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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 phenytoin

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Abstract This study addressed some of the controversial issues surrounding the anticonvulsant effect of phenytoin, and the predictive validity of the guinea-pig kindling model for the screening of anticonvulsant drugs. Following an intraperitoneal injection of either 50 or 75 mg/kg phenytoin, we analysed plasma concentrations of phenytoin at various time intervals. Behavioural toxicity was assessed at 0.5 h postinjection using quantitative locomotor tests, as well as scores on a sedation/muscle relaxation rating index. The anticonvulsant efficacy of phenytoin was evaluated from measurements of afterdischarge threshold (ADT), afterdischarge duration (ADD) and behavioural seizure severity at three phases of kindling: non-kindled, kindling acquisition (early and late) and kindled (50+ ADs). ADD and seizure severity were also measured in response to both threshold and suprathreshold kindling stimulation. Plasma levels of phenytoin corresponded to the human therapeutic range at the time of behavioural testing and kindling. Phenytoin did not exert significant adverse effects in guinea-pigs on both the behavioural tests and rating index. Phenytoin increased ADT in non-kindled and kindled guinea-pigs and effectively reduced ADD and seizure severity, indicating that the guinea-pig model correctly predicted phenytoin's anticonvulsant effect. Phenytoin produced reliable anticonvulsant activity in the guinea-pig at threshold stimulation but a somewhat reduced efficacy on seizure severity at suprathreshold stimulation intensities. Kindling in the guinea-pig is a valid model of human partial seizures.

Keywords Epilepsy · Antiepileptic drugs · Afterdischarge threshold · Behavioural toxicity

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Introduction

Partial (focal) seizures are the most common type of seizures in adults (Engel 1998). While anticonvulsant drugs (ACDs) remain the primary treatment for reducing seizure frequency and severity, at least 30% of those afflicted have seizures that are resistant to treatment with the currently available drugs (Löscher and Schmidt 1994). Thus, the identification of new ACDs remains the most obvious and important avenue for therapeutic advancement. Since Merritt and Putnam discovered phenytoin in 1938, the identification of ACDs has and will likely continue to depend on animal models for screening. Electrical kindling provides an excellent in vivo model for determining the basic mechanisms underlying the genesis and progression of partial seizures as well as for the screening of potential ACDs (Teskey 2001). The kindled limbic focus appears to provide the only validated animal model of partial seizures (Albright and Burnham 1980).

Our laboratory has examined the electrical kindling phenomenon in the guinea-pig (Teskey et al. 1995, 1996, 1999). With repeated stimulation guinea-pigs show some initial growth of the electrographic indices and behavioural seizures. Unlike rats, guinea-pigs become arrested at partial seizures and even after hundreds of stimulations do not progress to fully generalised convulsions during regular single-site kindling, allowing researchers to evaluate the partial seizures for extended periods of time. Thus, guinea-pig kindling shares a common feature with the majority of human partial epileptics, that is the failure to progress to fully generalised convulsions (Engel 1998). Furthermore, guinea-pig seizures mimic human seizure behaviour and have a similar EEG pattern for both ictal and interictal events. The next logical step is the evaluation of the pharmacological profile of the guinea-pig kindling model. The effect of phenytoin in guinea-pig kindling serves as an excellent test case for this model of partial seizures.

Phenytoin is one of the most extensively studied ACDs and is still one of the first drugs of choice today

for the treatment of partial epilepsy (Graves and Ramsay 1996; Tunnicliff 1996). Phenytoin has been reported to exert variable anticonvulsant effects in the kindling model and there has been the suggestion that phenytoin may be the exception to the general observation that ACDs that suppress clinical symptoms also suppress kindled seizures (McNamara et al. 1989; Lothman et al. 1991). It has been reported that phenytoin can have no anticonvulsant effects (Rundfeldt et al. 1990; Rundfeldt and Löscher 1993; Morimoto et al. 1997), proconvulsant effects (Callaghan and Schwark 1980; Schmutz et al. 1988; Ebert et al. 1997), anticonvulsant effects at toxic doses (Albright and Burnham 1980; Ehle 1980; Mace and Burnham 1987; Morimoto et al. 1997; Otsuki et al. 1998), as well as anticonvulsant effects at non-toxic doses (Albright and Burnham 1980; McNamara et al. 1989; Löscher et al. 1998b). Factors such as route of administration (McNamara et al. 1989), gender (Ebert et al. 1994), genetic differences (Löscher and Rundfelt 1991; Ebert and Löscher 1999), stimulus intensity (Rundfeldt et al. 1990; Morimoto et al. 1997) and amount of kindling (Löscher et al. 1998a) have been touted as being responsible for the lack of concordance in the results.

This study extended the results of preliminary work (Gilbert et al. 1998) in an attempt to resolve some of the controversy surrounding the anticonvulsant effect of phenytoin, as well as determine the potential usefulness of the guinea-pig kindling model for the screening of ACDs. We took serial measurements of serum phenytoin after intraperitoneal (i.p.) administration of 50 and 75 mg/kg phenytoin to determine both the level and rate of elimination of the drug. Following phenytoin administration, potential behavioural toxic effects were examined using bracing, righting, and swimming tests and scores on a general sedation index. The effects of ACDs can be categorised into those that elevate threshold and those that inhibit propagation. In the kindling model, afterdischarge threshold (ADT) serves as an index of the drug effect on seizure threshold, whereas duration of afterdischarge (AD) and the duration and manifestation of clonic motor seizures serve as indices of drug effects on seizure propagation. Thus, we measured the effect of phenytoin on ADT, AD duration (ADD) and seizure severity at 0.5 h postinjection. Since the intensity of stimulation appears to influence phenytoin's anticonvulsant effect (Rundfeldt et al. 1990; Morimoto et al. 1997), we measured ADD and seizure severity in response to both threshold and suprathreshold kindling stimulation. Since it has been reported that kindling can change the anticonvulsant properties of phenytoin (Löscher et al. 1998a), we measured seizure threshold and propagation at three phases of guinea-pig kindling including non-kindled, kindling acquisition (early and late) and kindled (50+ ADs).

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 8.00 a.m. All testing was done during the light phase. They were maintained and handled according to the guidelines set by the Canadian Council on Animal Care.

Drug

Phenytoin (Sigma Laboratories) was freshly dissolved in a vehicle consisting of propylene glycol, ethanol and water (40:10:50) before administration. The drug and vehicle were given in a volume of 1 ml/kg and delivered i.p.

Phenytoin plasma concentrations

Blood samples were repeated collected from experimentally naive guinea-pigs (n=5 or 6, per time and per group) via cardiac puncture, in the absence of anaesthesia, at 0.25, 0.5, 1, 2, 24, 48 and 72 h after i.p. injection of either 50 or 75 mg/kg phenytoin, between 9.00 a.m. and 12:00 noon. Plasma was separated from whole blood via centrifugation, stored at 4°C for 1–24 h and then analysed by Calgary Laboratory Services. The AxSYM phenytoin assay (Abbott Laboratories), based on fluorescence polarisation immunoassay technology (Jolley 1981), was utilised for phenytoin detection. This assay utilises a four parameter logistic curve fit method (4PLC, Y weighted) to generate a calibration curve. This curve is stored in memory and concentrations of drug in controls and unknown samples are calculated from this curve using polarisation values generated. The AxSYM phenytoin assay is reported to have variability of less than 5% and a sensitivity calculation of 0.50 µg/ml, defined as the lowest measurable concentration that can be distinguished from zero with 95% confidence (Jolley 1981).

Behavioural toxicity

Potential behavioural toxic effects of phenytoin were assessed at 0.5 h postinjection using three behavioural 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), that 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 familiarised with all three tasks on each of 2 days prior to testing. They were then exposed to the fol-

lowing rotation design: day 1, exposure to all three tasks in succession without drug and each task was repeated once immediately, except a 2-min rest interval was allowed between water task trials; day 2, same as above, except 0, 50 or 75 mg/kg phenytoin was administered 0.5 h prior to testing; days 3 and 4 were rest days. This schedule was repeated until all guinea-pigs received all drug doses.

In addition to the quantitative behavioural tests, guinea-pigs (n=8) were observed in an open-field and scored on a five-point index of sedation and muscle relaxation (modified from Hönack and Löscher 1989). Sedation/muscle relaxation was classified as follows: 0 – normal forward locomotion, no decrease in neck and abdominal muscle tone; 1 – slightly reduced forward locomotion, slight decrease of muscle tone; 2 – reduced locomotion with rest periods in between (eyes partly closed), further decrease of muscle tone; 3 – reduced locomotion with more frequent rest periods, more pronounced decrease in muscle tone; 4 – no forward locomotion, animal sits quietly with eyes closed, total loss of muscle tone. Muscle tone was evaluated by palpation and observation. The guinea pigs were lifted out of cage and tilted slightly backwards to observe the exertion made to keep head up. The amount of leg movement and torso twisting was also observed.

Surgery

Guinea-pigs were anaesthetised 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 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 connected to a strand of insulated stainless steel wire was secured to the skull and served as the ground/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 phenytoin was evaluated from measurements of ADT, ADD and behavioural seizure stage. ADTs 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 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. Seizure severity was graded according to Teskey et al. (1995): stage 1, chewing, and/or pronounced salivation, and/or facial automatisms; stage 2, head-jerking and/or circling towards side of stimulation; stage 3, unilateral forelimb clonus on ipsilateral side; 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 naive and kindled guinea-pigs

The anticonvulsant effects of phenytoin on ADT and ADD were first determined in a group of non-kindled (i.e. naive) guinea-pigs. Following recovery from surgery, we determined ADT 0.5 h following i.p. injection of 0 (n=8), 50 (n=8) or 75 (n=8) mg/kg phenytoin. ADT was also redetermined 1 week later, but each animal received a different drug dose in a counterbalanced fashion.

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=9) were operationally defined as "kindled." They were then exposed to a rotation design to investigate the anticonvulsant efficacy levels. This design consisted of the following schedule: day 1, guinea-pigs were given threshold stimulation or suprathreshold (i.e. 200 µA greater than ADT) stimulation; day 2, guinea-pigs were given either drug or vehicle, followed 0.5 h later by ADT determination or suprathreshold stimulation; days 3 and 4 were rest days (i.e. no stimulation, drug or vehicle). This schedule was repeated until all guinea-pigs received all drug doses.

Anticonvulsant assessment during kindling acquisition

During the *kindling acquisition* phase, initial thresholds were determined in a separate group of guinea-pigs followed by stimulation once a day at an intensity 200 µA greater than ADT. Guinea-pigs were divided into three groups: 0 mg/kg phenytoin (n=6), 50 mg/kg phenytoin (n=6) and 75 mg/kg phenytoin (n=5). Kindling stimulation was applied once daily until 50 ADs were evoked from the amygdala. Phenytoin was administered i.p. 0.5 h 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). Data were grouped as such because Teskey et al. (1995) had 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 emerged, Teskey et al. (1995) also reported 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 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 three groups (0, 50 and 75 mg/kg phenytoin) and subsequently compared statistically.

Histology

Following all experimentation, the animals were deeply anaesthetised with sodium pentobarbital 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 thionin to verify electrode placements.

Statistical analyses

Paired t-tests were used to compare performance on behavioural 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 seizure severity 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.

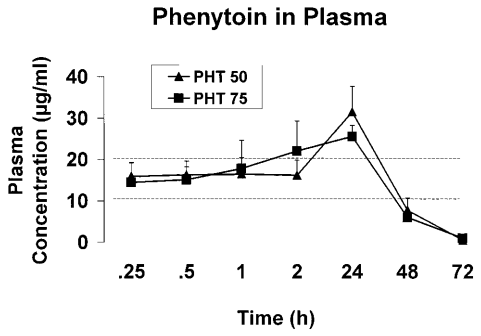

Fig. 1 Effect of a single intraperitoneal (i.p.) administration of 50 mg/kg (*triangles*) and 75 mg/kg (*squares*) phenytoin, in guinea-pigs, on mean (± SEM) plasma concentrations at 0.25, 0.5, 1, 2, 24, 48 and 72 h postinjection. Plasma levels corresponding to the human therapeutic range (10–20 µg/ml) are represented by the *horizontal dashed lines*. Note that at the time of behavioural testing and anticonvulsant assessment (0.5 h postinjection) phenytoin plasma levels were within the human therapeutic range

Results

Plasma concentrations

Plasma levels of phenytoin corresponded to the human therapeutic range of 10–20 µg/ml (Graves and Ramsay 1996) at the time of behavioural testing and kindling (Fig. 1). Plasma levels corresponding to 75 mg/kg phenytoin were within the human therapeutic range at 0.25, 0.5 and 1 h postinjection and just above the range at 2 and 24 h. Plasma levels corresponding to 50 mg/kg phenytoin were within the human therapeutic range at 0.25, 0.5, 1 and 2 h postinjection and above the range at 24 h. Phenytoin plasma levels corresponding to both doses were below the human therapeutic range at 48 h and returned to zero levels, within measurement error, by 72 h postinjection. Plasma levels of phenytoin were maximum at 24 h following administration. At all time points, injections of either 50 or 75 mg/kg phenytoin yielded plasma concentration levels that were not significantly different from each other. It is possible that the two dosages of the drug resulted in different brain levels of phenytoin and this accounts for some of the results below.

Behavioural toxicity

At 0.5 h following either 0 (vehicle), 50 or 75 mg/kg phenytoin, guinea-pigs made bracing attempts on the sloping board at degrees of inclination (range: 35.2–37.0°) that were not significantly different from each other (Fig. 2a). Similarly, phenytoin did not significantly affect latency (range: 0.52–0.90 s) to righting (Fig. 2b). When tested on the swimming task, guinea-pigs in all three groups also displayed non-significant differences in their latency (range: 2.15–2.22 s) to reach the visible platform (Fig. 2c). When the guinea-pigs were scored for behavioural toxic effects in an open-field, little sedation and

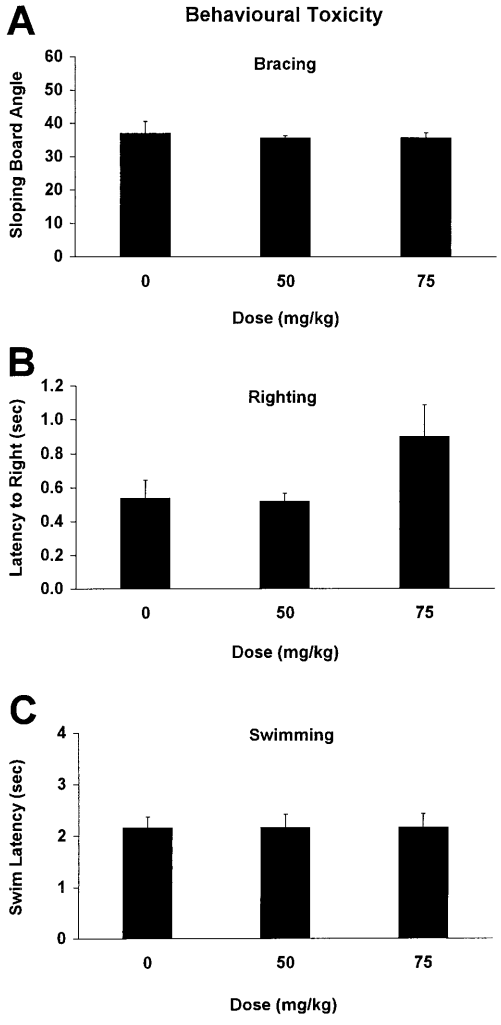

Fig. 2 Summary of the effect of a single i.p. administration of 0, 50 and 75 mg/kg phenytoin, at 0.5 h postinjection, on the degree of incline to brace (**a**), latency to right (**b**) and the time to swim to a visible platform (**c**). Phenytoin at both doses did not alter the performance of the guinea-pigs on any of the tasks as compared to controls. *Histobars* represent the mean (± SEM)

muscle relaxation was observed with either dose of phenytoin. The mean score for sedation/muscle relaxation was 0.125±0.125, 0.38±0.18 and 0.38±0.18 (0, 50 and 75 mg/kg phenytoin, respectively), and these differences were non-significant.

Afterdischarge threshold

In our non-kindled "naive" guinea-pigs, 75 mg/kg phenytoin significantly [$t(7)=-3.43$, $P<0.01$] raised ADT threefold compared to controls. However, 50 mg/kg phenytoin did not significantly increase ADT in the non-kindled animals (Fig. 3a). In our kindled guinea-pigs, the mean baseline ADT (i.e. before drug or vehicle testing commenced) was 225±21.98 µA.

Both 50 and 75 mg/kg phenytoin significantly [$t(8)=-2.68$, $P<0.03$ and $t(8)=-3.40$, $P<0.009$, respec-

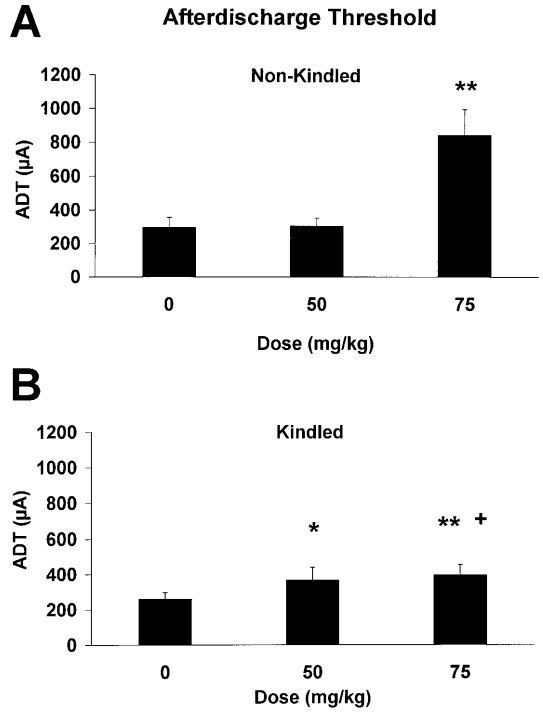

Fig. 3 Summary of the effect of a single i.p. administration of 0, 50 and 75 mg/kg phenytoin, at 0.5 h postinjection, on the afterdischarge threshold (ADT) in non-kindled guinea-pigs (**a**) and kindled guinea-pigs (**b**). Non-kindled guinea-pigs had significantly higher ADTs following 75 mg/kg phenytoin, while kindled guinea-pigs had significantly higher ADTs following both 50 and 75 mg/kg phenytoin. *Histobars* represent the mean (± SEM), * indicates significantly different at the 0.05 level from vehicle-injected controls, + indicates significantly different at the 0.01 level between ADTs in non-kindled and kindled guinea-pigs at 75 mg/kg phenytoin, ** indicates significantly different at the 0.01 level from vehicle-injected controls

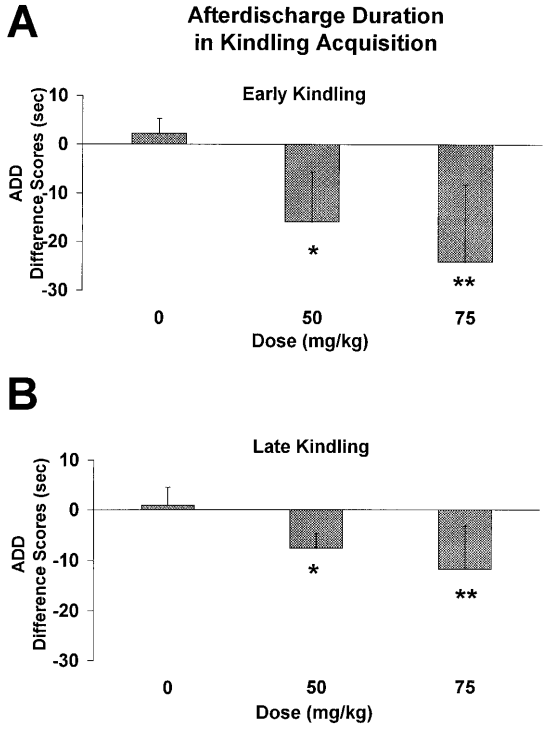

Fig. 4 Summary of the effect of a single i.p. administration of 0, 50 and 75 mg/kg phenytoin, at 0.5 h postinjection, on the afterdischarge duration (ADD; expressed as a difference score) at suprathreshold stimulation intensities during early kindling (first 15 ADs) in guinea-pigs (**a**) and during late kindling (ADs 25–50) in guinea-pigs (**b**). Note the significantly shorter ADDs with both 50 and 75 mg/kg phenytoin. *Histobars* represent the mean (± SEM), * indicates significantly different at the 0.05 level, ** indicates significantly different at the 0.01 level from vehicle-injected controls

tively] increased ADT over that of controls, but the amount of increase was only about 1.5-fold for both drug doses (Fig. 3b). Interestingly, the effectiveness of 75 mg/kg phenytoin in raising the ADT was significantly [$t(15)=2.66$, $P<0.01$] attenuated following kindling. We should note, that while we have observed a reduction in ADT with repeated kindling stimulation in non-drugged guinea-pigs (Gilbert and Teskey 2001), which is similar to observations in the rat (Racine 1972), we did not observe a reduction in ADT in this study.

Afterdischarge duration

Phenytoin did not significantly affect ADD on the first kindling trial in naive guinea-pigs (data not shown). The mean ADDs under phenytoin were 25.8±8.34, 23.87± 9.93 and 18.38±7.77 s (0, 50 and 75 mg/kg phenytoin, respectively). During the early phase of kindling acquisition 50 and 75 mg/kg phenytoin significantly [$F(1,23)=5.27$, $P<0.03$ and $F(1,23)=20.39$, $P<0.0002$, respectively] reduced the ADD compared to controls (Fig. 4a). This general result was also observed during the late phase of kindling acquisition with both 50 and 75 mg/kg phenytoin significantly [$F(1,23)=4.57$, $P<0.04$ and $F(1,23)=8.87$, $P<0.007$, respectively] reducing the ADDs compared to controls (Fig. 4b). While there was a trend that phenytoin's effectiveness declines in late kindling, such differences were not significant. That is, when comparing ADD reduction between same drug dosages (Fig. 4a, b) in early versus late kindling, only non-significant differences were found ($P=0.65$ for 50 mg/kg phenytoin; $P=0.45$ for 75 mg/kg phenytoin).

Kindled guinea-pigs that received threshold stimulation had a mean predrug baseline ADD of 31.71±12.19 s. Administration of both 50 and 75 mg/kg phenytoin significantly [$t(8)=2.32$, $P<0.05$ and $t(8)=2.31$, $P<0.05$, respectively] reduced ADDs compared to controls (Fig. 5a). Kindled guinea-pigs that received suprathreshold stimulation had a mean predrug baseline ADD of 37.2±9.14 s, indicating that suprathreshold stimulation did not result in significantly longer ADDs in kindled guinea-pigs as compared to threshold stimulation. Administration of both 50 and 75 mg/kg phenytoin to guinea-pigs receiving suprathreshold stimulation significantly [$t(8)=3.47$, $P<0.04$ and $t(8)=4.40$, $P<0.002$, respec-

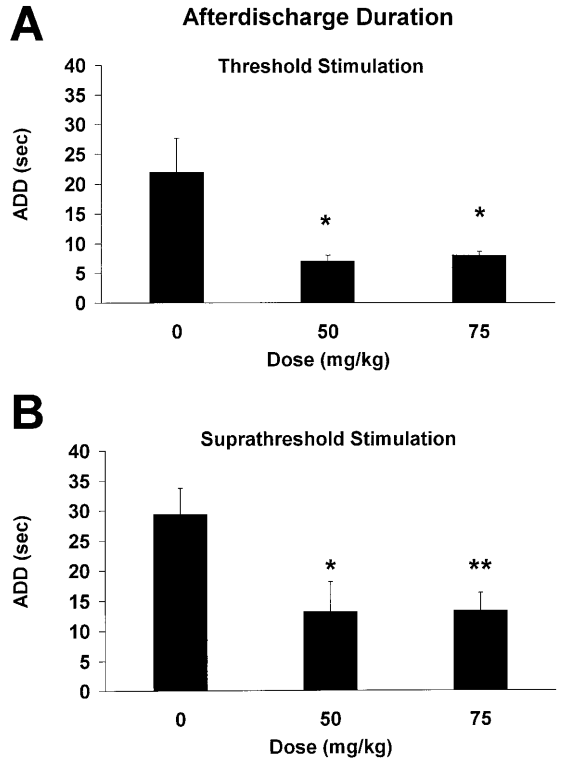

Fig. 5 Summary of the effect of a single i.p. administration of 0, 50 and 75 mg/kg phenytoin, at 0.5 h postinjection, on the ADD at threshold stimulation intensities (**a**) and suprathreshold stimulation intensities (**b**) in kindled guinea-pigs. Kindled guinea-pigs receiving threshold and suprathreshold stimulation had significantly shorter ADDs following both 50 and 75 mg/kg phenytoin. *Histobars* represent the mean (± SEM), * indicates significantly different at the 0.05 level, ** indicates significantly different at the 0.01 level from vehicle-injected controls

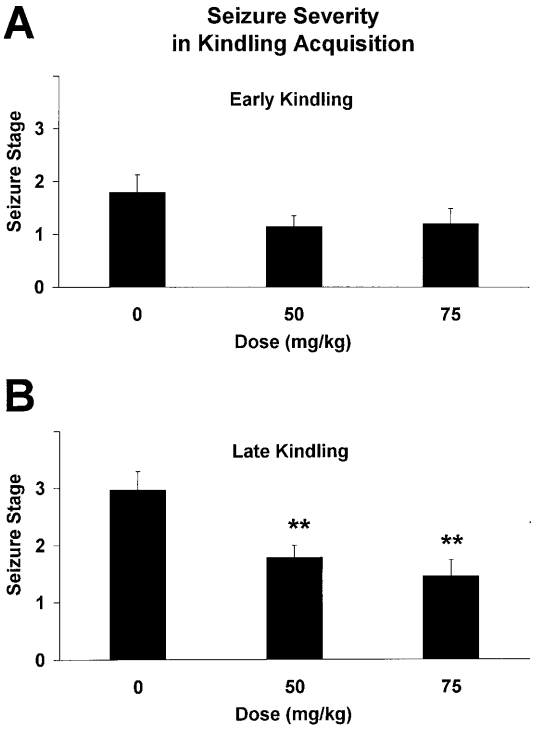

Fig. 6 Summary of the effect of a single i.p. administration of 0, 50 and 75 mg/kg phenytoin, at 0.5 h postinjection, on seizure severity at suprathreshold stimulation intensities during early kindling (first 15 ADs) in guinea-pigs (**a**) and during late kindling (ADs 25–50) in guinea-pigs (**b**). Note the significantly reduced seizure severity scores with both 50 and 75 mg/kg phenytoin in the late-kindled animals. *Histobars* represent the mean (± SEM), ** indicates significantly different at the 0.01 level from vehicle-injected controls

tively] reduced ADDs over that of controls and in a manner comparable to threshold stimulation levels (Fig. 5b).

Seizure severity

During the early phase of kindling acquisition 50 or 75 mg/kg phenytoin did not significantly reduce seizure severity (Fig. 6a). However, during the late phase of kindling acquisition both 50 and 75 mg/kg phenytoin significantly (U=63.5, P<0.004 and U=29.5, P<0.008, respectively) reduced seizure severity (Fig. 6b).

Those guinea-pigs in the rotation designs that did not display behavioural seizures (i.e. only stage 0) even following 50+ ADs were dropped from the analysis of seizure stage because the anticonvulsant efficacy of phenytoin could not be assessed. Kindled guinea-pigs that received threshold stimulation had a mean predrug baseline seizure stage of 2.83±0.28. A Wilcoxon test revealed that both 50 and 75 mg/kg phenytoin significantly reduced seizure severity (Fig. 7a) over that of controls (T=0, N=5, P<0.04 and T=0, N=6, P<0.03, respectively). Kindled guinea-pigs that received suprathreshold stimulation had a mean predrug baseline seizure stage of 2.17±0.31. As Fig. 7b shows, 50 mg/kg phenytoin did not significantly reduce seizure severity over that of controls but 75 mg/kg phenytoin did (T=0, N=5, P<0.04), indicating that phenytoin was slightly less effective at suprathreshold stimulation.

Discussion

The present results have shown that following a single i.p. injection, phenytoin was absorbed from the peritoneum and yielded plasma concentration levels comparable to those observed in humans (Graves and Ramsay 1996), at the time of experimental testing. When plasma levels were within the human therapeutic range phenytoin did not have any significant adverse behavioural effects in guinea-pigs. Additionally, the results demonstrated that phenytoin acted as an effective anticonvulsant by both increasing ADT and reducing seizure propagation. Phenytoin was generally effective at reducing ADD and seizure severity with both threshold and suprathreshold kindling stimulation, although a loss of effectiveness at the low dose of phenytoin was observed with suprathreshold stimulation intensity on seizure severity. Col-

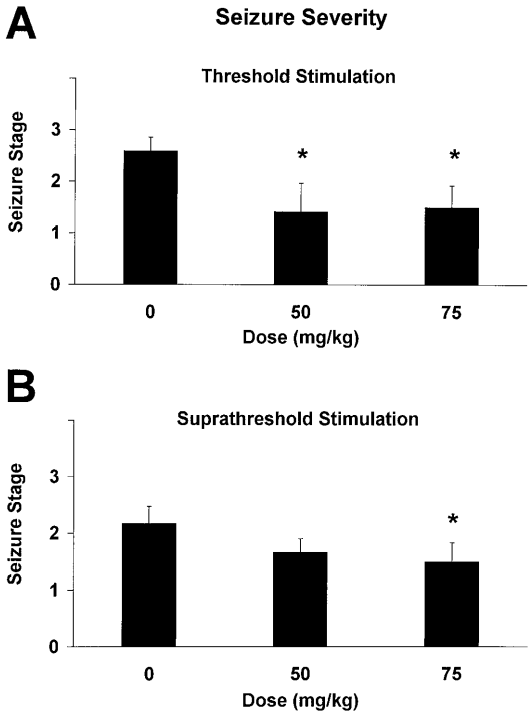

Fig. 7 Summary of the effect of a single i.p. administration of 0, 50 and 75 mg/kg phenytoin, at 0.5 h postinjection, on seizure severity at threshold stimulation intensities (**a**) and suprathreshold stimulation intensities (**b**) in kindled guinea-pigs. Note that both 50 and 75 mg/kg phenytoin significantly reduced seizure severity when kindled guinea-pigs were stimulated at threshold intensities, but only 75 mg/kg phenytoin significantly reduced seizure severity when kindled guinea-pigs were stimulated at suprathreshold intensities. *Histobars* represent the mean (± SEM), * indicates significantly different at the 0.05 level from vehicle-injected controls

lectively, the data presented here indicate that the guinea-pig kindling model correctly predicted the anticonvulsant effects of phenytoin at dosages that were comparable to the human therapeutic range and did not result in behavioural toxicity.

Studies that have examined the anticonvulsant efficacy of phenytoin using the rat kindling model have yielded variable results. It has been suggested that some of this variation may be accounted for by the fact that because phenytoin is only water soluble at a pH of more than 10, it may be poorly absorbed following an i.p. injection (McNamara et al. 1989). However, in this study we observed that 50 mg/kg phenytoin was absorbed into the blood and yielded plasma levels equivalent to the human therapeutic range between 0.25 and 2 h following administration. These results are in general agreement with Lothman et al. (1991) and Löscher et al. (1998a) who demonstrated that i.p. injections in the rat can result in phenytoin plasma levels within the human therapeutic range. However, Lothman et al. (1991) and Löscher et al. (1998a) reported that the plasma levels peaked 1.5 and 0.5 h, respectively, postinjection whereas our data show that plasma phenytoin peaked 24 h postinjection. This discrepancy may be explained by the vehicle in which the phenytoin was dissolved and a species difference. Lothman et al. (1991) administered 50 mg/kg phenytoin in distilled water and polyethylene glycol (70:30) to male Sprague-Dawley rats and found the peak concentration (30 µg/ml) to occur at approximately 1.5 h postinjection and a concentration of approximately 5 µg/ml at 24 h postinjection. On the other hand, Löscher et al. (1998a) administered 50 mg/kg phenytoin in isotonic saline with NaOH to female Wistar rats and found the peak concentration (ca 28 µg/ml) to occur within 0.5 h postinjection and a near zero concentration 8 h postinjection. While Löscher et al. (1998b) have reported a sex difference in plasma levels (higher in females), the most likely account of the difference between the two studies is the vehicle and strain differences. In this current study we used guinea-pigs and administered phenytoin in a vehicle consisting of propylene glycol, ethanol and water (40:10:50) similar to McNamara et al. (1989) and found the peak concentration (ca 31 µg/ml) to occur 24 h after administration. Thus, the related vehicles, polyethylene glycol and propylene glycol, appear to slow the rate of absorption of phenytoin and extend its time in plasma. Our results did yield plasma levels within the human therapeutic range although the pharmacokinetic profile differed from rats. This most likely resulted from the properties of the vehicle and a species difference.

In this study we also measured plasma levels of phenytoin following a single i.p. injections of 50 and 75 mg/kg phenytoin. Somewhat surprisingly we found that both doses yielded statistically equivalent plasma levels. This is most likely due to the slow absorption of phenytoin dissolved in propylene glycol, independent of concentration, from a large “reservoir” located in the peritoneum. While both doses resulted in equivalent plasma levels it is also possible that this may not reflect the effective phenytoin concentration in the brain. Accordingly, it is possible that 75 mg/kg phenytoin resulted in higher brain levels of the drug and thus might explain the few differences in anticonvulsant effects observed between the two doses of phenytoin (see below).

This is the first study on guinea-pigs to use both a detailed battery of quantitative behavioural tests and a qualitative scoring system to illuminate potential behavioural toxic properties of an ACD. While some studies employing behavioural analysis of ACDs in the rat kindling model have used quantitative measures of motor impairment, like the rotarod test (see, for example, Hönack and Löscher 1989), many have usually relied on either descriptions of the animal's behaviour and/or a qualitative scoring system of sedation and ataxia (Albertson et al. 1980; Ehle 1980; McNamara et al. 1989; Löscher et al. 1998a, b; Otsuki et al. 1998). While objective quantitative tests may allow for greater inter-observer reliability and are potentially more sensitive to the behavioural impairment of an ACD, we did not find behavioural impairment following either 50 or 75 mg/kg administration of phenytoin on both the quantitative tasks and qualitative scoring system. While there was a trend to increase righting latencies after 75 mg/kg phe-

nytoin, this was not statistically significant. Our results are in agreement with studies in rats that reported minimal, if any, adverse effects of phenytoin (McNamara et al. 1989; Rundfeldt et al. 1990; Löscher et al. 1998a; Otsuki et al. 1998).

Research using the rat kindling model has convincingly demonstrated the ability of phenytoin to raise the ADT (Ehle 1980; Albright 1983; Rundfeldt et al. 1990; Löscher and Rundfeldt 1991; Löscher et al. 1993, 1998a; Ebert et al. 1994, 1997; Cramer et al. 1998). The results from this study also confirm a threshold increase under phenytoin and extend the observation to the guinea-pig kindling model. While 50 mg/kg phenytoin was ineffective in non-kindled guinea-pigs, it was able to significantly raise ADT in kindled guinea-pigs. We report a threefold increase in ADT with 75 mg/kg phenytoin in the non-kindled guinea-pigs and a 1.5-fold ADT increase in kindled guinea-pigs, suggesting that kindling may alter the effects of an ACD. The question of whether kindling itself can lead to an alteration of phenytoin-induced ADT increase was previously explored by Löscher et al. (1998a) in the rat. They reported that before kindling 75 mg/kg markedly raised the ADT, while after kindling the threshold raising effect was totally lost in male rats and threshold was even decreased in female rats. In a related study, Ebert et al. (1997) reported that partially kindled animals had their threshold raised to a greater degree than fully kindled animals. These data suggest that kindling itself can dramatically alter the anticonvulsant efficacy of phenytoin, as measured by ADT. The present results support the view that kindling perhaps shifts the dose-response curve of phenytoin to the left, so that high dosages may become less effective after kindling. This is relevant in that our results support the proposal that kindling may model the mechanisms leading to intractability of temporal lobe epilepsy (Löscher et al. 1998a). Proconvulsant effects of high doses of phenytoin have been reported in patients (Lerman 1986; Oscorio et al. 1989; Elger et al. 1998) and may be explained by an epileptogenesis-induced shift in drug responses that can be modelled with kindling. Accordingly, our results from the guinea-pig kindling model support the view that although phenytoin is able to suppress focal activity by raising thresholds, this effect is reduced at higher doses of phenytoin with kindling.

Studies using the rat kindling model in which stimulation was delivered at or just above ADT reported that phenytoin resulted in a reduction in ADD (Howe et al. 1980; McNamara et al. 1989; Rundfeldt et al. 1990; Rundfeldt and Löscher 1993; Standley et al. 1994; Voits and Frey 1994; Morimoto et al. 1997; Otsuki et al. 1998). However, when stimulation intensities well above threshold were used, phenytoin resulted in either no effect on ADD (Rundfeldt et al. 1990; Rundfeldt and Löscher 1993; Morimoto et al. 1997) or an increase in ADD (Callaghan and Schwark 1980; Schmutz et al. 1988; Ebert et al. 1997), with one exception reporting reduction in ADD at stimulation intensities which were likely well above threshold (Albertson et al. 1980). Together, the data from the rat kindling model indicate that phenytoin apparently reduces ADD (i.e. an anticonvulsant effect) at or near threshold stimulation, but has either no effect or results in an increase in ADD (i.e. a proconvulsant effect) at stimulation intensities well above threshold. This seems to indicate, at least in the rat amygdala kindling model, that phenytoin most likely raises seizure thresholds but perhaps does not mediate its anticonvulsant action through suppressing seizure propagation. Therefore, findings from the rat kindling model indicate that the intensity of stimulation is a critical variable when assessing phenytoin's ability to reduce ADD. The results from this study indicate that stimulation intensity did not affect phenytoin's ability to reduce ADD in the guinea-pig. We observed that both 50 and 75 mg/kg phenytoin were able to reduce ADD during kindling acquisition and following 50+ kindling stimulations. Furthermore, while suprathreshold stimulation resulted in slightly longer ADDs, both doses of phenytoin were quite effective at reducing ADD. Our results demonstrate that phenytoin reliably inhibits ADD at both threshold and suprathreshold stimulation intensity in the guinea-pig kindling model.

Studies which have examined the effects of phenytoin on seizure severity in the rat kindling model have also indicated variable effects. Many studies have reported a decrease in seizure severity (Howe et al. 1980; Renfrey et al. 1989; Rundfeldt and Löscher 1993; Standley et al. 1994; Voits and Frey 1994); one exception was a study that reported an increase in seizure severity in animals expressing partial seizures (Ebert et al. 1997). Other studies have reported decreased seizure severity, but only at high, behaviourally toxic dosages (Albright and Burnham 1980; Mace and Burnham 1987; Morimoto et al. 1997; Otsuki et al. 1998). Similar to the data on ADD, there appears to be a greater reduction in seizure severity when rats are stimulated at or near ADT (Rundfeldt et al. 1990; Rundfeldt and Löscher 1993; Voits and Frey 1994) and less of a reduction in seizure severity when rats are stimulated well above ADT (Rundfeldt et al. 1990; Rundfeldt and Löscher 1993; Morimoto et al. 1997). The results from the present study indicate that phenytoin was able to reduce seizure severity in guinea-pigs during late kindling acquisition and in guinea-pigs that had received 50+ stimulations. Although we did not observe a reduction in seizure severity in non-kindled and early kindling acquisition guinea-pigs, this was probably because the seizures were not severe enough to allow the effect of phenytoin to be measured. However, during late kindling acquisition and in guinea-pigs that had received 50+ stimulations, 75 mg/kg phenytoin reduced the severity of partial seizures at both threshold and suprathreshold stimulation intensities. While 50 mg/kg phenytoin effectively reduced seizure severity in kindled guinea-pigs with threshold stimulation, and in guinea-pigs during late acquisition, a significant reduction in seizure severity to suprathreshold stimulation in kindled guinea-pigs was not observed, indicating a slight effect of stimulation intensity.

In sum, phenytoin in the guinea-pig kindling model produces consistent anticonvulsant activity by increasing ADT and reducing seizure duration and severity that does not appear to be largely dependent on stimulation intensity. These anticonvulsant effects occur at concentrations that are within the human therapeutic range and that do not cause significant behavioural impairment. The results reported here indicate that phenytoin in the guinea-pig exerts a consistent anticonvulsant action, further suggesting that guinea-pig kindling, with its extended period of partial seizures, functions as an advantageous model system for human partial seizures.

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