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RESEARCH ARTICLE

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Conventional anticonvulsant drugs in the guinea pig kindling model of partial seizures: effects of acute phenobarbital, valproate, and ethosuximide

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Abstract This study addressed the anticonvulsant effects of phenobarbital, valproate, and ethosuximide in the amygdala of kindled guinea pigs to further validate this model for the screening of anticonvulsant drugs. Behavioral toxic effects were assessed at 30 min following drug administration using quantitative locomotor tests, as well as scores on a sedation and muscle relaxation rating index. The anticonvulsant efficacy of the drugs were evaluated from measurements of afterdischarge threshold (ADT), afterdischarge duration (ADD), and behavioral seizure severity (SS) during early and late phases of kindling acquisition, and in kindled guinea pigs. ADD and SS were also measured in response to both threshold and suprathreshold kindling stimulation. All drugs exerted slight to moderate sedative effects in guinea pigs on both the behavioral tests and rating index. We found that phenobarbital exhibited effective anticonvulsant properties in guinea pigs by consistently reducing ADD and SS in response to both threshold and suprathreshold kindling stimulation. Valproate exhibited effective anticonvulsant properties at threshold stimulation and less effective properties at suprathreshold stimulation. Lastly, we found that ethosuximide lacked effective anticonvulsant action at either threshold or suprathreshold kindling stimulation. Our results indicate that the guinea pig kindling model correctly predicted the actions of phenobarbital, valproate, and ethosuximide in the treatment of partial seizures. Guinea pig amygdala kindling appears to serve as a useful and valid model for partial epilepsy.

Keywords Epilepsy · Seizures · Antiepileptic drugs · Afterdischarge threshold · Behavioral toxicity · Guinea pig

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Introduction

Kindling is an in vivo model of complex partial epilepsy and refers to the progressive development of epileptiform activity and associated behavioral convulsions in response to low-intensity electrical stimulation of the brain (Goddard et al. 1969; Racine 1972a, 1972b). Electrical kindling provides an excellent avenue for determining the basic mechanisms underlying the genesis and progression of partial seizures as well as for the screening of potential anticonvulsant drugs (ACDs; Albright and Burnham 1980; Teskey 2001). The kindling phenomenon has previously been described in the guinea pig (Teskey et al. 1995, 1996, 1999). With repeated stimulation, guinea pigs show some initial growth of the electrographic indices and behavioral seizures. However, unlike rats, guinea pigs stop at partial seizures and, even after hundreds of stimulations, do not progress to fully generalized convulsions during regular, single-site kindling. Thus, guinea pig kindling shares a common feature with the majority of people with partial epilepsy: the failure to progress to fully generalized convulsions (Engel 1998). Indeed, partial (focal) seizures are the most common seizure type experienced by patients with chronic epilepsy (Löscher and Schmidt 1994; Engel 1998). While ACDs continue to be the fundamental remedy for reducing seizure frequency and severity, many of those afflicted have seizures that are resistant to treatment with the currently available drugs (Löscher and Schmidt 1994). Consequently, the identification of new ACDs is essential in the pursuit of therapeutic advancement and will undoubtedly continue to depend on valid animal models for screening.

A well-documented feature of kindling that supports its validity as a model of human epilepsy is the general observation that ACDs that suppress partial seizures in people with epilepsy also suppress kindled seizures (Sato et al. 1990). Phenobarbital (PB) is the oldest of the currently prescribed ACDs, and, although the use of PB has been decreasing because it has more undesirable side-effects than most other ACDs, it is still a major drug in

treating epileptic disorders (Bourgeois 1996). While PB has been shown to have at least some level of efficacy against every seizure type except absence seizures, it is mainly used for generalized convulsive and partial seizures. Valproate (VPA) has been established as one of the most important major ACDs. Although VPA has been shown to effectively control absence, myoclonic, and tonic-clonic seizures of the primary generalized epilepsies, it can be effective for refractory focal seizures as well (Dean 1996). Ethosuximide (ESM) is the drug of choice for the control of childhood absence epilepsy and is not effective against partial seizures (Wolff 1996).

We have just recently shown that the guinea pig kindling model correctly predicts the acute anticonvulsant actions of phenytoin (Gilbert et al. 2001) and carbamazepine (Gilbert and Teskey 2001). This study attempted to determine the pharmacological and behavioral profiles of three common ACDs (PB, VPA, and ESM) in guinea pig kindling, a novel model of human partial epilepsy. Following drug administration, potential behavioral toxic effects were analyzed via quantitative behavioral tests and scores on a general sedation index. In the kindling model, afterdischarge threshold (ADT) serves as an index of the drug effect on seizure threshold, whereas duration of AD and the duration and manifestation of clonic motor seizures serve as indices of drug effects on seizure propagation. Accordingly, we measured the effects of PB, VPA, and ESM on AD threshold, AD duration (ADD), and behavioral seizure severity (SS) at 30 min postinjection. Since the intensity of stimulation can potentially influence an ACD's efficacy (Rundfeldt et al. 1990; Voits and Frey 1994; Morimoto et al. 1997), we measured ADD and SS in response to both threshold and suprathreshold kindling stimulation. Finally, since it has been reported that kindling itself can also change an ACD's efficacy (Löscher et al. 1998), we measured seizure propagation at both early and late phases of kindling.

Materials and methods

Subjects

Male and female mixed-strain guinea pigs, weighing 600–1,200 g, housed individually or in same-sex groups, served as subjects. Food (Laboratory Rabbit Diet 531; PMI) and vitamin C-enriched water was available ad libitum. The guinea pigs were maintained on a 12-h light/12-h dark cycle with lights on at 08:00 a.m.. They were maintained and handled according to the guidelines set by the Canadian Council on Animal Care.

Drugs

PB (Abbott Laboratories) was freshly dissolved in a vehicle consisting of propylene glycol, ethanol, and water (40:10:50). VPA and ESM (Sigma Laboratories) were freshly dissolved in saline. All drugs and vehicles were given in a volume of 1 ml/kg and delivered intraperitoneally (i.p.) 30 min prior to behavioral and anticonvulsant testing. This 30-min time interval was chosen based on previous studies that obtained appropriate results with similar drugs in guinea pigs (Gilbert et al. 2001) and rats (Silver et al. 1991).

Behavioral toxicity

Potential behavioral toxic effects of PB, VPA, and ESM were assessed at 30 min postinjection using three behavioral tasks (bracing, righting, and swimming), which yielded quantitative end point measures. The bracing task was assessed using a sloping board (Morrissey et al. 1989), which consisted of a cork board (32.5×21 cm) hinged to plywood of the same dimensions. A piece of Plexiglas, which served as a protractor with degrees of inclination denoted on its surface, was attached vertically to the edge of the plywood. The dependent measure was the slope of the cork board, in degrees, at which the guinea pig made a bracing attempt. The animal was placed onto the cork surface facing the hinged end of the board. The opposite end of the board was raised slowly until the animal made a bracing attempt. A bracing attempt included lowering of the back end and straightening of the hindlimbs, turning laterally with an attempt to move upward, and/or jumping off. In the righting task (Pellis 1996), the guinea pig was placed on its back by the experimenter and quickly released. Under slow motion video replay, a righting score was determined by measuring the latency from release until all four limbs came in contact with the bench surface. The swimming task was assessed using an aquarium (35×42.5×121.5 cm) filled to a depth of approximately 24 cm with 20–21°C water. A visible wire-mesh platform (20×20 cm), which extended approximately 1.5 cm above the surface of the water, was placed at the far end of the aquarium. The animal was released at one end of the aquarium and the latency to reach the visible platform was measured.

The guinea pigs (n=7) were familiarized with all 3 tasks on each of 2 days prior to testing. They were then exposed to the following rotation design:

1. Day 1: exposure to all 3 tasks in succession without a drug; each task was repeated once immediately, except a 2-min rest interval was allowed between water task trials
2. Day 2: same as day 1, except drug or vehicle was administered 30 min prior to testing
3. Days 3 and 4: rest days

This schedule was repeated until all guinea pigs received all drug dosages.

In addition to the quantitative behavioral tests, guinea pigs (n=8) were observed in an open field and scored on a 4-point index of sedation and muscle relaxation (modified from Hönack and Löscher 1989). Sedation and muscle relaxation was classified as follows:

1. Normal forward locomotion, no decrease in neck and abdominal muscle tone: 0
2. Lightly reduced forward locomotion, slight decrease in muscle tone: 1
3. Reduced locomotion with rest periods in between (eyes partly closed), further decrease in muscle tone: 2
4. Reduced locomotion with more frequent rest periods, more pronounced decrease in muscle tone: 3
5. No forward locomotion, animal sits quietly with eyes closed, total loss of muscle tone: 4

All behavioral testing took place at the same time each day (between 08:30 a.m. and 12:00 p.m.).

Surgery

Guinea pigs were anesthetized with an intramuscular injection of ketamine (85% at 58.83 mg/kg) and xylazine (15% at 0.5 ml/kg). The electrodes consisted of twisted, Teflon-insulated stainless steel wire 127 μm in diameter and were implanted bilaterally into the basolateral amygdala nucleus using standard stereotaxic techniques. Using coordinates from Luparello (1967), the amygdala was targeted at 1.5 mm posterior to bregma, 7.0 mm lateral to midline, and 8.0 mm ventral from the brain surface. A surgical screw

Table 1 Effects of phenobarbital, valproate, and ethosuximide on the degree of incline to brace (*Bracing*), latency to right (*Righting*), and the latency to swim to a visible platform (*Swimming*). Behavioral testing commenced 30 min after drug administration. Values represent the mean (± SEM)

Drug	Dose (mg/kg)	Bracing (deg)	Righting (s)	Swimming (s)
PB	0	37.0±1.61	0.54±0.11	2.15±0.22
	25	33.0±1.32	2.27±1.09	2.77±0.62
	40	32.4±1.39	3.26±1.74	3.02±0.52
VPA	0	34.4±0.48	0.51±0.06	2.19±0.24
	200	35.1±0.99	2.06±1.19	2.30±0.16
	300	35.1±0.89	2.34±1.28	2.24±0.27
ESM	0	34.4±0.48	0.51±0.06	2.19±0.24
	150	34.6±1.22	1.87±1.36	2.04±0.25
	300	35.6±0.80	1.87±1.36	

All comparisons from control were nonsignificant (P=0.5–0.15)

connected to a strand of insulated stainless steel wire was secured to the skull and served as the ground or reference electrode. The electrodes were secured and anchored to the skull using three additional surgical screws and dental acrylic. Amphenol pins that were attached to each electrode were inserted into a plastic pedestal that was permanently secured to the animal's head. All animals were given a period of at least 7 days for recovery.

Kindling and anticonvulsant measures

The anticonvulsant efficacy of PB, VPA, and ESM was evaluated from measurements of ADT, ADD, and SS. Afterdischarge thresholds were determined by delivering one set of 50-μA stimulation trains, consisting of balanced, biphasic square-wave pulses, each 1.0 ms in duration, at 60 Hz for a total duration of 2 s. Failure to elicit a discharge resulted in increasing the current in 50-μA steps and restimulating at 60 s intervals, until the threshold was surpassed. The lowest intensity of stimulation that induced 4 s of AD was arbitrarily defined as threshold (ADT). ADD, a measure of electrographic seizure activity, was the total duration of spikes in the EEG, with an amplitude of at least twice that of baseline EEG and a frequency greater than 1/s. SS was graded according to Teskey et al. (1995):

1. Stage 1: chewing, and/or pronounced salivation, and/or facial automatisms
2. Stage 2: head-jerking and/or circling toward side of stimulation
3. Stage 3: unilateral forelimb clonus on ipsilateral side
4. Stage 3.5: unilateral forelimb clonus, alternating between ipsilateral and contralateral sides during a single AD

EEG signals were amplified and filtered at 1 Hz (high pass) and 100 Hz (low pass) with Grass Model 12 EEG amplifiers. Animals were stimulated daily with current intensities 200 μA above threshold, and polygraph records were recorded.

Anticonvulsant assessment in kindled guinea pigs

Once initial threshold was determined, the guinea pigs were stimulated once daily at an intensity 200 μA greater than ADT until 50 ADs were evoked from the amygdala. These guinea pigs (n=8) were operationally defined as "kindled." They were then exposed to a counterbalanced rotation design to investigate the anticonvulsant efficacy levels. This design consisted of the following schedule:

1. Day 1: guinea pigs were given threshold stimulation
2. Day 2: guinea pigs were given, in a counterbalanced fashion, either drug or vehicle, followed 30 min later by ADT determination
3. Days 3–4 were rest days (i.e., no stimulation, drug, or vehicle).

This schedule was repeated until all guinea pigs received all drug dosages with at least a 1-week interval between different drugs. All anticonvulsant testing took place at the same time each day (between 08:30 a.m. and 12:00 p.m.).

Anticonvulsant assessment during kindling acquisition

During the *kindling acquisition* phase, initial threshold was determined in guinea pigs followed by stimulation once a day at an intensity 200 μA greater than ADT (i.e., suprathreshold stimulation). The animals were placed in 8 groups: 0 mg/kg (n=5), 25 mg/kg (n=5), and 40 mg/kg (n=5) PB; 0 mg/kg (n=7) 200 mg/kg (n=7), and 300 mg/kg (n=4) VPA; 150 mg/kg (n=6) and 300 mg/kg (n=6) ESM. Kindling stimulation was applied once daily until 50 ADs were evoked from the amygdala. The drugs were administered i.p. 30 min prior to kindling stimulation once weekly during kindling development.

For purposes of analysis, kindling was divided into two phases: *early* (the first 15 ADs) and *late* (ADs 25–50) kindling. Data were grouped as such because Teskey et al. (1995) have shown that during single-site kindling in guinea pigs there is an early phase of kindling, with increasing ADD, and a later phase, with stable ADD. Although these general trends have emerged, Teskey et al. (1995) also report relatively high day-to-day electrographic variability in guinea pigs, even within an individual guinea pig. In an attempt to compensate for this variability, we computed difference values between ADD on the day of drug administration (drug day) and ADD on the preceding, drug-free day for each animal. This was calculated by subtracting the ADD on the drug-free day from the ADD on the drug day, which would give a net negative value if ADD was reduced. Mean difference scores were computed for each of the groups and subsequently compared statistically.

Histology

Following all experimentation, the animals were deeply anesthetized with pentobarbital sodium and transcardially perfused with saline and formalin. The brains were removed and fixed in 10% formalin with sucrose. Frozen coronal sections 40 μm thick were taken and stained with thionine to verify electrode placements.

Statistical analyses

Paired t-tests were used to compare performance on behavioral tasks and to compare ADT and ADD in previously kindled guinea pigs (i.e., in the rotation designs). Repeated-measures ANOVA was used to compare ADD during kindling acquisition. Statistical analysis of SS was calculated by a Wilcoxon signed-rank test for the rotation design and by a Mann-Whitney test for kindling acquisition. All tests were performed two-sided and P<0.05 was considered significant.

Results

Behavioral toxicity

The effects of PB, VPA, and ESM on the degree of incline to brace, the latency to right, and the latency to swim to a visible platform are shown in Table 1. At 30 min after either 0 mg/kg (vehicle), 25 mg/kg, or 40 mg/kg PB, guinea pigs made bracing attempts on the sloping board at degrees of inclination (range 25.0–58.0°) that were nonsignificantly different from each other. While there was a trend that PB increased righting latencies (range 0.32–10.0 s), such differences were not significant. When tested on the swimming task, guinea pigs in all three groups also displayed nonsignificant differences in their latency (range 1.58–7.47 s) to reach the visible platform. When the guinea pigs were scored for behavioral toxic effects in an open-field, slight to moderate sedation and muscle relaxation was observed with both dosages of PB. The mean scores were 0±0, 1.38±0.18, and 2.13±0.30 (0 mg/kg, 25 mg/kg, and 40 mg/kg PB, respectively). A Wilcoxon test revealed that both 25 mg/kg and 40 mg/kg PB dosages were significantly different (T=0, N=8, P<0.012; T=0, N=8, P<0.012, respectively) from controls.

At 30 min following either 0 mg/kg, 200 mg/kg, or 300 mg/kg VPA, guinea pigs made bracing attempts on the sloping board at degrees of inclination (range 28.0–40.0°) that were also nonsignificantly different from each other. While there was a trend that VPA increased righting latencies (range 0.30–10.0 s), such differences were not significant. When tested on the swimming task, guinea pigs in all three groups also displayed nonsignificant differences in their latency (range 1.44–4.15 s) to reach the visible platform. When the guinea pigs were scored for behavioral toxic effects in an open-field, slight sedation and muscle relaxation was observed with both dosages of VPA. The mean scores were 0±0, 1.13±0.30, and 1.4±0.32 (0 mg/kg, 200 mg/kg, and 300 mg/kg VPA, respectively). A Wilcoxon test revealed that both 200 mg/kg and 300 mg/kg VPA dosages were significantly different (T=0, N=6, P<0.03; T=0, N=6, P<0.03, respectively) from controls.

At 30 min following either 0 mg/kg, 150 mg/kg, or 300 mg/kg ESM, guinea pigs made bracing attempts on the sloping board at degrees of inclination (range 30.0–41.0°) that were also nonsignificantly different from each other. While there was a trend that ESM increased righting latencies (range 0.28–10.0 s), such differences were not significant. When tested on the swimming task, guinea pigs in all three groups also displayed nonsignificant differences in their latency (range 1.41–4.11 s) to reach the visible platform. When the guinea pigs were scored for behavioral toxic effects in an open-field, slight sedation and muscle relaxation was observed with both dosages of ESM. The mean scores were 0±0, 0.63±0.18, and 0.63±0.18 (0 mg/kg, 150 mg/kg, and 300 mg/kg ESM, respectively). A Wilcoxon test revealed that both 150 mg/kg and 300 mg/kg ESM dosages were significantly different (T=0, N=5, P<0.04; T=0, N=5, P<0.04, respectively) from controls.

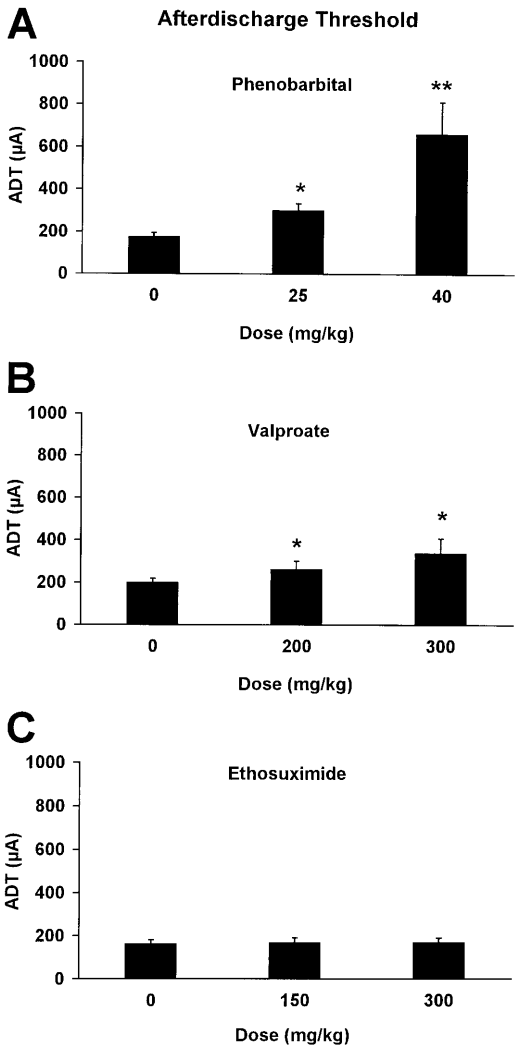

Fig. 1A–C Summary of the effect of a single i.p. administration of phenobarbital (**A**), valproate (**B**), and ethosuximide (ESM) (**C**) at 30 min postinjection on the afterdischarge threshold (*ADT*) in kindled guinea pigs. Note that guinea pigs had significantly higher ADTs following both PB and VPA administration, but not after ESM administration. Histobars represent the mean (± SEM); *P=0.05 and **P=0.01, significantly different from vehicle-injected controls

Afterdischarge threshold

The mean baseline ADT before PB administration commenced was 163±18 μA. Both PB dosages significantly raised ADT in a dose-dependent manner (t_7=–3.03, P<0.02; t_7=–3.30, P<0.01; 25 mg/kg and 40 mg/kg PB, respectively; Fig. 1A). The mean increase was 71% for 25 mg/kg and 279% for 40 mg/kg PB. The mean baseline ADT before VPA administration commenced was 213±23 μA. Both VPA dosages significantly raised ADT in a dose-dependent manner (t_7=–2.38, P<0.05;

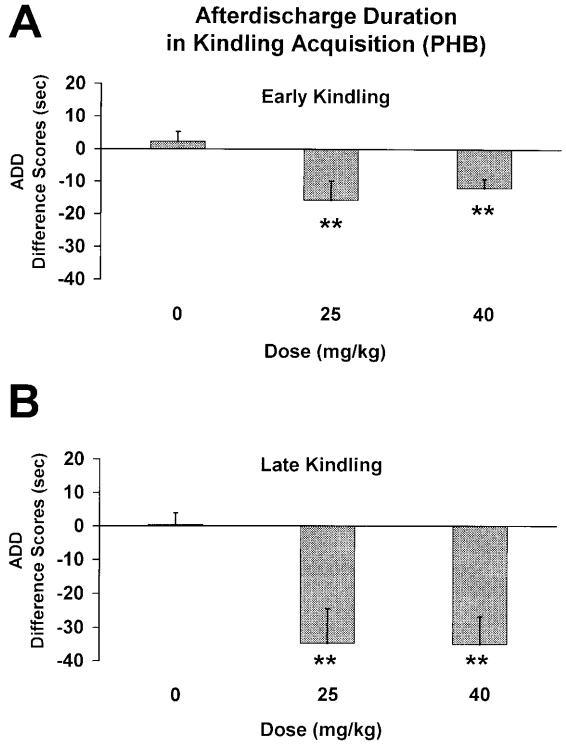

Fig. 2A, B Summary of the effect of a single i.p. administration of 0 mg/kg, 25 mg/kg, and 40 mg/kg phenobarbital (*PB*) at 30 min postinjection on the afterdischarge duration (*ADD*; expressed as a difference score) at suprathreshold stimulation intensities during early kindling (**A**) and late kindling (**B**) in guinea pigs. Note that both dosages of PB effectively reduced ADD during the two kindling phases. Histobars represent the mean (± SEM); $^{**}P$=0.01, significantly different

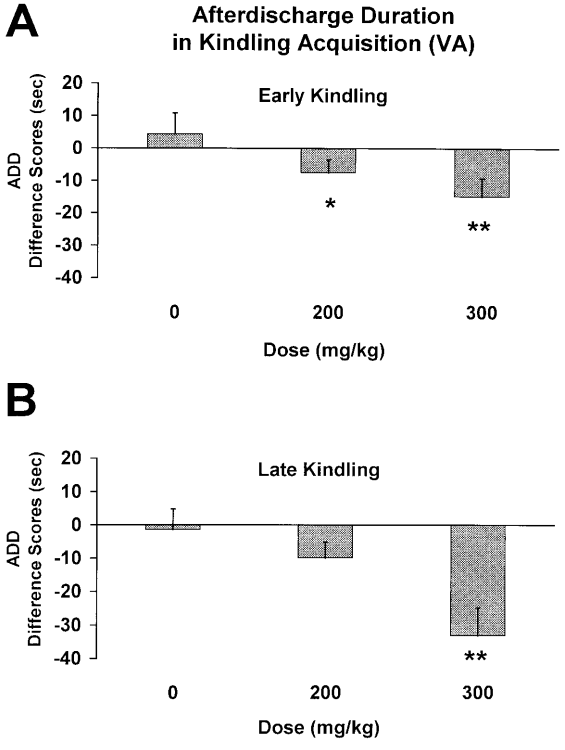

Fig. 3A, B Summary of the effect of a single i.p. administration of 0 mg/kg, 200 mg/kg, and 300 mg/kg valproate (*VPA*) at 30 min postinjection on the afterdischarge duration (ADD; expressed as a difference score) at suprathreshold stimulation intensities during early kindling (**A**) and late kindling (**B**) in guinea pigs. Note that the high dosage of VPA effectively reduced ADD during the two kindling phases. Histobars represent the mean (± SEM); $^{*}P$=0.05 and $^{**}P$=0.01, significantly different from vehicle-injected controls

t_7=–2.31, P<0.05; 200 mg/kg and 300 mg/kg VPA, respectively; Fig. 1B). The mean increase was 31% for 200 mg/kg and 69% for 300 mg/kg VPA. The mean baseline ADT before ESM administration commenced was 163±18 μA. ESM did not have any significant effects on ADT (Fig. 1C).

Afterdischarge duration

In response to suprathreshold stimulation during the early phase of kindling acquisition, 25 mg/kg and 40 mg/kg PB significantly reduced the ADD compared with controls ($F_{1,\ 20}$=30.71, P<0.0001; $F_{1,\ 20}$=29.86, P<0.0001, respectively; Fig. 2A). This general result was also observed during the late phase of kindling acquisition with both 25 mg/kg and 40 mg/kg PB significantly reducing the AD durations compared with controls ($F_{1,\ 20}$=58.52, P<0.0001; $F_{1,\ 20}$=51.23, P<0.0001, respectively; Fig. 2B). The larger dose of PB appeared more effective with kindling, but such differences were nonsignificant. That is, when comparing ADD reduction between same-drug dosages in early versus late kindling (Fig. 2A, B), significant differences were not found (P=0.20 and P=0.09; 25 mg/kg and 40 mg/kg PB, respectively).

In response to suprathreshold stimulation during the early phase of kindling acquisition, 200 mg/kg and 300 mg/kg VPA significantly reduced the ADD compared with controls ($F_{1,\ 20}$=8.62, P<0.008; $F_{1,\ 20}$=16.13, P<0.0007, respectively; Fig. 3A). This general result was also observed during the late phase of kindling acquisition, but with only the high dosage significantly reducing the ADD compared with controls ($F_{1,\ 20}$=14.84, P<0.001; Fig. 3B). Similar to PB, it appeared that the effectiveness of the high dosage of VPA increased in late kindling; however, such differences were nonsignificant (P=0.13).

ESM did not have a significant (P=0.4 and P=0.35) effect on ADD during the early and late stages of kindling and therefore the data are not graphically represented here. The mean ADD difference scores for early kindling were 2.6±5.3, –4.6±5.8, and –6.6±8.2 s, while for late kindling they were 3.9±7.1, –6.2±11.9, and –11.2±10.5 s (0 mg/kg, 150 mg/kg, and 300 mg/kg ESM, respectively).

Kindled guinea pigs that received threshold stimulation and vehicle had an ADD of 45.9±9.70 s. Administration of both 25 mg/kg and 40 mg/kg PB significantly reduced AD durations compared with controls (t_7=3.63, P<0.008; t_7=3.82, P< 0.007, respectively; Fig. 4A). The mean baseline ADD before VPA administration com-

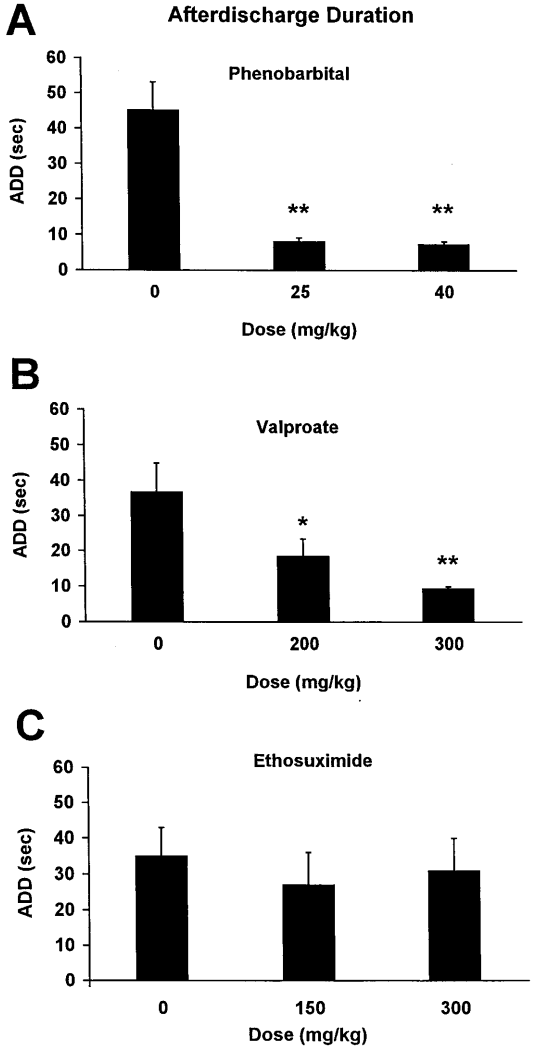

Fig. 4A–C Summary of the effect of a single i.p. administration of PB (**A**), VPA (**B**), and ESM (**C**) at 30 min postinjection on the ADD at threshold stimulation intensities in kindled guinea pigs. Note that guinea pigs had significantly reduced ADDs following both PB and VPA administration, but not after ESM administration. Histobars represent the mean (± SEM); **P*=0.05 and ***P*=0.01, significantly different from vehicle-injected controls

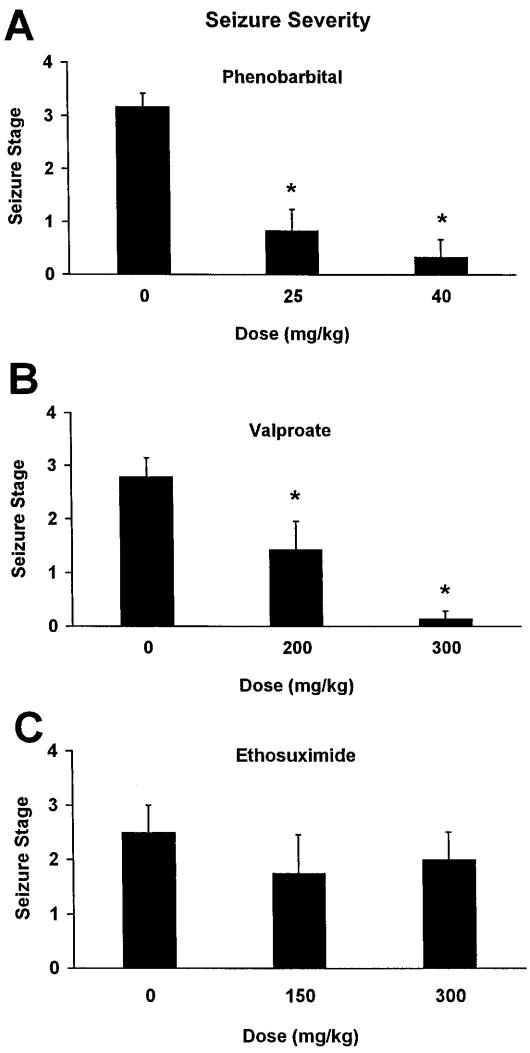

Fig. 5A–C Summary of the effect of a single i.p. administration of PB (**A**), VPA (**B**), and ESM (**C**) at 30 min postinjection on seizure severity at threshold stimulation intensities in kindled guinea pigs. Note that both PB and VPA were more effective than ESM at reducing seizure severity (SS). Histobars represent the mean (± SEM) and **P*=0.05, significantly different from vehicle-injected controls

menced in kindled guinea pigs that received threshold stimulation was 38.0±12.3 s. Administration of both 200 mg/kg and 300 mg/kg VPA significantly reduced AD durations compared with controls (t_7=2.41, P<0.05; t_7=3.38, P< 0.01, respectively; Fig. 4B). The mean baseline ADD before ESM administration commenced in kindled guinea pigs that received threshold stimulation was 38.3±7.4 s. Administration of both 150 mg/kg and 300 mg/kg ESM did not significantly reduce AD durations compared with controls (Fig. 4C).

Seizure severity

In the following analyses, guinea pigs that had not previously displayed behavioral seizures (i.e., at least stage 1) were dropped from the analysis of seizure severity. In response to suprathreshold stimulation and during the early phase of kindling acquisition, both 25 mg/kg and 40 mg/kg PB significantly reduced seizure severity compared with controls (U=19.5, P<0.01; U=13.0, P<0.0007, respectively; data not illustrated). The mean seizure scores were 1.6±0.27, 0.38±0.38, and 0.1±0.1 (0 mg/kg, 25 mg/kg, and 40 mg/kg PB, respectively). Similarly, during the late phase of kindling acquisition, both 25 mg/kg and 40 mg/kg PB significantly reduced seizure severity compared with controls (U=40.5, P<0.05;

U=21.5, P<0.0003, respectively). The mean seizure scores were 2.6±0.36, 1.4±0.34, and 0.3±0.19 (0 mg/kg, 25 mg/kg, and 40 mg/kg PB, respectively).

In response to suprathreshold stimulation and during the early phase of kindling acquisition, only the high dosage (300 mg/kg) of VPA significantly reduced seizure severity compared with controls (U=15.0, P<0.001; data not illustrated). The mean seizure scores were 1.6±0.29, 0.90±0.31, and 0.1±0.1 (0 mg/kg, 200 mg/kg, and 300 mg/kg VPA, respectively). Similarly, during the late phase of kindling acquisition, only the high dosage of VPA significantly reduced seizure severity compared with controls (U=17.0, P<0.0002). The mean seizure scores were 2.2±0.21, 1.8±0.29, and 0.4±0.24 (0 mg/kg, 200 mg/kg, and 300 mg/kg VPA, respectively). Similar to the results with ADD, we found that both 150 mg/kg and 300 mg/kg ESM did not significantly reduce seizure severity during late kindling compared with controls (data not illustrated). The mean seizure scores were 1.9±0.44, 1.5±0.33, and 1.3±0.37 (0 mg/kg, 150 mg/kg, and 300 mg/kg ESM, respectively).

Kindled guinea pigs that received threshold stimulation and vehicle had a mean seizure stage of 3.1±0.42. A Wilcoxon test revealed that both 25 mg/kg and 40 mg/kg PB significantly reduced seizure severity over that of vehicle-injected controls (T=0, N=6, P<0.03; T=0, N=6, P<0.03, respectively; Fig. 5A). The mean baseline seizure stage before VPA administration commenced in kindled guinea pigs that received threshold stimulation was 2.8±0.51. A Wilcoxon test revealed that both 200 mg/kg and 300 mg/kg VPA significantly reduced seizure severity over that of vehicle-injected controls (T=0, N=6, P<0.03; T=0, N=7, P<0.02, respectively; Fig. 5B). The mean baseline seizure stage before ESM administration commenced in kindled guinea pigs that received threshold stimulation was 2.5±0.41. ESM did not significantly reduce seizure severity over that of vehicle-injected controls (Fig. 5C).

Discussion

The present study has demonstrated that, after a single i.p. injection, PB and VPA acted as efficient anticonvulsants by both increasing ADT and reducing seizure propagation. While PB exerted consistent anticonvulsant actions in response to both threshold and suprathreshold kindling stimulation, VPA exhibited effective anticonvulsant properties at threshold stimulation but variable effects at suprathreshold stimulation. Additionally, we found that ESM lacked effective anticonvulsant properties at both threshold and suprathreshold kindling stimulation. The present results generally indicate that the guinea pig kindling model correctly predicted the actions of PB, VPA, and ESM at dosages that yielded slight to moderate behavioral toxic effects.

We used a set of behavioral tests to illuminate potential behavioral toxic effects of PB, VPA, and ESM in the guinea pig. Quantitative tests potentially allow for greater interobserver reliability and are potentially more sensitive to the behavioral impairment of an ACD. However, in the present study we observed that guinea pigs did not display significant behavioral impairment on the quantitative tasks following any of the drug administrations, although all drugs resulted in a trend toward increased righting latencies. However, we did find significant differences on the qualitative scores with all of the drug dosages tested. Nevertheless, our results are in general agreement with previous studies that have reported minimal to moderate PB-induced adverse effects (Löscher and Hönack 1989; Silver et al. 1991; Voits and Frey 1994; Otsuki et al. 1998), VPA-induced adverse effects (Silver et al. 1991; Dziki et al. 1992; Voits and Frey 1994), and ESM-induced adverse effects in rats (Albright and Burnham 1980; Albertson et al. 1980).

There are only two studies that have looked at the effects of PB on ADT. Silver et al. (1991) have reported a nonsignificant effect of 40 mg/kg PB 30 min after administration in fully kindled rats, while Löscher (1998) have reported a 300% increase in ADT following 30 mg/kg. In the present study, we found that PB dose-dependently increased ADT. Previous studies investigating the anticonvulsant efficacy of VPA using the rat kindling model have reported consistent ADT elevating effects (Dziki et al. 1992; Mori and Ohta 1992; Löscher and Hönack 1993). Our results also confirm the ability of VPA to increase threshold and extends the observation to the guinea pig kindling model. To our knowledge, there are no documented reports that have looked at the effects of ESM on ADT in the rat kindling model. Because ESM is generally not clinically effective against partial seizures, we would predict that it would have little or no effect in the kindling model and thus serve as a control in determining the predictive validity of kindling. In the present study we have demonstrated that ESM does not affect ADT in the guinea pig.

PB has been shown to exert consistent effects in reducing seizure propagation in the rat kindling model. Many studies have reported decreases in afterdischarge duration (Mace and Burnham 1987; Löscher and Hönack 1989; Hönack et al. 1991; Silver et al. 1991; Voits and Frey 1994) and reductions in both seizure severity and seizure duration (Callahan and Schwark 1980; Löscher and Hönack 1989; Hönack et al. 1991; Silver et al. 1991; Voits and Frey 1994). It is important to note that these studies used suprathreshold stimulation. However, since the intensity of stimulation can potentially influence an ACD's efficacy (Voits and Frey 1994; Morimoto et al. 1997), we measured ADD and SS in response to both threshold and suprathreshold kindling stimulation. We found that stimulation intensity did not affect PB's ability to reduce ADD or SS in the guinea pig. Both 25 mg/kg and 40 mg/kg PB were able to reduce seizure propagation during kindling acquisition in which guinea pigs were given suprathreshold stimulation and after more than 50 kindling stimulations (in the rotation design) in which guinea pigs were given threshold kindling stimulation. Moreover, since it has been reported that kindling itself

can also change an ACD's efficacy (Löscher et al. 1998), we measured seizure propagation at early and late phases of kindling acquisition. Although there was a trend toward enhanced anticonvulsant effectiveness with PB as kindling progressed, this was not significant.

In the rat kindling model, VPA has been reported to consistently reduce both ADD (Löscher et al. 1989; Pallini et al. 1989; Hönack et al. 1991; Löscher and Hönack 1993; Voits and Frey 1994; Otsuki et al. 1998) and SS (Löscher et al. 1989; Pallini et al. 1989; Hönack et al. 1991; Mori and Ohta 1992; Löscher and Hönack 1993; Voits and Frey 1994; Otsuki et al. 1998). A general trend that emerged from these studies was that when animals were stimulated at threshold intensities a lower dose of the drug was effective at reducing ADD and SS. At intensities well above threshold, more drug was required to find significant reductions in the seizure propagation measures. In the present study VPA tended to act in a similar fashion, whereby it exerted consistent anticonvulsant action at threshold stimulation and less effective action at suprathreshold stimulation. Only the higher dose of VPA (300 mg/kg) was able to reduce ADD and SS in kindled guinea pigs at suprathreshold intensities. Similar to PB, VPA did not significantly enhance its anticonvulsant effectiveness as kindling progressed, although a trend did emerge. ESM has been reported to result in some suppression of generalized convulsions in the rat, but only at behaviorally toxic doses (Albertson et al. 1980; Albright and Burnham 1980). In our study ESM did not reduce ADD in guinea pigs at either threshold or suprathreshold stimulation intensities. ESM did not reduce SS at threshold or suprathreshold stimulation intensities.

The present study has shown that the guinea pig model correctly predicted PB's effective anticonvulsant action, VPA's somewhat variable anticonvulsant effects, and ESM's deficiency of anticonvulsant action in the clinical treatment of partial seizures. These results extend more recent reports from our lab, whereby the guinea pig model correctly predicted both phenytoin's (Gilbert et al. 2001) and carbamazepine's (Gilbert and Teskey 2001) anticonvulsant effects. Together these data expand the significance of guinea pig kindling by demonstrating that this model can act as a useful screening tool for future ACDs. Testing of new ACDs requires animal models that can accurately predict both anticonvulsant efficacy against specific seizure types and adverse or toxic effects at anticonvulsant doses. Valid models of partial seizures, the most frequent type of seizure in humans, should be used to test the anticonvulsant efficacy against partial epileptic activity. Guinea pig amygdala kindling appears to serve as a valuable and valid model for partial epilepsy, because guinea pigs display repeated partial seizures over weeks and months without full generalization, similar anticonvulsant action to conventional anticonvulsant medications, and all the other positive attributes of the kindling model (Teskey 2001).

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