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ORIGINAL PAPER

Effect of the Anti-depressant Sertraline, the Novel Anti-seizure Drug Vinpocetine and Several Conventional Antiepileptic Drugs on the Epileptiform EEG Activity Induced by 4-Aminopyridine

Maria Sitges[1] · Blanca Irene Aldana[2] · Ronald Charles Reed[3]

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Abstract Seizures are accompanied by an exacerbated activation of cerebral ion channels. 4-aminopyridine (4-AP) is a pro-convulsive agent which mechanism of action involves activation of Na^+ and Ca^{2+} channels, and several antiepileptic drugs control seizures by reducing these channels permeability. The antidepressant, sertraline, and the anti-seizure drug vinpocetine are effective inhibitors of cerebral presynaptic Na^+ channels. Here the effectiveness of these compounds to prevent the epileptiform EEG activity induced by 4-AP was compared with the effectiveness of seven conventional antiepileptic drugs. For this purpose, EEG recordings before and at three intervals within the next 30 min following 4-AP (2.5 mg/kg, *i.p.*) were taken in anesthetized animals; and the EEG-highest peak amplitude values (HPAV) calculated. In control animals, the marked increase in the EEG-HPAV observed near 20 min following 4-AP reached its maximum at 30 min. Results show that this epileptiform EEG activity induced by 4-AP is prevented by sertraline and vinpocetine at a dose of 2.5 mg/kg, and by carbamazepine, phenytoin, lamotrigine and oxcarbazepine at a higher dose (25 mg/kg). In contrast, topiramate (25 mg/kg), valproate (100 mg/kg) and levetiracetam (100 mg/kg) failed to prevent the epileptiform EEG activity induced by 4-AP. It is concluded that 4-AP is a useful tool to elicit the mechanism of action of anti-seizure drugs at clinical meaningful doses. The particular efficacy of sertraline and vinpocetine to prevent seizures induced by 4-AP is explained by their high effectiveness to reduce brain presynaptic Na^+ and Ca^{2+} channels permeability.

Keywords Carbamazepine · Lamotrigine · Levetiracetam · Oxcarbazepine · Phenytoin · Topiramate · Valproic acid

✉ Maria Sitges
sitges@biomedicas.unam.mx

[1] Departamento de Biología Celular y Fisiología, Instituto de Investigaciones Biomédicas, Universidad Nacional Autónoma de México, A.P. 70228, Ciudad Universitaria, 04510 Mexico, DF, Mexico

[2] Department of Drug Design and Pharmacology, Faculty of Health and Medical Sciences, University of Copenhagen, Copenhagen, Denmark

[3] Department of Pharmacy Practice, Husson University, One College Circle, Bangor, ME 04401-2999, USA

Introduction

During seizures an exacerbated neuronal excitability takes place. Therefore epilepsy is a neurological disease in which voltage sensitive Na^+ and Ca^{2+} channels are particularly activated. Na^+ and Ca^{2+} channels controlling neurotransmitter release, including glutamate (Glu), that is the most concentrated and important excitatory neurotransmitter in the brain, are particularly abundant in cerebral nerve endings. By reducing Na^+ and Ca^{2+} channels permeability, several antiepileptic drugs inhibit Glu release induced by depolarization [1–3]. In contrast, the K^+ channel blocker 4-aminopyridine (4-AP), that induces tonic-clonic seizures and epileptiform EEG activity in the animal in vivo [4–9], increases brain Na^+ and Ca^{2+} channels permeability and induces Glu release in cerebral isolated nerve endings [3, 10–13].

One of the most common co-morbidities in patients with epilepsy is depression [14–23]. Sertraline is a drug frequently prescribed as an antidepressant [24]. Interestingly, in hippocampal isolated nerve endings sertraline acted as an efficient inhibitor of Na^+ channel mediated responses including Glu release [25]. Also the synthetic derivative of vincamine, vinpocetine (ethyl apovincamine-22-oate), that

is a natural product contained in the leaves of the periwinkle plant *Vinca minor*, and a novel third generation anti-seizure drug [26], inhibited the rise in cytoplasmic Na^+ and Glu release induced by depolarization in brain isolated nerve endings [1–3, 27–29].

Brain ion channels are among the most important targets of various antiepileptic drugs. In previous studies we have demonstrated the anti-seizure capability of both sertraline and vinpocetine [5, 9, 30]. In the present study, the effects of these drugs on the epileptiform activity induced by 4-AP were compared with the effects of an array of classic and newer antiepileptic drugs, including carbamazepine, phenytoin, valproic acid, oxcarbazepine, lamotrigine, topiramate and levetiracetam.

Materials and Methods

Source of Materials

4-AP was from Sigma Chemical Co. (St. Louis, MO). Sertraline, vinpocetine (eburnamenine-14-carboxylic acid ethyl ester), carbamazepine, lamotrigine, topiramate oxcarbazepine, phenytoin (5,5-diphenylhydantoin sodium salt), magnesium valproate and levetiracetam were donated by Psicofarma S.A. de C.V. (México).

Approval of the Study Protocol

Animal experiments were carried out in compliance with the Guidelines for Animal Experimentation and with the approval of the "Laboratory Animals Care and Use Committee".

Experimental Animals

Sixty-five male Wistar rats weighting 309 ± 9 g were used to obtain the results presented here. Control animals were injected (*i.p.*) either with saline, acidified saline, 70 % saline containing 30 % DMSO or DMSO alone, which were the vehicles used to dissolve the drugs tested. To verify that the 4-AP solution was capable to induce the usual epileptiform activity, in each experiment a control animal administered with one of the vehicles used was included. Since from previous studies we knew that none of the vehicles used here was able to prevent the appearance of the characteristic epileptiform activity induced by 4-AP only 11 animals were used as controls. In one experiment, we studied an average of four rats per experiment. Thus, in some of the last experiments, the capability of the 4-AP solution to induce the usual epileptiform activity was verified in an animal administered with an anti-epileptic drug at a low dose, which we knew does not prevent the epileptiform activity to 4-AP.

The experimental animals were injected with the drug to be tested at a specific dose. The relevance of the doses chosen is explained in Tables 2, 3 and 4. Experimental animals were pre-administered with sertraline at a dose of 0.75 mg/kg or at a dose of 2.5 mg/kg 4 h before exposure to 4-AP. We chose this time because sertraline has been reported to reach a high concentration in rat brain tissue after this time [31]. The animals pre-administered with vinpocetine (2.5 mg/kg) were injected with this drug 1 h before exposure to 4-AP. The other experimental animals pre-administered either with phenytoin, lamotrigine or oxcarbazepine at a dose of 2.5 mg/kg; carbamazepine, phenytoin, lamotrigine, oxcarbazepine or topiramate a dose of 25 mg/kg, and valproate or levetiracetam at a dose of 100 mg/kg were injected 2 h before exposure to 4-AP.

The animals pre-administered with the vehicle used to dissolve sertraline (70 % saline/30 % DMSO) were injected 4 h before exposure to 4-AP. The animals pre-administered with the acidified saline (pH 3.5), which was the vehicle used to dissolve vinpocetine were injected 1 h before exposure to 4-AP. The animals pre-administered with saline or DMSO, that were the other two vehicles used to dissolve the rest of the drugs tested were injected 2 h before exposure to 4-AP.

All the drugs were administered *i.p.* at a small volume. Namely, 0.3 ml per 300 g of animal. In the case of the animals injected with the antiepileptic drugs that were dissolved in the organic solvent, DMSO, the volume used was smaller (i.e. 0.15 ml/300 g of animal).

EEG Recordings

The cortical excitability was evaluated with a Nihon-Kohden Neuropack IV Mini (MEB-5304K) system. To get the EEG recordings, the animals were first anaesthetized with ketamine/xylazine (50/10 mg/kg *i.p.*) to restrain movement, stress and muscular activity. Then needle electrodes were rapidly placed subcutaneously over the left temporal area (reference electrode) and over the left frontal area between the midline and the arched portion of the orbital crest (active electrode). Once the 2 min EEG baseline recording was obtained, the animals were gently twisted and injected *i.p.* with 4-AP (2.5 mg/kg). Then three additional recordings, each one of 2 min, starting at minutes 9, 19 and 29 following 4-AP were obtained in sets of twelve EEG recordings (10 s each). The recordings of each animal were stored in a floppy disk for further analysis.

EEG Highest Peak Amplitude Value (HPAV)

In order to quantify the changes in the EEG before and after the injection of the convulsive agent in all the animals pre-administered with vehicle or the drug to be tested at a

specific dose, the EEG-HPAV was measured. Four HPAV were obtained in each animal. One baseline value before 4-AP and three at three time intervals (i.e. min 9–11, min 19–21 and min 29–31) following 4-AP. These intervals were chosen because for the first half-hour following 4-AP all animals exhibit similar EEG changes and do not leave the anesthetized state. For obtaining the EEG-HPAV the main positive to negative non interrupted peaks (in μV) obtained in twelve consecutive EEG recordings (10 s each) for the 2 min were averaged. This practical and objective method initially reported in Nekrassov and Sitges [7] with mild modifications that improved it, allows comparison of the EEG changes using exactly the same experimental paradigm in all the animals.

Statistical Analysis

One-way ANOVA followed by a post hoc Tukey test was used for the statistical evaluations. The criterion for statistical significance was $p < 0.05$.

Results

Effects of Sertraline, Vinpocetine and Antiepileptic Drugs on the EEG Epileptiform Activity Induced by 4-AP

Representative EEG recordings taken before and at the three intervals following 4-AP in the animals pre-administered with the different vehicles or with a specific drug at a specific dose are shown in Figs. 1, 2, 3 and 4.

A representative recording of the epileptiform EEG activity induced by 4-AP in a control animal pre-administered with 70 % saline/30 % DMSO, that was the vehicle used to dissolve sertraline, is shown in Fig. 1a. Representative recordings of the cortical EEG activity before and following 4-AP in animals pre-treated with 0.75 or 2.5 mg/kg sertraline are shown in Fig. 1b, c, respectively. In these recordings the effectiveness of sertraline at the dose of 2.5 mg/kg to suppress the epileptiform EEG activity induced by 4-AP contrasts with the failure of the lower sertraline dose (0.75 mg/kg) to do so.

In Fig. 2 representative recordings of the epileptiform EEG activity induced by 4-AP in animals pre-administered with vinpocetine, phenytoin, lamotrigine or oxcarbazepine at a dose of 2.5 mg/kg are shown. Notice that at this dose only vinpocetine was able to prevent the epileptiform EEG activity induced by 4-AP. The other three clinical established antiepileptic drugs failed to prevent the epileptiform EEG activity induced by 4-AP.

The epileptiform activity induced by 4-AP in a control animal pre-administered with DMSO, that was the vehicle used to dissolve carbamazepine, phenytoin, oxcarbazepine and topiramate, is shown in Fig. 3a. The other recordings included in Fig. 3 show the cortical activity induced by 4-AP in animals pre-administered with carbamazepine, phenytoin, lamotrigine, oxcarbazepine or topiramate at a dose of 25 mg/kg. At this dose, carbamazepine, phenytoin, lamotrigine and oxcarbazepine effectively prevented the epileptiform EEG activity induced by 4-AP; however, topiramate did not.

The epileptiform activity induced by 4-AP in a control animal pre-administered with saline, that was the vehicle used to dissolve valproic acid, levetiracetam and lamotrigine, is shown in Fig. 4a. Representative recordings in animals pre-administered with 100 mg/kg valproic acid or

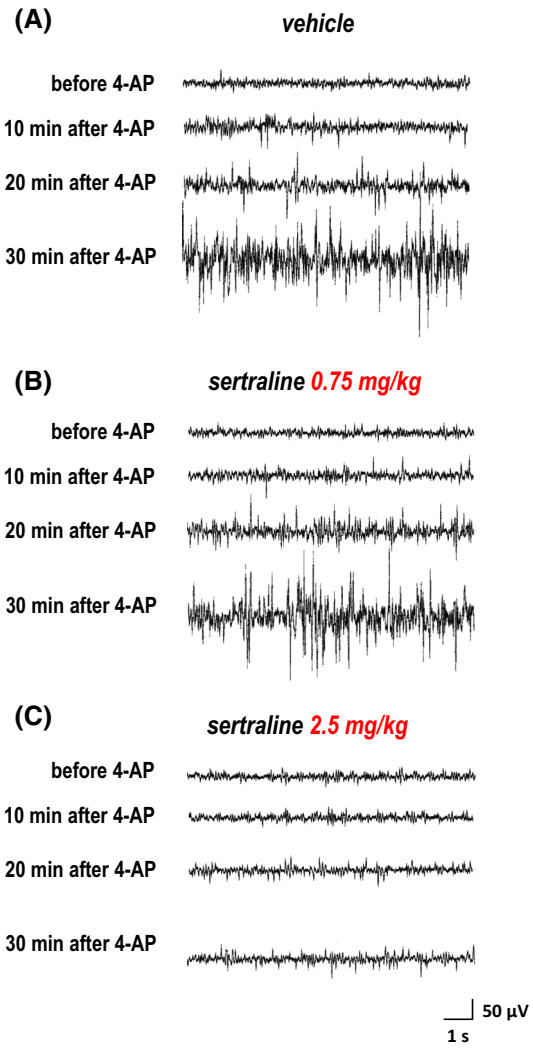

Fig. 1 Effect of sertraline on the epileptiform EEG activity induced by 4-AP in the rat. EEG recordings before and at the indicated times following 4-AP administration at the convulsive dose (2.5 mg/kg *i.p.*) in representative animals pre-administered with: **a** vehicle (70 % saline/30 % DMSO), **b** 0.75 mg/kg sertraline and **c** 2.5 mg/kg sertraline

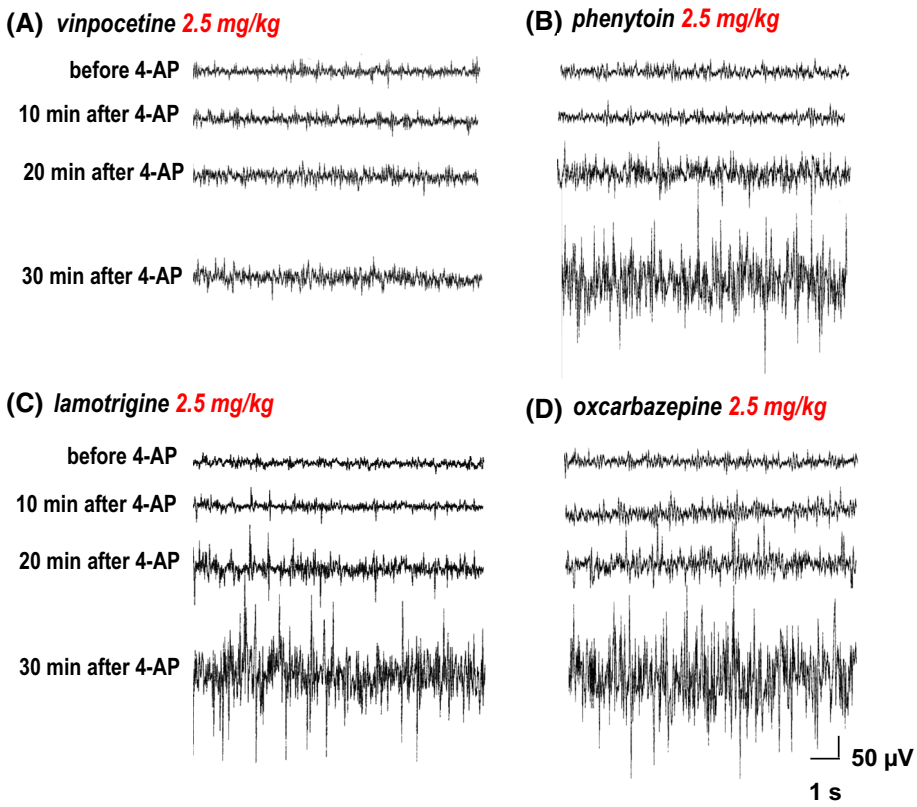

Fig. 2 Effects of vinpocetine and several antiepileptic drugs at a dose of 2.5 mg/kg on the epileptiform activity induced by 4-AP. EEG recordings before and at the indicated times following 4-AP in representative animals pre-administered with 2.5 mg/kg of: **a** vinpocetine, **b** phenytoin, **c** lamotrigine or **d** oxcarbazepine

levetiracetam are shown in Fig. 4b, c, respectively. At this dose these antiepileptic drugs failed to prevent the epileptiform EEG activity induced by 4-AP.

A recording of an animal injected with acidified saline, that was the vehicle used to dissolve vinpocetine, was not shown, but is similar to the recordings taken in control animals injected with the other vehicles.

Effects of Sertraline, Vinpocetine and the Antiepileptic Drugs on the Changes in the EEG-HPAV Induced by 4-AP

In order to obtain an objective measure of the effect of each drug on the EEG changes induced by 4-AP in the different animal groups studied, the mean ± SEM of the EEG-HPAV obtained in the recordings taken before and at the three intervals following 4-AP were calculated in the 65 animals. Table 1 shows that in the animals pre-administered with vehicles, the EEG-HPAV only increased slightly at the first interval (min 9 to 11) following 4-AP. At the second interval (min 19–21) following 4-AP, the increase in EEG-HPAV was marked, and at the third interval (min 29–31) following 4-AP reached a maximum. In the animals pre-administered with 0.75 mg/kg sertraline, 2.5 mg/kg phenytoin, 2.5 mg/kg lamotrigine or 2.5 mg/kg oxcarbazepine, 25 mg/kg topiramate, 100 mg/kg valproate or 100 mg/kg levetiracetam, this pattern was practically unchanged. In contrast, in the animals pre-administered with 2.5 mg/kg sertraline, 2.5 mg/kg vinpocetine, 25 mg/kg carbamazepine, 25 mg/kg phenytoin, 25 mg/kg lamotrigine or 25 mg/kg oxcarbazepine, the EEG-HPAV at the three intervals following 4-AP was maintained around the respective baseline control value, indicating a strong anti-seizure action.

Relevance of the mg/kg Doses Tested in the Rat to Control the 4-AP Induced Epileptiform EEG Changes with Regard to the Effective Doses Prescribed to Humans Calculated in mg/kg

In order to compare the doses in mg/kg administered here to the rat with the daily doses of antiepileptic drugs prescribed to epileptic patients to control seizures, or in the case of sertraline, the doses prescribed to psychiatric patients to control depression, we calculated the daily doses prescribed to humans in mg/kg for a 60 kg person. The first column in Tables 2 and 3 show the name of the drug. The second column shows the dose in mg/kg. The third column indicates whether, at that dose, the drug was able to prevent the epileptiform cortical activity induced by 4-AP, the fourth column in Tables 2 and 3 show the latency to the conspicuous epileptiform activity, usually observed near 20 min following 4-AP.

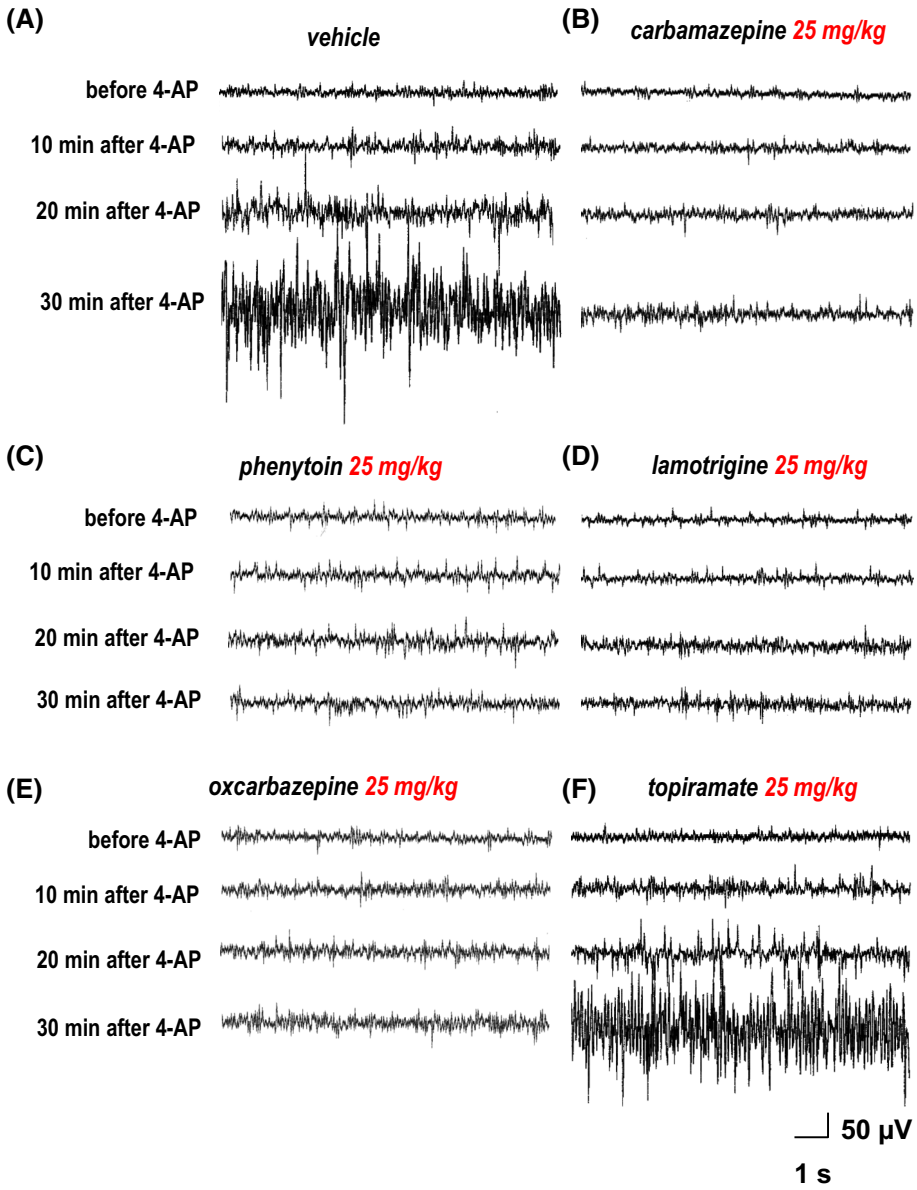

Fig. 3 Effects of several antiepileptic drugs at a dose of 25 mg/kg on the epileptiform activity induced by 4-AP. EEG recordings before and at the indicated times following 4-AP in representative animals pre-administered with: **a** vehicle (DMSO) or with 25 mg/kg of: **b** carbamazepine, **c** phenytoin, **d** lamotrigine, **e** oxcarbazepine or **f** topiramate

The supplementary table constructed (right side of Tables 2, 3) includes a reasonable clinical range of daily doses prescribed to patients in mg/day (for vinpocetine [32]). In the last column of this supplementary table, the doses prescribed to patients were calculated in mg/kg for a 60 kg person, so they can be compared with the mg/kg doses administered here to the rat.

Calculation of the Human Equivalent Doses

Extrapolation of an animal dose to a human equivalent dose (HED) by simple conversion based on body weight is reported to be less accurate than extrapolation based on weight and body surface area [33]. Thus, we calculated the HED using the body surface area normalization formula reported by Reagan-Shaw to convert the drug doses used in the animal to the human.

The first column of Table 4 shows the name of the drug administered to the rat, the second column the dose in mg/kg; the third column shows the values that arise from multiplying the mg/kg injected into the rat by 0.162, a constant representing the ratio of K_{rat} (6) to K_{human} (37), as described [33]. Finally, to obtain the HED (in mg per day) data in the previous column were multiplied by 60, since the formula was constructed for a 60 kg person.

In the last column at the right in Table 4 the range of doses prescribed to the patients were again included to facilitate comparison. Notice that using this method the doses of the drugs capable to prevent the epileptiform EEG changes in the present study were within or close to the

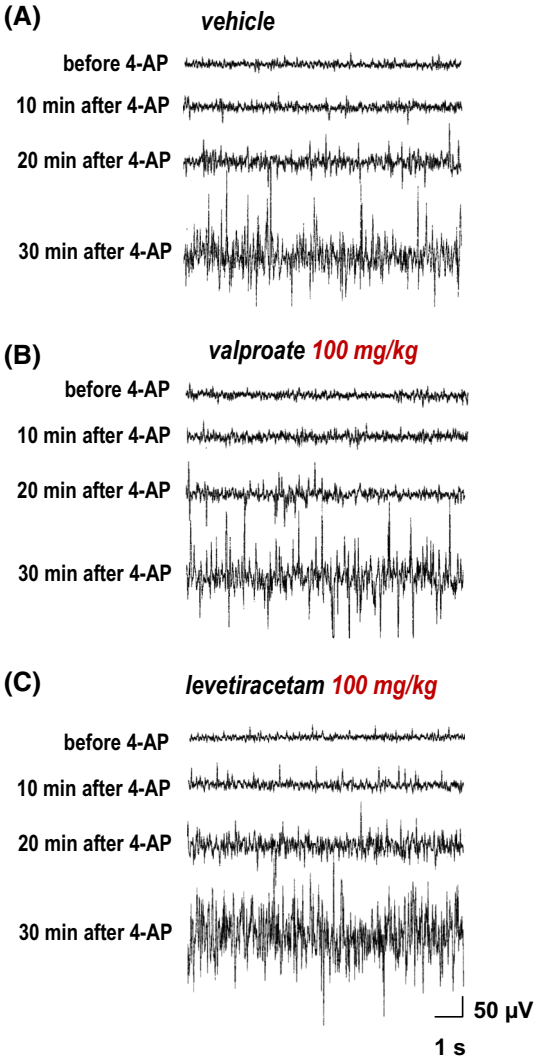

Fig. 4 Effects of valproic acid and levetiracetam on the epileptiform activity induced by 4-AP. EEG recordings before and at the indicated times following 4-AP in representative animals pre-administered with **a** vehicle (saline) or with 100 mg/kg of: **b** valproic acid or **c** levetiracetam

minimal dose prescribed to patients. Sertraline displayed anti-seizure efficacy even below the minimal antidepressant prescribed dose.

Discussion

In the present study the EEG epileptiform activity induced by 2.5 mg/kg 4-AP was used to compare the anti-seizure efficacy of the clinically established antidepressant sertraline, the new anti-seizure drug vinpocetine and several of the most commonly prescribed antiepileptic drugs at therapeutically meaningful doses, according to the extrapolated data supplied in Tables 2, 3 and 4.

The dose of 4-AP used here (2.5 mg/kg) has been selected on the basis of our previous work showing that at this dose, 4-AP is not lethal and induces reproducible EEG epileptiform changes for the first half-hour and before the animals begin to emerge from anesthesia [5, 9, 34]. This standardized experimental approach, that allowed to test the anti-seizure efficacy of anti-epileptic drugs at clinically meaningful doses on the same reproducible pattern of changes, revealed that not all the clinically established antiepileptic drugs were able to prevent the EEG epileptiform activity induced by 4-AP.

Here we calculated the HPAV from the EEG recordings obtained in the anaesthetized animal. HPAV provides a quantitative comparison of the effects of the different drugs on the epileptiform activity induced by the pro-convulsive agent 4-AP. However it is worthy to mention that in previous studies we found that some of the drugs that were effective inhibitors of the EEG epileptiform activity induced by 4-AP in the anesthetized animal, were also effective inhibitors of seizures induced by 4-AP and other pro-convulsive agents in the non-anesthetized animal [9, 34].

Based on the doses needed to inhibit the EEG epileptiform activity induced by 2.5 mg/kg 4-AP in the rat, the drugs tested here can be divided in three groups. One group composed of sertraline and vinpocetine, that effectively inhibited the EEG epileptiform activity induced by 4-AP at a dose of 2.5 mg/kg. A second group composed of lamotrigine, oxcarbazepine, carbamazepine and phenytoin that inhibit the epileptiform activity at a higher dose (25 mg/kg), and a third group composed of topiramate (25 mg/kg), and valproate and levetiracetam (100 mg/kg) that were unable to inhibit the EEG epileptiform activity induced by 4-AP. Nonetheless it is worthy to mention that topiramate, valproate and levetiracetam, that were unable to inhibit the EEG epileptiform activity induced by 4-AP are useful clinically. May be by diminishing the cerebral excitability induced by changes in other pro-convulsive neurochemical pathways not involved in the pro-convulsive mechanism of action of 4-AP.

The doses used in the present study are meaningful, because they are near or within the suggested ranges of doses daily prescribed to human patients [35], although we acknowledge the variability in dose ranges based upon factors such as refractoriness, new unset seizures, age, multi-therapy, etcetera. For instance, the dose of 2.5 mg/kg administered to the rat would be equivalent to prescribe 150 mg/day sertraline or vinpocetine to a human weighting 60 kg. The 25 mg/kg dose, to prescribe carbamazepine, phenytoin, lamotrigine, oxcarbazepine or topiramate at a dose of 1.5 g/day to a 60 kg person, and the highest dose of 100 mg/kg, that was used to test the effect of valproic acid

Table 1 Effect of 4-AP on the EEG highest peak amplitude value (HPAV) in animals pre-administered with vehicle (control) or with the indicated drugs at the specified doses

Drug	Dose in mg/kg	Baseline HPAV	HPAV following 4-AP			Number of animals	Weight (g)
			9–11 min	19–21 min	29–31 min		
Control[a]		35 ± 0.5	44 ± 0.9	167 ± 3.0	269 ± 4.6	(11)	307 ± 7
Sertraline	0.75	35 ± 0.6	42 ± 1.5	175 ± 3.0	282 ± 16	(4)	295 ± 5
	2.50	35 ± 0.9	37 ± 0.3*	43 ± 1.2*	52 ± 2.1*	(4)	303 ± 5
Vinpocetine	2.50	35 ± 0.6	36 ± 0.4*	44 ± 2.9*	55 ± 7.8*	(4)	312 ± 6
Carbamazepine	25.00	34 ± 0.7	38 ± 1.4*	41 ± 1.8*	50 ± 2.7*	(4)	314 ± 5
Phenytoin	2.50	33 ± 0.4	42 ± 0.6	168 ± 3.6	281 ± 7.7	(4)	314 ± 10
	25.00	34 ± 0.4	38 ± 1.4*	45 ± 1.2*	53 ± 0.4*	(4)	330 ± 8
Lamotrigine	2.50	37 ± 0.8	48 ± 1.1	163 ± 4.2	272 ± 15	(4)	306 ± 15
	25.00	35 ± 1.3	40 ± 1.0*	47 ± 1.4*	46 ± 0.6*	(4)	309 ± 13
Oxcarbazepine	2.50	33 ± 0.2	43 ± 0.5	160 ± 6.5	271 ± 4.1	(4)	312 ± 11
	25.00	33 ± 1.0	40 ± 1.6	46 ± 2.9*	48 ± 3.6*	(4)	311 ± 8
Topiramate	25.00	36 ± 0.2	47 ± 0.7	170 ± 15	271 ± 11	(4)	335 ± 5
Valproate	100.00	37 ± 1.1	46 ± 1.9	178 ± 4.9	277 ± 7.9	(5)	299 ± 6
Levetiracetam	100.00	35 ± 0.4	41 ± 1.8	161 ± 5.2	264 ± 8.3	(5)	305 ± 6

in μV

Baseline HPAV refers to the HPAV registered for 2 min before 4-AP administration

Results are the mean ± SEM values of the number of animals indicated in parenthesis

* $p < 0.05$ between control and the indicated experimental group, before and at the indicated time interval after 4-AP

[a] Animal group injected with vehicles used to dissolve the anticonvulsive drugs

Table 2 Effectiveness of the doses used (in mg/kg) to prevent the 4-AP-induced epileptiform EEG activity in the rat

Drug doses used in rats exposed to 4-AP				
Drug	mg/kg	Prevention of the EEG epileptiform changes to 4-AP	Latency in min to the epileptiform activity	Number of animals
Vehicles		No	20 ± 2	(11)
Sertraline	0.75	No	20 ± 3	(4)
	2.5	Yes	No EEG changes	(4)
Vinpocetine	2.5	Yes	No EEG changes	(4)
Carbamazepine	25	Yes	No EEG changes	(4)
Phenytoin	2.5	No	26 ± 4	(4)
	25	Yes	No EEG changes	(4)
Lamotrigine	2.5	No	24 ± 2	(4)
	25	Yes	No EEG changes	(4)
Oxcarbazepine	2.5	No	25 ± 2	(4)
	25	Yes	No EEG changes	(4)
Topiramate	25	No	22 ± 3	(4)
Valproate	100	No	23 ± 2	(5)
Levetiracetam	100	No	25 ± 2	(5)

No EEG changes refer to no conspicuous changes in the EEG and absence of epileptiform activity

and levetiracetam, would be equivalent to prescribe 6 g to a 60 kg patient per day.

In a previous study, we found that the EEG epileptiform changes induced by pentylenetetrazole in the guinea pig were effectively prevented by valproate even at a lower (30 mg/kg) dose [35], than the dose of 100 mg/kg that here failed to prevent the epileptiform EEG activity induced by 4-AP in the rat. The different mechanisms underlying the

pro-convulsive action of 4-AP and pentylenetetrazole might explain this apparent controversy. 4-AP pro-convulsive action involves changes in several ion channels permeability [12, 36]. The pro-convulsive mechanism of action of pentylenetetrazole is linked to a decrease in GABAergic transmission [37], and valproic acid anti-seizure mechanism of action is mainly due to an increase in GABAergic transmission [38, 39].

In previous studies the effect of various antiepileptic drugs, including some of those tested here, has been also tested on lethality induced by 4-AP [4, 40]. However, when 4-AP is administered at an extremely high dose, the antiepileptic drugs have to be administered to the animal at higher doses than the therapeutic equivalent doses prescribed to humans in order to detect their effects. The value of measuring the epileptiform activity induced by a non-lethal 2.5 mg/kg 4-AP dose to unmask the participation of ionic channels in the mechanism of action of an anti-seizure drug becomes strengthened when the more accurate daily HED, which takes into account the body surface area to normalize comparison between animal and human doses, is used (see Table 4). When this technique was used to calculate the doses administered to the rat, the effectiveness of sertraline, vinpocetine, carbamazepine, phenytoin, lamotrigine and oxcarbazepine to inhibit the epileptiform activity induced by 4-AP at doses below the minimal doses prescribed to epileptic patients becomes evident. In contrast, topiramate, valproate and levetiracetam were ineffective at HED within the range prescribed to epileptic patients.

In agreement with the present study, when administered at doses of 100–300 mg/kg, valproate also failed to inhibit seizures elicited by the injection of 4-AP to the CA3 hippocampal region of the rat. However, at doses quite higher than those prescribed to epileptic patients, valproic acid had some effect on seizures triggered by 4-AP [41]. Thus, to determine whether the inhibition of presynaptic ion channels is particularly involved in the anti-seizure mechanism of action of a drug, it is important to test the drug at a therapeutic relevant dose.

The present experimental method also revealed that only those drugs that were shown to inhibit cerebral presynaptic Na^+ and/or Ca^2 channels mediated responses in cerebral isolated nerve endings [1–3, 13, 25], were able to prevent the EEG epileptiform activity induced by 2.5 mg/kg 4-AP.

Table 3 Daily human equivalent Dose calculated in mg/kg for a human weighting 60 kg

Drug dose used in epileptic patients		
Drug	mg/day	mg/kg/day (60 kg subject)
Sertraline[a]	50–200	0.8–3
Vinpocetine[b]	120	2
Carbamazepine	400–1600	7–27
Phenytoin	200–600	3–10
Lamotrigine	200–1600	3–27
Oxcarbazepine	600–1200	10–20
Topiramate	200–400	3–7
Valproate	600–2600	10–43
Levetiracetam	1000–3000	17–50

[a] Or in depressive patients for the case of Sertraline

[b] Daily effective dose in refractory epileptic patients

Table 4 Daily human equivalent doses calculated using the body surface area normalization method

	Rat (mg/kg)	$K_{rat}/K_{human} = 6/37$	HED (mg) 60 kg	Prevents EEG changes	HD mg/day
Sertraline	0.75	0.12	7		
	2.5	0.41	24	✔	50–200
Vinpocetine	2.5	0.41	24	✔	30–150
Carbamazepine	25	4.05	243	✔	400–1600
Phenytoin	2.5	0.41	24		
	25	4.05	243	✔	200–600
Lamotrigine	2.5	0.41	24		
	25	4.05	243	✔	200–1600
Ox-carbazepine	2.5	0.41	24		
	25	4.05	243	✔	600–1200
Topiramate	25	4.05	243		200–400
Valproate	100	16	973		600–2600
Levetiracetam	100	16	973		1000–3000

The HED in mg/kg = rat dose (mg/kg) × K_{rat}/K_{human}

HD range of human doses given to epileptic patients

HED (mg) human equivalent dose in mg

✔ Prevention of the EEG epileptiform activity induced by 4-AP in the rat

Moreover, there is a parallelism between the potencies of sertraline, vinpocetine, carbamazepine, phenytoin, lamotrigine and oxcarbazepine to inhibit glutamate release induced by activation of Na^+ and Ca^{2+} channels in cerebral nerve endings, and their potencies to inhibit the increase in the EEG epileptiform activity induced by 4-AP. This parallelism strongly suggests that a decrease in cerebral presynaptic Na^+ and Ca^{2+} channel permeability plays an important role in the capability of these drugs to prevent seizures. In addition, our findings that the epileptiform activity induced by 4-AP was insensitive to antiepileptic drugs like valproate and topiramate, that were unable to inhibit presynaptic ion channels mediated responses in a wide range of concentrations [1, 2], indicate that the main mechanism of action underlying the anti-seizure action of these drugs is unrelated to a decrease in presynaptic Na^+ and/or Ca^{2+} channels permeability.

4-AP main mechanism of action is associated with inhibition of delayed rectifier voltage-dependent K^+ channels. In cerebral isolated nerve endings the decrease in K^+ channels permeability induced by 4-AP is accompanied by an increase in presynaptic Na^+ and Ca^{2+} channels permeability [12]. Consistently the epileptiform activity induced by 4-AP is particularly sensitive to drugs whose mechanism of action involves changes in channels permeability. Nevertheless, since the increase in brain excitability triggered by other means, like a reduced GABAergic transmission, also may activate Na^+ channels indirectly, drugs like vinpocetine and sertraline, that are potent inhibitors of cerebral presynaptic Na^+ channels, although at higher doses, also prevent seizures induced by pentylenetetrazole [9, 30, 42]. In contrast, present data indicate that antiepileptic drugs whose mechanism of action does not involve a direct change in brain presynaptic channels permeability at clinically meaningful doses are unable to prevent the epileptiform EEG changes induced by 4-AP.

In summary, present results indicate that the antidepressant sertraline and the anti-seizure drug vinpocetine are particularly effective in preventing seizures triggered by alterations in cerebral presynaptic voltage sensitive ion channels. Another implication that arises from present findings is that the sensitivity of the epileptiform activity induced by 4-AP to a drug, is a valuable experimental tool to determine whether changes in channels permeability, and particularly in cerebral presynaptic Na^+ and/or Ca^{2+} channels, underlie the anti-seizure action of a drug.

Conclusion

The present study demonstrates, firstly that the epileptiform activity induced by a non-lethal dose of the pro-convulsive agent 4-AP can be used to unmask the participation of cerebral presynaptic ionic channels in the anti-seizure mechanism of action of new anti-epileptic drugs. Secondly, that the HPAV can be used as an objective method to compare the effects of anti-seizure drugs at human equivalent doses. Finally, using this approach the anti-depressant sertraline and the novel anti-epileptic drug vinpocetine were revealed to inhibit the epileptiform activity induced by 4-AP in the animal in vivo at lower doses than several classic antiepileptic drugs.

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Compliance with Ethical Standards

Conflict of interest The authors declare that they have no conflicts of interest.

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