and 30 mg/kg LCM, 20 mg/kg LTG, and both 15 and 30 mg/kg CBZ significantly decreased the Racine score compared with vehicle.

When the stimulation intensity was increased to 3xADT, LCM and CBZ at 30 mg/kg significantly decreased the Racine scores to 4.5 and 4.0, respectively, compared with vehicle (Fig. 1C and E). The only AED that suppressed seizures to a Racine score < 4 was PER, at a dose of 1.5 mg/kg; the Racine score was 2.1 (Fig. 1A).

3.2. Effects of AEDs on S_4L

Upon stimulation at the ADT, PER, LEV, and LTG significantly prolonged S_4L compared with vehicle. PER significantly increased S_4L to 215.2% of the baseline value at a dose of 0.4 mg/kg and to 274.4% at

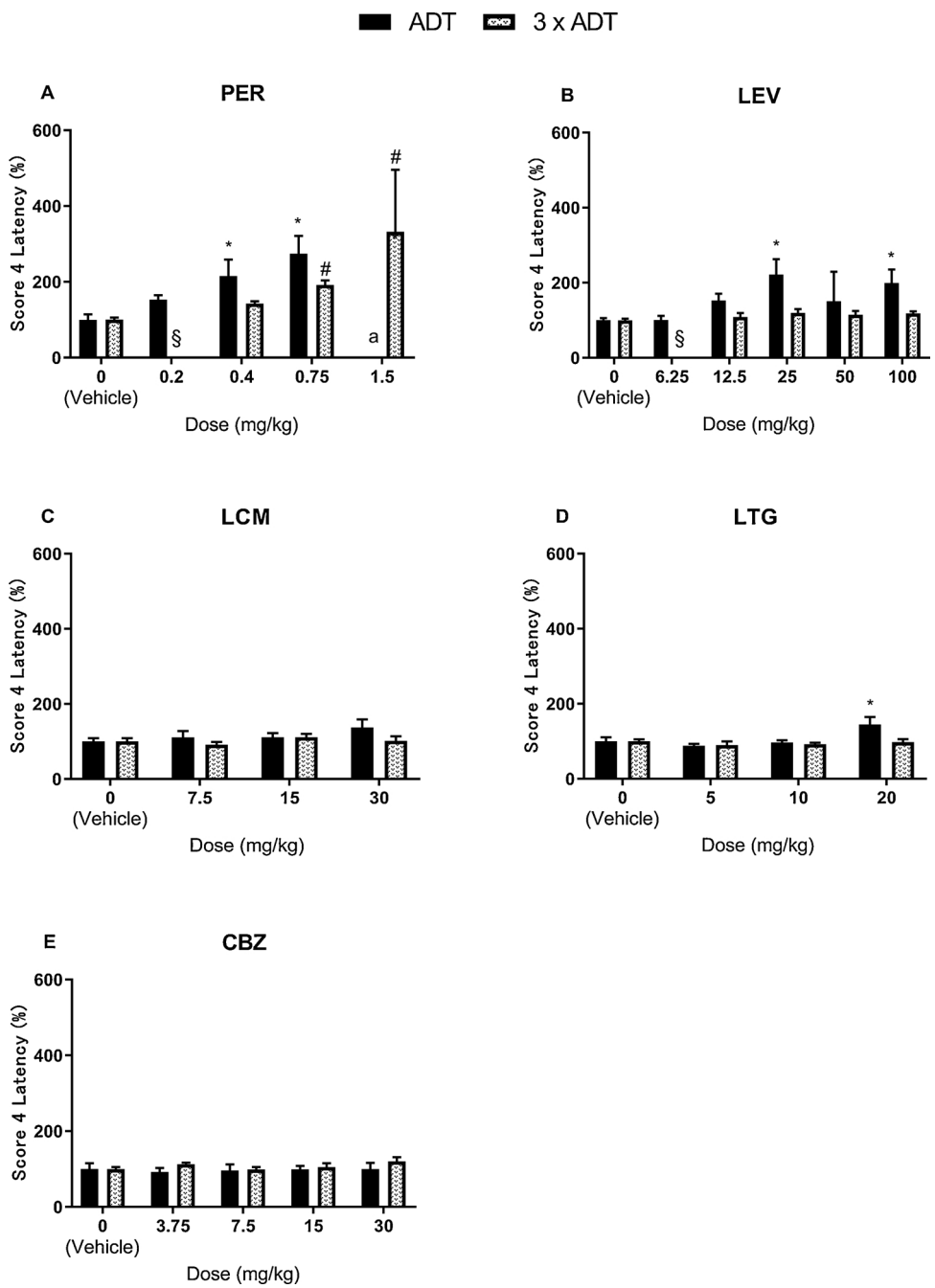

Fig. 2. Effects of (A) PER, (B) LEV, (C) LCM, (D) LTG, and (E) CBZ on latency to Racine score 4 generalized seizure with stimulation at the ADT and at 3xADT. Data are presented as percentages of baseline values.

*$P < 0.05$ compared with vehicle (stimulation at the ADT); #$P < 0.05$ compared with vehicle (stimulation at 3xADT); one-way ANOVA followed by Dunnett's multiple comparison test.

[§]Not tested.

[a]There were not enough animals with Racine seizure scores > 4 for statistical analysis, because of the potent seizure-inhibitory effect of PER at this dose.

0.75 mg/kg (Fig. 2A). For PER at a dose of 1.5 mg/kg, there were not enough animals with Racine seizure score ≥4 for statistical analysis, probably because of the potent seizure-inhibitory effect of PER at this dose. LEV significantly increased S_4L to 221.6% of the baseline value at 25 mg/kg and to 199.2% at 100 mg/kg (Fig. 2B). LTG significantly increased S_4L to 144.9% at 20 mg/kg (Fig. 2D). LCM and CBZ did not significantly affect S_4L (Fig. 2C and E). When the stimulation intensity was increased to 3xADT, PER at 0.75 mg/kg and 1.5 mg/kg significantly prolonged S_4L to 191.6% and 332.1%, respectively, compared with vehicle (Fig. 2A). The effect of PER on S_4L was dose-dependent at both the ADT and at 3xADT. LEV, LCM, LTG, and CBZ had no significant effects on S_4L at 3xADT.

3.3. Effects of AEDs on ADD

Upon stimulation at the ADT, PER, and LEV significantly reduced the ADD compared with vehicle. PER significantly shortened the ADD, in a dose-dependent manner, to 55.5% of the baseline value at 0.75 mg/kg and to 35.9% at 1.5 mg/kg (Fig. 3A). LEV significantly decreased the ADD to 45.4% at 50 mg/kg, but did not at 100 mg/kg (Fig. 3B). CBZ decreased the ADD to 64.5% at 15 mg/kg and to 69.4% at 30 mg/kg, but the effect was not significant at either dose (Fig. 3E). LCM and LTG did not affect ADD (Fig. 3C and D). When the stimulation intensity was increased to 3xADT, only PER at a dose of

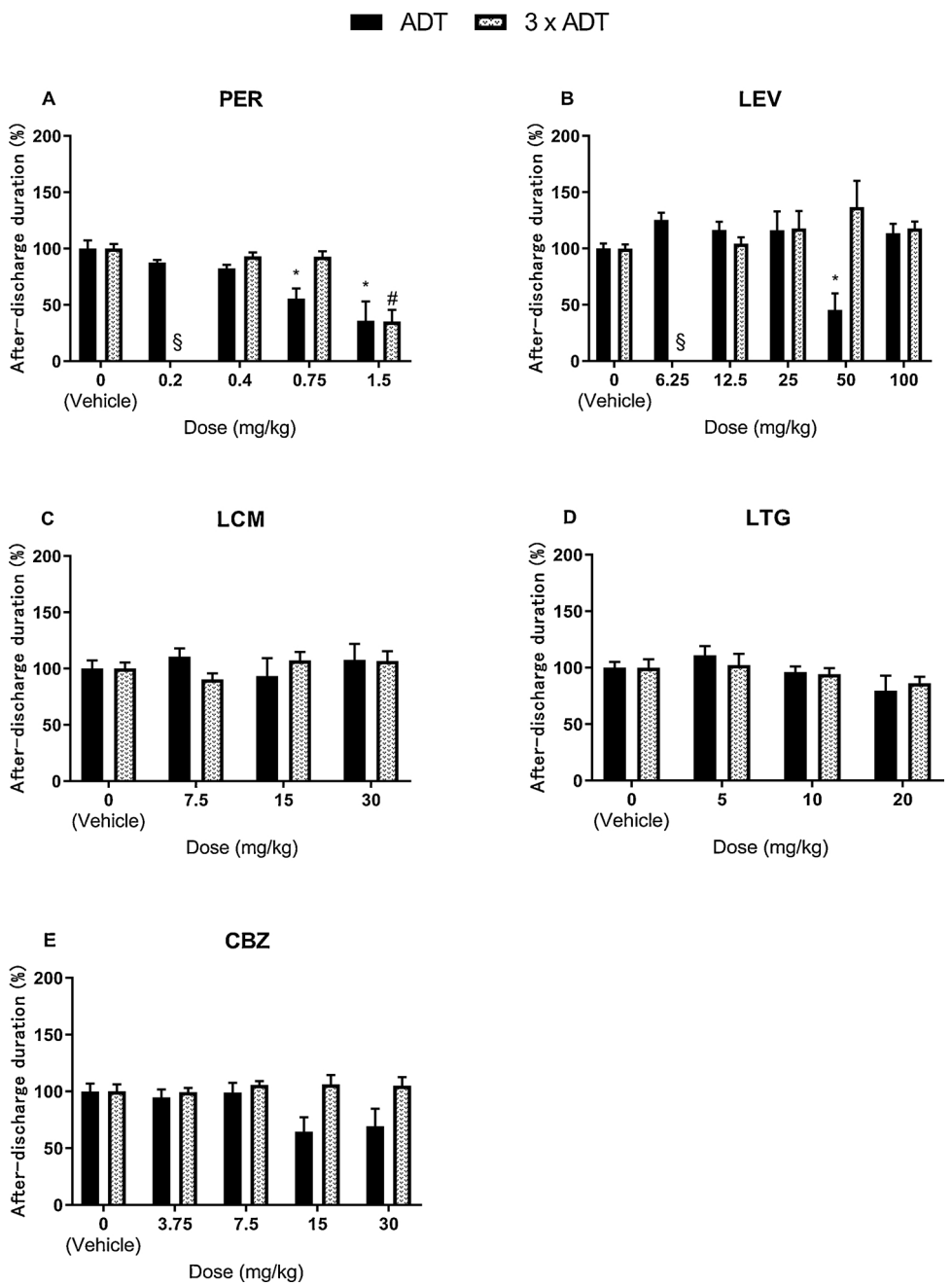

Fig. 3. Effects of (A) PER, (B) LEV, (C) LCM, (D) LTG, and (E) CBZ on after-discharge duration with stimulation at the ADT and at 3xADT.
Data are presented as percentages of baseline values.
*$P < 0.05$ compared with vehicle (stimulation at the ADT); #$P <$ 0.05 compared with vehicle (stimulation at 3xADT); one-way ANOVA followed by Dunnett's multiple comparison test.
§Not tested.

1.5 mg/kg significantly reduced the ADD, to 35.1% (Fig. 3A). LEV, LCM, LTG, and CBZ had no significant effects on ADD at 3xADT.

4. Discussion

The kindling model is a well-established animal model of focal to bilateral tonic-clonic seizures (McNamara, 1984). Here, to identify the mode of seizure inhibition of PER, LEV, LCM, LTG, and CBZ, we evaluated the effects of each AED on seizure score, S_4L, and ADD in the rat amygdala kindling model.

All AEDs tested significantly reduced seizure score with stimulation at the ADT. Some of the rats showed decreases in Racine score to ≤3, corresponding to suppression of seizure severity from focal impaired-awareness seizures to seizure free. The mean minimum Racine seizure scores with LCM or LTG were > 3, whereas those with PER, LEV, or CBZ were ≤3. This difference between these two groups of AEDs was due mainly to differences in the numbers of rats receiving each AED with seizure scores of 0. The doses of each AED were normalized according to data describing elevation of the ADT with various doses of AEDs, as described previously (Wu et al., 2014). In that study, PER, LEV, LTG, and CBZ demonstrated a dose-dependent effect on the ADT, although the dose response of LEV was shallow compared with the other AEDs. The effect of LCM on the ADT was similar to that of PER, LTG, and CBZ. LCM at a dose of 30 mg/kg showed a sedative effect, as

did PER and CBZ (data not shown). The doses utilized in this study were either the maximum-tolerated dose or a sufficiently high dose to elicit maximum pharmacological effect. Therefore, the higher proportions of rats with Racine score 0 in the PER, LEV, and CBZ groups may have been due to differences in the mechanisms of seizure control of these three AEDs compared with those of LCM and LTG. In the present study, the highest dose of LEV (100 mg/kg) did not have a significant effect on Racine score. Similar to our results, Löscher and Hönack (1993) evaluated LEV in the kindling model and observed strong efficacy at a dose of 54 mg/kg; however, efficacy was reduced at 108 mg/kg. Although Doheny et al. (1999) reported a dose-dependent increase in LEV concentration in the cerebrospinal fluid between doses of 40 and 80 mg/kg, LEV was also found to differentially inhibit both excitatory and inhibitory neurotransmission (Meehan et al., 2012). Therefore, discussion of the lack of dose response of LEV in the kindling model should take into consideration the mechanistic aspects of seizure inhibition, as well as pharmacokinetic aspects.

To further characterize the modes of seizure inhibition by the five AEDs, we evaluated additional parameters, namely S_4L and ADD. PER, LEV, and LTG all increased S_4L. Dose-dependent increases in S_4L were observed upon treatment with PER, and for both PER and LEV, the minimum dose that increased S_4L after stimulation at the ADT was lower than that which suppressed the seizure score, suggesting that the anti-seizure effects of PER and LEV are in some part due to their prolongation of S_4L. Since a Racine seizure score of 4 is indicative of generalized seizures (Beheshti Nasr et al., 2013; Cheng et al., 2015), this increase in S_4L suggests that PER and LEV could affect seizure propagation by inhibiting synaptic transmission (Golomb and Amitai, 1997). PER is an AMPA-type glutamate receptor antagonist that inhibits excitatory transmission at the post-synaptic membrane (Hanada et al., 2011), whilst the target of LEV is SV2A, a presynaptic vesicle protein (Lynch et al., 2004). Therefore, it is possible that the prolongation of S_4L by PER and LEV may occur through the modulation of synaptic transmission. In contrast, the common mode of action among LCM, LTG, and CBZ is sodium channel blockade (Brodie, 2017). LCM and CBZ did not significantly increase S_4L, whereas LTG exerted a small but significant prolongation of latency at the same dose as that which reduced seizure severity, suggesting that LTG might have a different mode of seizure inhibition from LCM and CBZ. This finding may also be related to the different clinical anti-seizure spectrum of LTG from those of other sodium channel blockers; for example, LTG has demonstrated efficacy in absence seizures, whereas other sodium channel blockers have not (Nonino, 2008; Brodie, 2017). LTG also exerts effects on other channels, such as the hyperpolarization-activated cyclic-nucleotide-gated and A-type potassium (Hainsworth et al., 2003) channels, and this may explain its prolongation of S_4L.

PER and LEV significantly decreased ADD (Fig. 3). Given that the recording was made at the focus of the seizure, a change in ADD is indicative of an effect on the neuronal circuits at the seizure focus (Jones and Lambert, 1990); the decrease in ADD caused by PER and LEV suggests that these AEDs influenced neuronal hypersynchronism at the focus. The initial dose of both AEDs that resulted in a decrease in ADD was higher than that which initially prolonged S_4L. Stronger manipulation of synaptic transmission is likely required to reduce synchronized activity in the neuronal circuits at the seizure focus. In one simulation study, attenuation of AMPA receptor activity reduced the synchronization of synaptic activity (Traub et al., 1993); our results for S_4L and ADD suggested that PER and LEV exerted their anti-seizure effects by modulating synaptic transmission and neuronal synchronized activity at the seizure focus (Margineanu and Klitgaard, 2000; Rogawski, 2013; Unichenko et al., 2015).

Stimulation at 3xADT was used to further characterize the influence of each AED. This high stimulation intensity was selected to eliminate the possibility of some AEDs completely inhibiting seizure propagation at the focus by increasing the seizure threshold. The anti-seizure effects of LTG and CBZ on kindled seizures are explained by their elevation of the seizure-triggering threshold at the kindling focus (Morimoto et al., 1997; Otsuki et al., 1998). In our previous study, the reductions of seizure score and EEG seizure duration caused by LEV, LTG, CBZ, and valproate (VPA) were diminished with stimulation at 3xADT, whereas the effects of PER were not (Wu et al., 2014), suggesting that this type of stimulation is a useful paradigm for studying the influence of each AED on seizure propagation and on brain areas other than the seizure focus. In fact, at 3xADT, ADD at the focus was not shortened by any AEDs other than PER. PER, LCM, and CBZ all significantly reduced the seizure score at 3xADT, but seizure scores < 3 were not observed with either LCM or CBZ. The efficacy of LCM and CBZ at 3xADT could be attributable to a reduction in neuronal excitability throughout the whole brain; this is supported by the finding that S_4L was not prolonged by any AEDs other than PER. This limited efficacy of the sodium channel blockers at 3xADT suggests that they mainly act by elevating the seizure threshold at the focus, and only exert mild effects on seizure propagation (Morimoto et al., 1997; Otsuki et al., 1998). In contrast, PER at a dose of 1.5 mg/kg reduced the seizure score (2.1 ± 0.48) at 3xADT (Fig. 1A), and this was comparable with its effect with stimulation at the ADT (seizure score 1.8 ± 0.80). However, consistent with the results of our previous study (Wu et al., 2014), LEV did not demonstrate similar effects at different stimulation intensities (Fig. 1B; Table A1 in Supplementary material). Similarly, in a 6-Hz seizure model, PER had comparable anti-seizure effects at 32 mA and 44 mA stimulation intensities (Hanada et al., 2011). Moreover, consistent with a previous kindling and 6-Hz seizure model, the efficacy of LEV was strongly diminished when the stimulus intensity was increased (Barton et al., 2001; Wu et al., 2014).

PER had significant effects on S_4L and ADD at 3xADT stimulation, but LEV failed to affect these parameters (Figs. 2 and 3). The persistent effect of PER on these parameters may be explained by its non-competitive antagonism of AMPA receptors (Hanada et al., 2011; Rogawski and Hanada, 2013). Strong stimulation of an epileptic focus engages an increased number of neuronal synapses, resulting in high glutamate concentrations at the post-synaptic receptors. Non-competitive antagonism could reduce the influence of these elevated glutamate levels, and accordingly, direct inhibition of the AMPA receptor efficiently reduces the synaptic activity of the neuronal network (Traub et al., 1993). In a recent clinical trial, PER reduced bilateral tonic-clonic seizures and focal impaired awareness seizures but did not reduce focal aware seizures (Nishida et al., 2018). These results suggest that PER suppresses seizures to a less severe form. PER has also been shown to terminate experimental status epilepticus and reduce neuronal degeneration more strongly in the seizure relay area (the thalamus) than at the seizure focus (the hippocampus and piriform cortex) (Wu et al., 2017), which may also be explained by non-competitive AMPA receptor antagonism by PER. In contrast to PER, LEV partially reduces the synaptic potential in a frequency-dependent manner (Yang et al., 2007). This partial response of synaptic transmission to LEV may have been the cause of the loss of effect of this drug at 3xADT in the present study.

We demonstrated that each AED had different effects on seizure parameters in a kindling model. PER, LEV, and LTG may affect seizure propagation (S_4L) by delaying synaptic transmission at ADT stimulation. These three AEDs have shown broad-spectrum anti-seizure effects in animal models and clinical studies (Klitgaard et al., 1998; Choi and Morrell, 2003; Crepeau and Treiman, 2010; Hanada et al., 2011; Potschka and Trinka, 2018), and they have demonstrated efficacy in generalized seizures in humans (Mikati and Holmes, 1997; Krauss et al., 2003; French et al., 2015). Given that VPA is also known to be effective in generalized seizures, assessment of the effect of VPA in this experimental paradigm may help to clarify the mechanism of effects on seizure inhibition. Three of the AEDs we examined in the present study acted by increasing the ADT through attenuation of synaptic transmission. Among them, only PER elicited a response regardless of the stimulus intensity, implying that it affected seizure propagation both at the seizure focus and throughout the seizure propagation pathway. In

contrast, LCM and CBZ appeared to exert their anti-seizure effects primarily by elevating the seizure threshold through a reduction in neuronal excitability at the seizure focus. LCM and CBZ have effects on resting, fast, and slow inactivation, with their contribution to each being different (Hebeisen et al., 2015). It is therefore possible that LCM and CBZ may share a similar mode of seizure inhibition, as this may explain their similar effects on seizure parameters observed in this study. PER, LEV, and LTG may have reduced both neuronal excitability and the efficiency of neuronal synchronization at the focus.

Over 20 AEDs are currently used in clinical treatment. Rational drug selection of both monotherapy and adjunctive therapy is required. In general, the mode of action of each AED is the first consideration during appropriate drug selection. However, our results indicate that AEDs with the same theoretical mode of action in fact had different effects in the kindling model of focal impaired awareness and focal to bilateral tonic-clonic seizures. Consideration of the effects of each AED on various seizure parameters, in addition to each drug's molecular target and spectrum of anti-seizure effects, may aid rational drug selection.

5. Conclusion

We confirmed that each AED studied had different effects on seizure generation and propagation. PER, LEV, and LTG affected seizure propagation, whereas LCM and CBZ appeared to exert their anti-seizure effects mainly by reducing the excitability of neurons. Further evaluation of other AEDs is warranted to clarify modes of seizure inhibition, in order to support rational drug selection.

Author disclosures

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.eplepsyres.2019.03.011.

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