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Does brain slices from pentylenetetrazole-kindled mice provide a more predictive screening model for antiepileptic drugs?

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ABSTRACT

The cortical wedge is a commonly applied model for *in vitro* screening of new antiepileptic drugs (AEDs) and has been extensively used in characterization of well-known AEDs. However, the predictive validity of this model as a screening model has been questioned as, *e.g.*, carbamazepine has been reported to lack effect in this model. The neuroplastic changes induced in acute and chronic animal models of epilepsy are known to affect the pharmacological profile of AEDs *in vivo*. Hence, we investigated whether brain slices from pentylenetetrazole (PTZ)-kindled animals could provide a more predictive screening model for AEDs. To this end, we compared the *in vitro* and *in vivo* pharmacological profile of several selected AEDs (phenobarbital, phenytoin, tiagabine, fosphenytoin, valproate, and carbamazepine) along with citalopram using the PTZ-kindled model and brain slices from naïve, saline-injected and PTZ-kindled mice. Our data suggest that the use of slices from PTZ-kindled mice in the cortical wedge does not increase the predictive validity of the model as an *in vitro* screening model for AEDs. Traditionally, the incidence of certain seizure types is widely used as a measure to characterize drug action in animal models of epilepsy. In our study, the anticonvulsant effect of the AEDs was investigated *in vivo* using several observational parameters (*i.e.*, incidence and duration of convulsions, latency to clonic convulsions, and severity of convulsions). We found that including the observational parameter "severity" offered important additional information about the drug profile that would otherwise be lost if only a single parameter as "incidence" was used.

1. Introduction

Animal models of epilepsy play a key role in identifying drugs used for the treatment of epilepsy. Besides possessing anticonvulsant properties, a good drug candidate should ideally not possess side effects, have a good bioavailability, and reach the target area, *i.e.*, penetrate the blood brain barrier. *In vitro* models of epilepsy cannot substitute for the use of animals. However, the use of appropriate *in vitro* models may provide valuable information in the early phase of identifying new drug candidates, which may then reduce the amount of animal experiments needed. A vital requirement for an *in vitro* model to serve such a purpose is that it is predictive in identifying candidates with an antiepileptic potential. In particular, tissue from hippocampus has been extensively used as *in vitro* models of epilepsy either by addition of acute convulsants such as picrotoxinin to slices (Jefferys, 2003), as cell cultures (Deshpande et al., 2008) or in the form of organotypic cultures (Noraberg et al., 2005).

The cortical wedge preparation has successfully been used to study the effect of several antiepileptic drugs (AEDs) such as lamotrigine, phenobarbital, phenytoin and valproate (Ngo Bum et al., 2003; Phillips et al., 1997). In addition, the model has been used as a screening model for ligands acting directly at both ionotropic and metabotropic glutamate receptors (Johansen et al., 1994, 1998; Sheardown, 1992), $GABA_A$ receptors and voltage-sensitive ion channels (Burton et al., 1987; Robichaud et al., 1994), though not all compounds being efficacious in the model necessarily display anticonvulsant activity in animal models of epilepsy. Moreover, Phillips et al. (1997) questioned the predictivity of the model as a screening model for AEDs based on the lack of efficacy of carbamazepine.

Traditionally, slices used in the cortical wedge model are obtained from naïve animals. Work on acute and chronic animal models of epilepsy has demonstrated that there may be major differences in the pharmacological profile of AEDs in terms of potency and efficacy, depending on which type of model is used (Hansen et al., 2004; Honack and Loscher, 1995; Jefferys, 2003; Loscher, 2002). During the process of epileptogenesis, several changes occur in the brain, *i.e.*, neuronal cell death, alteration in gene expression, changes in plasticity, receptor density and stoichiometry (Meldrum, 2001). Hence, these neuroplastic changes may differ from one model to

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another. Using slices from epileptic animals or patients may therefore provide valuable new information and have a higher predictive value (Albensi et al., 2007; Moddel et al., 2005). The pentylenetetrazole (PTZ) kindling model is a commonly used animal model of epilepsy used among others for screening purposes (Kupferberg, 2001). We therefore set out to test whether brain slices obtained from PTZ-kindled animals could provide a more predictive screening model for AEDs. For this purpose, we compared the *in vitro* and *in vivo* pharmacological profile of several selected AEDs: phenobarbital (PB), phenytoin (PHT), tiagabine (TGB), fosphenytoin (FosPHT), valproate (VPA), and carbamazepine (CBZ) along with citalopram (CTP) using the PTZ-kindling model and brain slices from naïve, saline-injected and PTZ-kindled mice. The AEDs used in the present study were chosen based on they each have a unique pharmacological profile and target the main mechanisms of action of AEDs (Kwan et al., 2001; White, 1999) and were therefore considered appropriate for the purpose of model characterization. Further, CTP was included based on contradictory reports of its antiepileptic potential.

2. Materials and methods

2.1. Animal handling and care

Three-week-old male NMRI mice (Taconic, DK) were housed in plastic home cages (macrolon, type II) with a maximum of 10 mice per home cage under a 12 h light–dark cycle (lights on at 6 a.m.). Standard rodent pellet food (Altromin 1314, Møllegården, DK) and tap water were available *ad libitum*. The mice were allowed one week to acclimatize before the start of experimentation. Ethical permission for the studies was granted by the Animal Welfare Committee, appointed by the Danish Ministry of Justice, and all animal procedures were carried out in compliance with the EC Directive 86/609/EEC and with the Danish law regulating experiments on animals.

2.2. PTZ-kindling

Mice were injected with PTZ (Sigma, 43 mg/kg) three times per week for four weeks at which time the mice were fully kindled, *i.e.*, all mice had clonic convulsions upon PTZ injections 2 out of 3 times during the 4th week. Following this procedure 85% of the mice were up-kindled during the 4th week. Mice that failed to be kindled were sacrificed.

For anticonvulsant testing, PTZ-kindled mice were subsequently allocated to groups of two to be used in a modified Roman square design, so that every dose level (D1–4) was represented on every day of testing, according to the following scheme:

Gr/Day	Mon	Wed	Fri	Mon	Wed	Fri	Mon	Wed	Fri
1	P + veh	P + D1	P	P + D2	P + veh	P + D4	P	P + D3	P + veh
2	P + veh	P + D3	P	P + D4	P + veh	P + D2	P	P + D1	P + veh
3	P + veh	P + D2	P	P + D3	P + veh	P + D1	P	P + D4	P + veh
4	P + veh	P + D4	P	P + D1	P + veh	P + D3	P	P + D2	P + veh

In total, eight kindled animals were used per dose level, the number of animals used being based on previous experience of number of animals needed in this model to show an effect. In general this kindling protocol was well tolerated. In a few instances, mice had to be sacrificed during the drug testing due to bite wounds because male mice become more aggressive as they mature. The regular testing of whether the mice were still responding to PTZ (or PTZ + vehicle) ensured that they had not lost sensitivity or had hangover effects from the compounds. Thereby, the effect of PTZ was tested twice and the effect of PTZ + vehicle was tested three times on each mouse. Data were only included if the whole data set was valid, *i.e.*, PTZ injections with or without vehicle elicited clonic convulsions in mice. To avoid artificially increasing N in the control groups, an average score for PTZ and PTZ + vehicle was calculated and included in the final data analysis. All compounds, except CTP, were tested in 4 different doses (n = 8), so that the highest dose employed did not produce motor impairment as determined in a rotarod model (ENV 575M, Med Associates, VT, USA). CTP was only tested in three different doses, because mice on the highest dose level had toxic effects appearing one day after dosing. Drugs at the selected dose levels were in general well tolerated, however, CTP-treated mice at the highest dose level (n = 8) had to be sacrificed the following days. In the highest PHT dose group, two mice experienced status epilepticus and died subsequently. Furthermore, eight PHT-treated mice used for initial experiments (data not shown) were sacrificed due to adverse events.

Upon each PTZ injection mice were observed for 30 min for incidence, duration of, and latency to clonic convulsions. In addition, the severity of the convulsions was scored according to a modified Racine scale: 0 = no response, 1 = 1–3 myoclonic jerks and/or facial twitching and/or axial waves going through the body, 2 = more than 3 myoclonic jerks, 3 = clonic convulsion with forelimb clonus without loss of postural control, 4 = clonic convulsion with loss of postural control, turning to the side and/or rearing, and 5 = clonic convulsion with loss of righting reflex and/or bouncing, two or more clonic convulsions, tonic convulsion or status epilepticus (Racine, 1972).

Mice used in the cortical wedge were allocated upon arrival to any of the three groups, *i.e.*, naïve, saline-treated or PTZ-kindled. After a week of acclimatization, the mice were given either saline- or PTZ-injections using the protocol described above. The naïve mice were handled in an identical manner as if they were injected. At the fourth week, the mice receiving PTZ-injections were observed for their kindling status, only fully kindled mice in the PTZ group were used for further studying.

2.3. Mice cortical wedge preparation

The cortical wedge preparation was carried out according to previously published methods (Ebert et al., 2002; Harrison and Simmonds, 1985). In this work, eight-week-old male NMRI mice (Taconic, Denmark) were decapitated and the brain rapidly removed and placed in ice-cold O_2/CO_2 (95%/5%) saturated artificial cerebrospinal fluid (aCSF). The composition of the aCSF was (in mM) NaCl 118, KCl 2.1, KH_2PO_4 1.2, D-glucose 11, $NaHCO_3$ 25, and $CaCl_2$ 2.5. Using a vibratome (Vibroslice model 752HA, Campden Instruments, Ltd., Leicester, U.K.), successive coronal sections were made and discarded until the corpus striatum was visible. Subsequently, 1–2 slices (400 μm thick) were cut and divided at the midline of the hemispheres. Parallel with the midline, a rod ("wedge") of brain tissue, approximately 1.5 mm thick, was cut from each hemisphere and the striatal tissue was trimmed off. Recordings were made from the remaining tissue, which consisted of the cingulate cerebral cortex and the secondary motor cortex. The recording arrangement consisted of two-compartment baths. The two compartments of each bath were separated by a wall supplied with a slot for placement of the wedge. The wedge was mounted across the slot, with the cortex part of the wedge situated in the left compartment and the corpus callosum part in the right compartment. The gap between the two compartments was insulated with grease. Ag/AgCl electrodes (Dri-Ref™, World Precision Instruments, Sarasota, Florida, USA) in each compartment were in contact with dishcloth tissue providing electrical contact with the wedge. The electrical potential difference between the electrodes was recorded on a chart recorder Yokogawa LR 4220E (Yokogawa Electric Corporation, Tokyo, Japan), which was connected to a PC via a RS-232C interface for digital sampling. The left and right compartments were independently and continuously superfused at 1 mL/min/compartment with aCSF (cortex part) and aCSF devoid of Ca^{2+} (corpus callosum part), respectively. The wedges were left for development of spontaneous epileptiform discharges (SEDs) for at least 2½ hours.

Both the frequency (number of SEDs during periods of 30 min) and the total depolarizing shift (the frequency times mean area of the SEDs) were studied initially. Both the total depolarizing shift and the frequency of the SEDs increased over time. Before drug application was initiated, the frequency of the SEDs was used to determine the degree of stability. Stability was defined as no greater than 10% variation in the basal activity (frequency) of the SEDs between two subsequent analyses. Characterization of drug effects on the SEDs was initiated when the frequency was ≥2.5 spikes/min and stable over a 40 min period. Drugs were applied in aCSF and superfused for 20 min over the wedges. The last 12 min of the drug application was used for data analysis. Concentration–response curves were constructed using cumulative concentrations with increase in concentration every 20 min.

2.4. Drugs

Fosphenytoin was a generous gift from Pfizer. Phenytoin and carbamazepine were purchased from Sigma-Aldrich (Brøndby, Denmark). Phenobarbital was purchased from Mecobenzon (Copenhagen, Denmark). *(S)*-Citalopram was obtained by dissolution of *(S)*-citalopram tablets in buffer. The concentration of the active ingredient (*(S)*-citalopram) was determined on LC-MS using standard solutions in the concentration range of 0.1 mM to 5 mM ($R^2 = 0.997$). Separation of citalopram was performed on a HPLC column from Waters (Xterra MSC 18, 3.5 μM particle size) and the mobile phase used was a mixture of 50/50% v/v mobile phase A (95% H_2O containing 0.2% HCOOH and 5% methanol) and mobile phase B (5% H_2O containing 0.2% HCOOH and 95% methanol). The detector used was a single quadrupole mass spectrometer (MSD) from Agilent operating in a positive ionization mode (ESI+) acquiring at single ion monitoring of *(S)*-citalopram (m/z 325).

2.5. Data and statistical analysis

The frequency and area of the SEDs were determined using the Mini Analysis Program 6.0.3 (Synaptosoft Inc.). The total depolarizing shift (mean area × frequency) during the 12 min time period was estimated by the product of the mean area per SED and the total number of the SEDs during the 12 min period. The relative frequency (in percent), was calculated as the ratio between SEDs in the presence and absence (basal activity) of drug, respectively; likewise for the relative total depolarizing shift. Data for concentration–response curves were analyzed using the non-linear curve fitting program Prism 4.02 (GraphPad Software, Inc.) and the logistic equation:

$$E = \frac{[A]^n}{IC_{50}^n + [A]^n}$$

where E is the observed drug response at a specific agonist concentration [A], IC_{50} is the concentration, which reduces the basal activity by 50%, and n is the Hill coefficient. The effect of CTP and AEDs on incidence and severity of clonic convulsions in PTZ-kindled mice were likewise fitted to the logistic function above. For the *in vivo* data constraints were used to obtain realistic fits. The constraints were bottom = 0 and top ≤ vehicle for all data sets except for CBZ severity where the constraints were 0 < bottom < 2.

Data were analyzed using SigmaStat (version 3.11, Systat software, Inc.). IC_{50}-values were assumed to follow a log Gaussian distribution and are presented as means followed by 95% confidence intervals in square brackets. Data for latency to- and duration of clonic convulsions are given as mean ± S.E.M. One-way analysis of variance (ANOVA) was used for comparisons.

3. Results

3.1. Anticonvulsant effects of test compounds

The effect of several AEDs and CTP was tested on various observational parameters in PTZ-kindled mice. Besides seizure severity and incidence of clonic convulsions, a possible drug effect on the latency to- and the duration of clonic convulsions was investigated. The latency to clonic or tonic convulsions has previously been used as measure for seizure threshold in, *e.g.*, the intravenous PTZ-threshold model. The rationale is that the longer the latency to clonic convulsions, the higher is the threshold, as more PTZ needs to accumulate in the brain in order to cause seizures. As an example, the effect of carbamazepine on the four parameters is illustrated in Fig. 1 and Table 2. CBZ dose-dependently inhibited the incidence of clonic convulsions (Fig. 1A). However, only clonic convulsions were affected (stages 3–5) whereas CBZ had no effect on myoclonic convulsions (stages 1–2) (Fig. 1B). This example illustrates that the use of several observational parameters to characterize drug effects in a given disease model of epilepsy is beneficial as important information may otherwise be lost. In general, CBZ did not alter either the latency to or the duration of clonic convulsions at doses up to 15 mg/kg compared to PTZ only or vehicle-treated mice (Table 2). However, mice treated with 15 mg/kg CBZ had a significantly longer latency to seizures as compared to PTZ-treated mice. The significance of this finding should not be over-interpreted as it might be due to mass-significance, that is the chance of finding a false statistical difference when many statistical tests are performed.

Data for the incidence of clonic convulsions and seizure severity were fitted to a logistic function and the ED_{50} values are presented in Table 1. For all of the tested compounds, there is a good consistency

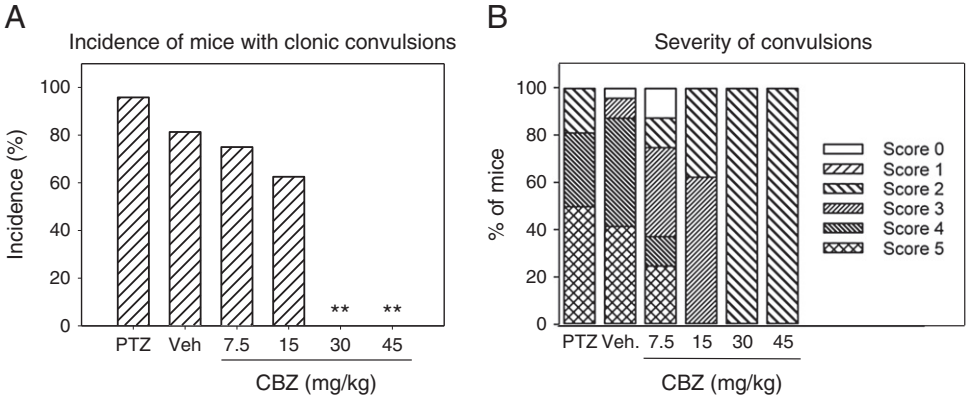

Fig. 1. Anticonvulsant properties of carbamazepine (CBZ). Mice were pre-treated with CBZ, vehicle or no pre-treatment (PTZ group) 30 min before PTZ injection (N = 6–8). **P < 0.01, Kruskal–Wallis one-way ANOVA on ranks with vehicle (veh) as control group with post hoc Dunn's method.

Table 1
The effect of CTP and of the AEDs (CBZ, PHT/FosPHT, VPA, TGB and PB). NE = no anticonvulsant effect in the tested dose range. The 95% confidence interval for the ED_{50} determination is given in square brackets.

	Incidence ED_{50} (mg/kg)	Severity ED_{50} (mg/kg)
CBZ	17	10.6
	[8–36]	[2.8–40.5]
PHT/FosPHT	NE	NE
VPA	225	234
	[152–335]	[183–298]
TGB	0.7	1.1
	[0.4–1.1]	[0.2–5.4]
PB	9	8
	[2–36]	[2–41]
CTP	NE	NE

between the ED_{50} values obtained from the two sets of data (Table 1). None of the tested compounds altered the latency to or the duration of clonic seizures (Table 2). CTP was without an effect on seizures evoked in PTZ-kindled mice. Unexpectedly, our initial experiments showed that PHT was without any anticonvulsant effect in PTZ-kindled mice. As PHT is only soluble in very basic solutions (pH $\geq$11), the lack of anticonvulsant effect could be due to a precipitation in the submucus following injection. FosPHT is a prodrug of PHT that is soluble at neutral pH. FosPHT is readily metabolized to PHT once in the body. Thus, FosPHT was used in the final experiments. Nevertheless, FosPHT also lacked anticonvulsant effects in PTZ-kindled mice (Table 1). Rather, both PHT (data not shown) and FosPHT had proconvulsive effects, as measured by the number of mice having more than one clonic convulsion following PTZ injection (Fig. 2). Twenty percent of the mice experienced status epilepticus at the highest dose employed, *i.e.*, 45 mg/kg FosPHT. None of the other drug treatments resulted in worsening of the seizures.

Table 2
Latency to and duration of clonic convulsions in mice administered with AEDs. None of the mice had clonic convulsions.

	Drug (mg/kg)	Latency (s)	Duration (s)
CBZ	PTZ	165 ± 19	14 ± 1
	Veh.	302 ± 62	13 ± 2
	7.5	293 ± 74	17 ± 2
	15	320 ± 23[a]	14 ± 1
	30	–	–
	45	–	–
PHT	PTZ	152 ± 29	15 ± 2
	Veh	114 ± 7	15 ± 1
	7.5	153 ± 32	15 ± 1
	15	102 ± 9	21 ± 1
	30	99 ± 14	14 ± 2
	45	113 ± 28	22 ± 1
VPA	PTZ	114 ± 7	24 ± 5
	Veh	133 ± 14	21 ± 4
	40	106 ± 22	50 ± 18
	80	110 ± 6	28 ± 5
	160	140 ± 6	13 ± 3
	320	–	–
TGB	PTZ	147 ± 20	20 ± 1
	Veh	155 ± 20	18 ± 1
	0.2	160 ± 15	17 ± 1
	0.4	189 ± 52	19 ± 2
	0.8	123 ± 22	15 ± 2
	1.6	–	–
PB	PTZ	174 ± 19	16 ± 1
	Veh	214 ± 42	16 ± 1
	3.5	221 ± 22	15 ± 3
	7	180 ± 13	13 ± 2
	15	262 ± 110	15 ± 5
	30	–	–
CPT	PTZ	145 ± 6	14 ± 1
	Veh	137 ± 14	15 ± 1
	10	118 ± 25	16 ± 3
	30	210 ± 65	15 ± 1
	60	119 ± 30	14 ± 2

[a] P<0.05 difference from PTZ alone.

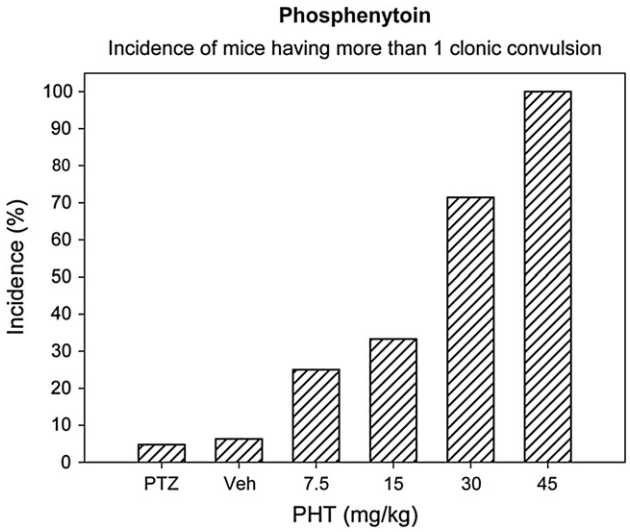

Fig. 2. Proconvulsive effect of fosphenytoin (FosPHT) measured by the incidence of mice experiencing more than one clonic convulsions following PTZ injection (n = 6–8). Several mice pre-treated with either 15 mg/kg FosPHT, 30 mg/kg FosPHT or 45 mg/kg FosPHT experienced more than 5 clonic convulsions. One mouse pre-treated with 45 mg/kg FosPHT had 10 consecutive clonic convulsions and another mouse in the same treatment group had repeated clonic convulsions lasting for more than 30 min.

3.2. Cortical wedge model

Both the frequency and the total depolarizing shift of the SEDs recorded from brain slices obtained from PTZ-kindled mice were significantly larger as compared to the SEDs recorded from slices obtained from naïve mice as determined by a binomial test (data not shown). This finding is in agreement with single-channel recordings from acutely isolated dentate gyrus granule cells showing large increases in mean open times, burst lengths, and cluster lengths of the NMDA channels in kindled neurons as compared to naïve control neurons (Kohr et al., 1993).

The effect of several antiepileptic drugs (CBZ, PB, PHT, TIA and VPA) and CTP on the SEDs was investigated in brain slices obtained from kindled, saline-treated and naïve animals. A representative example of an electrophysiological recording of the SEDs from a brain slice is shown in Fig. 3A in which the effect of CBZ on the SEDs was studied by conducting cumulative concentration–response curves. The inhibition curves for CBZ obtained by fitting to the pooled data set from several slices are shown in Fig. 3B.

The potency of the antiepileptic drugs varied significantly. Using the total depolarizing shift, the order of rank was starting with the most potent one CBZ > TGB > PB = PHT > > VPA (Table 3). A similar rank order was obtained using the frequency data (data not shown).

Neither of the drugs (including CBZ) shown profound differences in the frequency and the total depolarizing shift of the SEDs following PTZ-kindling (Table 3) although differences in the effect of the antiepileptic drugs on the SEDs were occasionally observed between tissues obtained from PTZ-kindled, saline-treated, and naïve mice (*e.g.*, inset Fig. 3B).

4. Discussion

Although the cortical wedge model can be used for the initial screening of the potency of epileptic drugs, our data suggest that this model has limited use for other purposes. Our data show that no additional information can be gained by using brain slices from

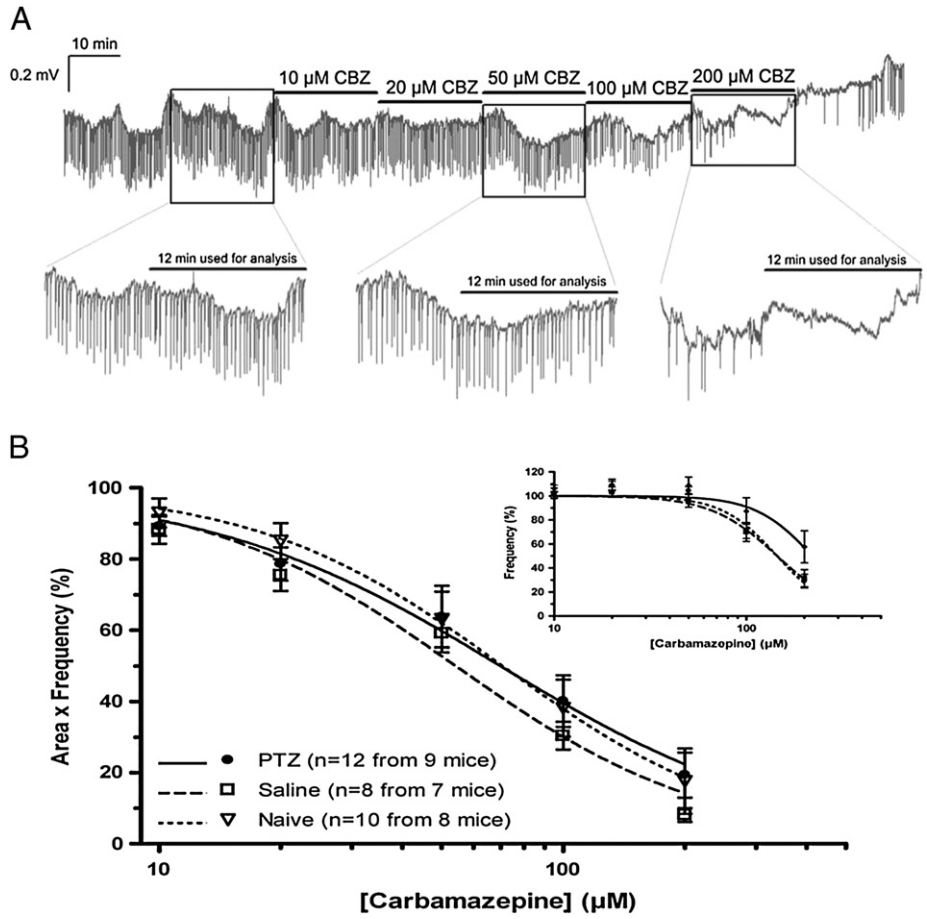

Fig. 3. The effect of carbamazepine (CBZ) on SEDs. (A) An electrophysiological recording of SEDs in a cerebral cortical brain slice obtained from a saline-treated mouse. Concentration–response curves were constructed using cumulative concentrations with increase in concentration every 20 min as shown in the upper trace (black bars). Selected parts of the upper trace (indicated by squares) are shown at a higher time-resolution in the lower traces. Only the last 12 min of the 20 min drug application was used for data analysis as indicated by the black bars in the lower traces. (B) The effect of CBZ on the total depolarizing shift (mean area × frequency) of the SEDs. Recordings were made from cerebral cortical brain slices obtained from PTZ-kindled (closed circles, n = 12), saline-treated (open squares, n = 8) and naïve mice (open triangles, n = 10). (Inset) The effect of CBZ on the frequency of the SEDs.

Table 3
The effect of the antidepressant CTP and the anticonvulsants CBZ, PB, PHT, TIA and VPA on the total depolarizing shift (mean area × frequency) of the SEDs. The IC_{50} values indicate the concentration of drugs, which was needed to reduce the basal activity of the SEDs to 50%. The IC_{50} values were obtained from curve fitting to the pooled data set and are shown as means followed by 95% confidence intervals in square brackets. Each group consists of 3–12 slices obtained from 3 to 9 animals.

Drug	Treatment	Mean area × frequency IC_{50} (μM)
CBZ	PTZ-kindled	70 [54;90]
	Saline-treated	54 [46;64]
	Naïve	71 [55;91]
PB	PTZ-kindled	233 [160;339]
	Saline-treated	209 [162;271]
	Naïve	370 [284;483]
PHT	PTZ-kindled	219 [152;314]
	Saline-treated	308 [174;547]
	Naïve	169 [141;202]
TIA	PTZ-kindled	128 [89; 183]
	Saline-treated	49 [29; 83]
	Naïve	121 [56; 262]
VPA	PTZ-kindled	6323 [4918; 8130]
	Saline-treated	16,360 [13,160; 20,350]
	Naïve	7789 [6739; 9003]
CPT	PTZ-kindled	>>100
	Saline-treated	>>100
	Naïve	>>100

PTZ-kindled mice. The reason for this can probably be explained by the high variability observed in the response to the epileptic drugs in this model. Thus, the cortical wedge model cannot be used for studying mechanistic effects of epileptic drugs on PTZ-kindled animals. Nevertheless, it cannot be ruled out that more useful information can be obtained in more sophisticated electrophysiological models (such as patch-clamp recordings in bran slices). However, such models are not suitable for screening purposes.

4.1. Evaluation of the in vivo efficacy parameters

The incidence of certain seizure types is widely used as a rough and fast measure to characterize drug action in animal disease models of epilepsy. The severity scale may be used to differentiate efficacy of the AEDs for different seizure types as exemplified with CBZ in the present study. Thus, CBZ was found to be effective against clonic convulsions, whereas it completely failed to inhibit myoclonic convulsions even at the highest dose (Fig. 1), a finding that would have been missed if only the incidence parameter was employed. The Racine scale was originally proposed to evaluate seizure severity in amygdala kindled rats (Racine, 1972) and has since been widely implemented to describe seizure severity in other models of epilepsy. Different brain areas are affected by PTZ-induced kindling as compared to electrical kindling of a particular locus (Szyndler et al., 2009). Given the well-known relation between activated anatomical location of the brain and the corresponding expressed behavior,

different modifications of the Racine scale have been proposed (Hellier et al., 1998; Luttjohann et al., 2009). Thus, the modified Racine scale used in the present study (as described in the Materials and methods section) is suitable to describe convulsive behavior in PTZ-kindled mice.

The fact that the duration of PTZ-evoked seizures is remarkably constant over time, regardless of drug treatments, litters *etc*. shows that the seizure duration is not a useful measure to characterize AEDs. Whereas seizure duration is a commonly employed measure in electrical kindling models, it is seldom used in chemical kindling models. Teskey et al. (2004) report a significant difference in total seizure duration between two strains of mice. However, this difference stems from a varying number of seizures and not the duration of a single seizure in accordance with the present findings (Teskey et al., 2004). The variability of latency measurements is too wide to be useful for experimental purposes.

4.2. Pharmacological considerations

Our study shows that both FosPHT and PHT had proconvulsive effects rather than anticonvulsant effects in PTZ-kindled mice. The incidence of clonic convulsions increased dose-dependently (Fig. 2). All mice administered 45 mg/kg FosPHT had two or more clonic convulsions. This may seem astounding at first given that PHT is a widely used AED *e.g.*, against different seizure types in the rat model of ischemia-induced epilepsy (Edmonds et al., 1996) and in the MES test in BDF1 mice (Kitani et al., 1984). In the study by File et al. (1985) chronic administration of PHT, but not single administration, decreased in the incidence of PTZ-induced seizures in mice. However, in line with our findings, Ebert et al. (1997) showed that at the initial electrical stimulation in the amygdala, PHT increased the threshold current for eliciting afterdischarges (*i.e.*, had an anticonvulsant effect in the focus) but did not prevent the spread of seizure activity (*i.e.*, the occurrence of a generalized seizure once focal activity was initiated) (Ebert et al., 1997). Rather both the seizure and afterdischarge duration were about three times higher in PHT-treated rats compared to saline-treated rats and 50% of the PHT-treated rats showed generalized seizures, which never occurred in saline-treated rats (Ebert et al., 1997). In fully kindled rats, PHT significantly increased after discharge threshold but had no effect on seizure severity or seizure duration. PHT did, however, increase the after discharge duration significantly (Ebert et al., 1997). Also, in epilepsy patients PHT may precipitate or aggravate generalized tonic clonic seizures or other generalized seizure types (Glauser et al., 2006). Along the same lines, Loscher et al. (1986) have repeatedly demonstrated varying anticonvulsant efficacy of PHT in the amygdala kindling model of epilepsy. Based on this finding, the authors proposed that PHT-resistant amygdala-kindled rats could constitute a model for drug-resistant focal epilepsy. Whether the PTZ-kindling model likewise could be used as a model of drug-resistance requires further characterization of this phenomenon.

CBZ potently inhibited SEDs to a similar degree in all treatment groups (*i.e.*, PTZ-kindled, saline-treated and naïve mice). Previously, CBZ (up to 100 μM) has been reported to have no effect on SEDs in cortical wedges prepared from naïve CD rats (Phillips et al., 1997). This disparity might be due to species differences. However, differences in the experimental conditions may provide a more likely explanation. Phillips et al. (1997) only applied the anticonvulsants for 10 min, which is considerably less than the 20 min of application used in this study, and may not have been sufficient time for diffusion through tissue to receptor sites. CBZ was an efficient anticonvulsant for clonic seizures but lacked effect on myoclonic seizures. CBZ has previously been shown efficacy in blocking MES-induced tonic extension and attenuating fully expressed kindled seizures (White, 1999). In accordance with the present findings, CBZ could only partially suppress myoclonic seizures in gerbils (Frey et al., 1983). In humans, CBZ has a broad spectrum of efficacy for the treatment of various epilepsy seizures and syndromes. It is currently the established initial monotherapy for adults with partial-onset epilepsy, and possibly effective for children and elderly with partial-onset seizures or with generalized tonic clonic seizures (Glauser et al., 2006).

PB inhibited SEDs to a similar degree in PTZ-kindled and saline-treated mice, $ED_{50} = 233$ μM and 209 μM, respectively, and less so in naïve mice ($ED_{50} = 370$ μM). Accordingly, PB effectively inhibited PTZ-induced convulsions in kindled mice. These findings are in agreement with the efficiency of PB in the MES model and on focal seizures in the electrical kindling model (White, 1999).

TGB inhibited SEDs to a similar degree in PTZ-kindled and naïve mice, ED_{50s} 128 and 121 μM, respectively, and even more potent in saline-treated mice ($ED_{50} = 49$ μM). *In vivo*, TGB was the most potent of the tested AEDs in line with that TGB has been demonstrated to be effective in several animal models of epilepsy (Dalby and Nielsen, 1997) and effective for the treatment of partial seizures (Stefan and Feuerstein, 2007).

VPA was the least efficient anticonvulsant both *in vitro* and *in vivo*. However, among standard AEDs, VPA has the broadest preclinical and clinical profile (White, 1999).

CTP is a commonly used antidepressant worldwide and was included in the study based on the neurobiological correlation between depression and epilepsy. Hence, CTP has also been reported to manage epilepsy clinically (Albano et al., 2006; Specchio et al., 2004) though generalized seizures are a recognized complication of CTP overdose (Waring et al., 2008). Intrahippocampal injections of 1 μM CTP almost completely abolished convulsant activity in pilocarpine-treated rats via 5-HT_{1A} receptors (Clinckers et al., 2004). The same study advocated for proconvulsant effects of CTP to be associated with concomitant hippocampal glutamate release. In the present study, CTP lacked effects both *in vivo* and *in vitro*. For the *in vitro* studies, concentrations ranging up to 100 μM were used, thus covering the concentrations used to demonstrate anticonvulsant effects in the study by Clinckers et al. (2004). Kabuto et al. (1994) found that acute administration of CTP to El-mice was without anticonvulsant effect whereas 2 weeks of daily administration prior to testing conferred anticonvulsant protection (Kabuto et al., 1994). In the PTZ kindling model, doses up to 60 mg/kg were employed and completely lacked anticonvulsant effects. In our preliminary studies, we also evaluated the dose level of 90 mg/kg CTP but the dose was not included due to toxicological signs one day after dosing. Thus, it is not likely that higher doses of CTP would have produced therapeutic anticonvulsant effects. With the design employed here, every mouse was dosed three times with varying doses of CTP over a two-week period and the number of drug administrations was not a confounding factor. However, it cannot be ruled out that longer term chronic dosing, like the dosing scheme used by Kabuto et al. (1994), is required to unravel anticonvulsant effects of CTP, although single dosing was sufficient to demonstrate anticonvulsant efficacy in the study by Clinckers et al. (2004).

5. Conclusion

The present study demonstrated that the use of slices from PTZ-kindled mice in the wedge model does not increase the predictive validity of the model as an *in vitro* screening model for AEDs. Our study shows that similar results can be obtained using brain slices from naïve animals. Hence, in view of the three Rs, the use of brain slices obtained from PTZ-kindled mice is presently unjustified, as it will not lead to an overall reduction, refinement or replacement of animal testing. Although no yet elucidated, the lack of predictability could be due to limited or highly variable PTZ-induced changes in the mouse cerebral cortex.

It was also shown that the inclusion of the observational parameter “severity” in animal models of epilepsy can provide important information about the drug profile that would otherwise be lost if only

"incidence" was used, which is typically the case when the animal models of epilepsy are used for drug screening. A better drug characterization will form a better foundation to judge the anticonvulsant potential of new drug candidates and ultimately better AEDs being developed. Finally, as new drug development is not feasible without the use of animals, ethical considerations should dictate that as much relevant information as possible is obtained from experiments involving animals.

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