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Antiepileptic Drugs—Their Effects on Kindled Seizures and Kindling-Induced Learning Impairments

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BECKER, A., G. GRECKSCH, AND M. BROSZ. *Antiepileptic drugs—Their effects on kindled seizures and kindling-induced learning impairments.* PHARMACOL BIOCHEM BEHAV **52**(3) 453–459, 1995.—Many epileptic patients suffer from cognitive impairments. These impairments may be a consequence of the epileptogenic process and/or antiepileptic medication. Kindling is considered a useful experimental model to investigate drug effects on both the convulsive component of epilepsy and related alterations at the behavioral level. In our experiments, kindling was induced by repeated injections of pentylenetetrazol (PTZ). To test the effect of antiepileptic drugs on kindled seizures and kindling-induced learning deficits we injected ethosuximide, dipropylacetate, and phenobarbital prior to each kindling stimulation or after kindling completion, and tested these animals in a shuttle-box paradigm. Dipropylacetate and phenobarbital suppressed the development of motor seizures and counteracted the learning deficit. Although ethosuximide had a clear effect on kindled seizures, the learning deficit occurred in kindled rats. This suggests that AEDs effects on kindled seizures are not correlated with the elemination of deficits in the field of cognition.

Kindling Pentylenetetrazol Learning Ethosuximide Dipropylacetate Phenobarbital Rats

HIGH rates of psychological, emotional, and educational alterations have been observed among individuals suffering from epilepsy. It was reported (24) that more than half of the epileptics they sampled had some sort of psychological or social problems with behavioral manifestations. Moreover, fewer than one in four individuals with epilepsy was free of intellectual, neurological, and behavioral problems. These abnormalities are related to multiple factors, including seizure type, age of onset, location of the focus, seizure frequency, and type of EEG pattern (6).

According to (5), generalised 3 Hz spike wave bursts lasting at least 3 s are most likely to produce demonstrable transitory cognitive impairments, but they can also be found during briefer and even focal discharges. Other investigations have shown that subclinical epileptiform discharges in the EEG of epileptics are accompanied by transitory cognitive impairments (17).

Another factor affecting cognition is antiepileptic drug (AED) medication. Although it is understood that the beneficial results of seizure suppression are of great clinical importance, there are indications of cognitive side effects of the substances administered at therapeutic doses (1,12,13,18). Nichols et al. (21) reported that AED-induced cognitive decline occurs especially during polytherapy. The authors concluded that cognitive side effects of AED medication are just beginning to be understood.

Kindling offers several unique opportunities to study pathophysiologic mechanisms of epileptogenesis. This animal model represents the convulsive component of epilepsy as well as secondary alterations in the field of cognition. Recently, it was demonstrated that two-way active avoidance learning (shuttle-box) was disrupted in pentylenetetrazol (PTZ)-kindled rats (2). The extent of this learning deficit was dependent on seizure severity, the number of stimulations, and the learning model. It was still detectable after a stimulation-free period of 4 weeks. Taken together, it might be speculated that these findings mirror aspects of human epilepsy underlining the clinical relevance of this experimental model.

The learning deficit in PTZ-kindled rats occurred even after seizure suppression with high-dosed diazepam (3). It was

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assumed that motor seizure suppression does not automatically result in the prevention of learning deficits. On the other hand, we have demonstrated that the learning deficits in kindled rats could be prevented by treatment with gangliosides (8) or the opiate antagonist naloxone (4) without any effect on seizure development. Therefore, it seems to be necessary to test common AED and potential AED for their effectiveness in seizure suppression without affecting mental processes.

In a first attempt we injected the clinically used AEDs dipropylacetate (DPA), ethosuximide (ETH), and phenobarbital (PB) in the course of kindling development (prior to each kindling stimulation) and after kindling completion (prior to each learning test), respectively, to study their effects on seizure occurrence and kindling-related learning deficits.

METHOD

Animals

Experiments were carried out with male Wistar rats (Mol : Wist(Shoe), Møllegaard Breeding Centre Deutschland GmbH) aged 8 weeks at the beginning of the experiments. The animals were kept under controlled laboratory conditions under a lighting regime of 12 L : 12 D (lights on at 0600 h), temperature 20 ± 2°C, and air humidity 55–60%. They had free access to commercial rat pellets (Altromin 1326) and tap water. The rats were housed in groups of five per cage.

Kindling Induction

For PTZ kindling an initially subconvulsant PTZ dose of 40 mg/kg body weight was injected intraperitoneally once every 48 h. After each injection the convulsive behavior was observed for 20 min. The resultant seizures were classified as follows—stage 0: no response; stage 1: ear and facial twitching; stage 2: myoclonic jerks without upright position; stage 3: myoclonic jerks, upright position with bilateral forelimb clonus; stage 4: clonic–tonic seizures; stage 5: generalised clonic–tonic seizures, loss of postural control.

The animals were considered to be kindled after having received 10 PTZ injections and after having reached at least three consecutive stage-4/5 seizures.

Control animals received the same number of saline injections.

Drugs

In order to test the effects of clinically used AEDs the following substances were administered: ethosuximide (Sigma E-7138) 50 mg/kg or 250 mg/kg; dipropylacetate (Arzneimittelwerk Dresden) 10 mg/kg or 100 mg/kg; phenobarbital (Lepinal®, Arzneimittelwerk Dresden) 12.5 mg/kg or 25 mg/kg.

Doses were injected according to their acute anti-PTZ (60 mg/kg IP) effect tested in a separate group of rats. For that purpose the AEDs were given 30 min prior to PTZ and the animals were observed for 20 min for the occurrence of clonic–tonic seizures. Further groups of rats were used to test drug effects on locomotor activity. Thirty minutes after application the animals were placed on an optoelectronic device for 20 min. Doses for the kindling experiment were selected in such a way that a dose without suppression of PTZ-induced seizures was used and one that was effective in suppressing this type of seizures and without effects on locomotion.

Drugs were injected at a volume of 10 ml/kg intraperitoneally 30 min prior to the convulsant (series A) or 30 min prior to each learning session (series B). Control rats were injected with the solvent physiological saline.

Experimental Design

To investigate the effect of AEDs on both kindling development and related learning deficits two sets of experiments were performed: a) the AED was administered prior to each convulsant injection and the learning performance was measured in a drug-free stage (determination of drug action on the development of kindling and related learning deficits); b) the animals were kindled and substance administration was 30 min prior to each learning session (determination of drug action on learning in kindled rats).

The learning experiment was performed 24 h after the last kindling stimulation.

Two-Way Active Avoidance Learning—Shuttle-Box

The automatic shuttle-box was divided into two compartments (0.25 × 0.25 × 0.6 m) separated by a 5 cm hurdle. The conditioned stimuli were light (40 W bulbs located on the central ceiling of each compartment) and a sound produced by a buzzer. The unconditioned stimulus was an electric foot shock of max. A 1 mA shock (shock levels were individually titrated for each animal, shock intensity was below vocalization threshold, 80% of the animals were trained at 0.5–0.7 mA) was delivered through stainless steel rods covering the floor. The conditioned stimuli–unconditioned stimulus interval was 4 s. One trial was limited to 20 s if the animal failed to react before that time. Each session consisted of 20 trials and was repeated on 4 consecutive days. Sessions were performed during the light part of the 12 L : 12 D cycles at about the same time ± 1 h. Prior to the first session, the rats were allowed to explore the box for 5 min, and on the following days, 1 min was provided.

The number of escapes (reaction time > 4 s) and conditioned reactions (reaction time < 4 s) was recorded for further evaluation.

Statistics

To evaluate the development of seizures in the course of kindling development, and the learning performance of the animals of the six experimental groups, the repeated measurement model was applied.

The basis of statistical decision was a significance level of 0.05. The calculations were carried out by means of SPSS/PC+ software (procedure ANOVA and MANOVA).

RESULTS

Repeated administration of initially subeffective PTZ doses resulted in increasing convulsive activity culminating in generalized clonic–tonic seizures (Fig. 1).

AEDs exerted dose-dependent effects on seizure activity. In comparison to controls, ETH suppressed kindled seizures significantly, $F(2, 37) = 361.22$, $p < 0.001$, and both doses differed in their strength of action, $F(18, 18) = 3.48$, $p = 0.002$, Greenhouse-Geisser adjusted (Fig. 1).

Equivalent effects were observed using DPA. Both doses had significant effects on kindled seizures, $F(2, 67) = 10.86$, $p < 0.001$, and the difference between both doses is $F(18, 25) = 2.54$, $p < 0.004$ (Fig. 1).

Similar to ETH, PB counteracted kindled seizures, $F(2, 34) = 103.56$, $p < 0.001$, and the difference between both doses of this substance is $F(18, 29) = 5.2929$, $p = 0.001$ (Fig. 1).

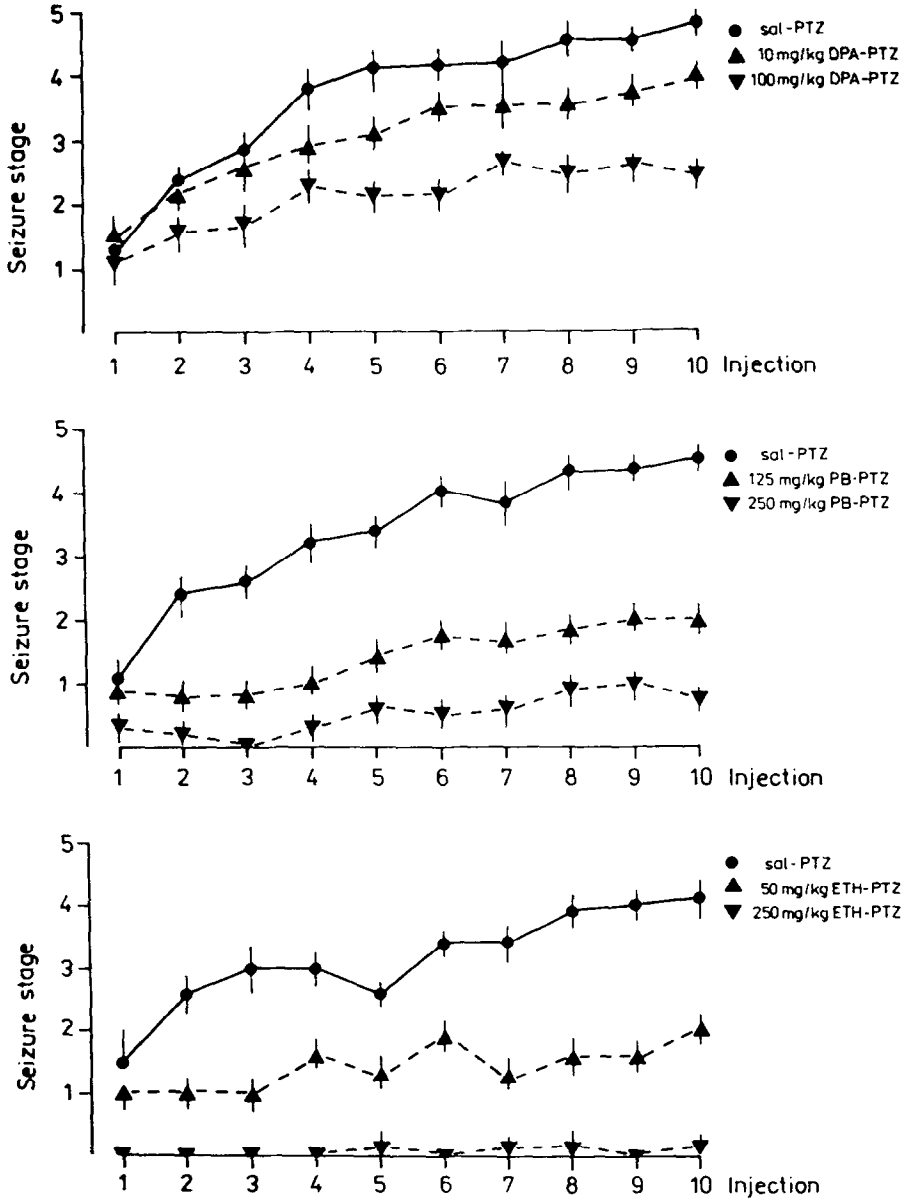

FIG. 1. The effect of Dipropylacetate (DPA), Phenobarbital (PB), and Ethosuximide (ETH) pretreatment on the development of pentylenetetrazol-kindled seizures. Substances were injected 30 min prior to the convulsant. Number of animals used, see Figs. 2-4. Mean ± SEM.

In comparison to saline-injected controls, in each experiment PTZ-kindled rats exhibited a significantly diminished performance in shuttle-box learning ($p < 0.05$). Interestingly, AEDs developed specific effects on learning, depending on both the substance as well as the administration schedule. The experimental groups did not differ in learning the instrumental reaction; the number of motor reactions (escape + avoidance reactions) was nearly identical in all experimental groups. That means that the poor learning performance in PTZ-kindled rats was not due to motor impairments or freezing behavior.

Experiments With ETH (Fig. 2)

Series A. In controls, ETH had no effect on learning performance, $F(2, 38) = 1.71$, $p = 0.195$, in comparison to saline-injected controls. In the kindled group, the learning deficit was eliminated, $F(2, 37) = 5.99$, $p = 0.006$, in comparison to saline-injected kindled animals.

Series B. ETH administered prior to each shuttle-box test had no effect, either in controls, $F(2, 35) = 0.39$, $p = 0.679$, in comparison to saline-injected animals, or in kindled rats, $F(2, 37) = 0.39$, $p = 0.486$, in comparison to saline-injected kindled animals.

Experiments With DPA (Fig. 3)

Series A. DPA administered in the course of the kindling procedure exerted no effect on controls, $F(2, 63) = 0.06$, $p = 0.942$, in comparison to saline-injected controls. Although there is a slight increase in learning performance in PTZ-

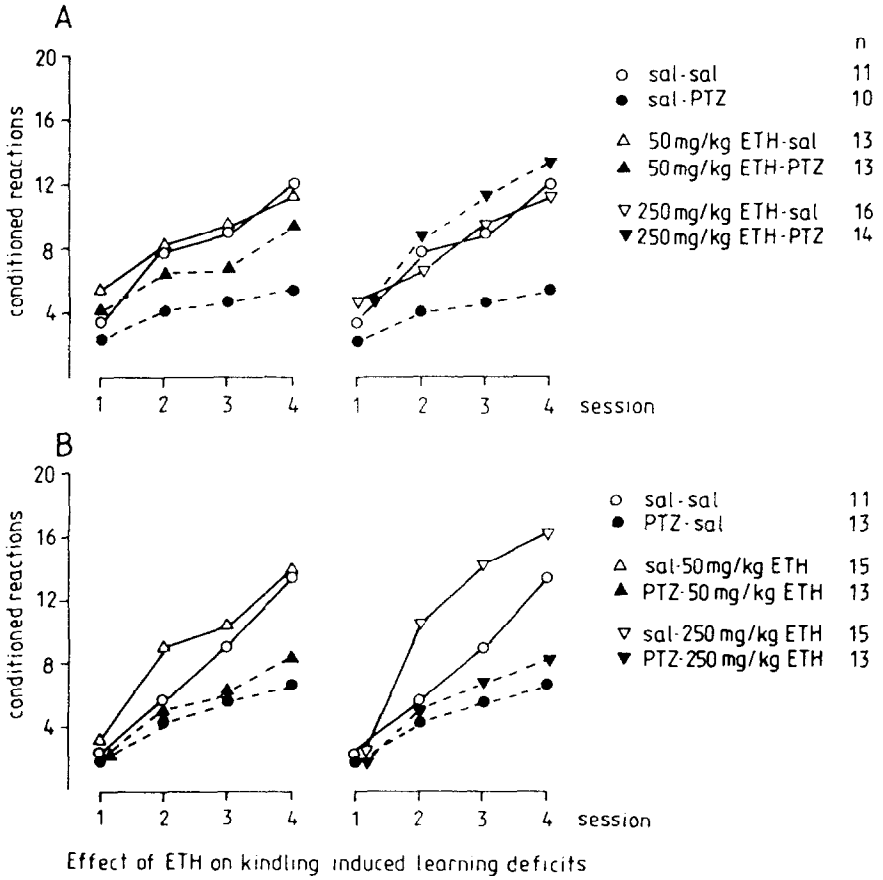

FIG. 2. Shuttle-box performance and number of conditioned reactions in saline-injected controls (means). Ethosuximide (ETH) was administered in doses of 50 mg/kg or 250 mg/kg. Series A: substance was administered prior to the kindling stimulation. Series B: substance was administered prior to the shuttle-box session.

kindled rats, this difference is insignificant, $F(2, 61) = 0.89$, $p = 0.417$.

Series B. In controls, DPA did not interfere with learning behavior, $F(2, 35) = 1.53$, $p = 0.231$. However, in kindled animals, DPA elevated the number of conditioned reactions significantly, $F(2, 29) = 3.64$, $p = 0.036$.

Experiments With PB (Fig. 4)

In both series, PB did not interfere with shuttle-box learning in controls [series A, $F(2, 27) = 0.06$, $p = 0.94$; series B, $F(2, 36) = 0.5$, $p = 0.608$]. In PTZ-kindled animals, shuttle-box learning was normalized [series A, $F(2, 40) = 3.69$, $p = 0.034$; series B, $F(2, 43) = 9.85$, $p = 0.001$, in comparison to saline-injected kindled animals].

The F-values represent overall effects of the treatment in the experimental groups. To provide an easy overview, the data are presented in the illustrations without SEM.

DISCUSSION

Our experimental data provide clear evidence that clinically used AEDs, administered in doses effective in suppressing PTZ-kindled seizures, exerted specific action on the learning deficit induced by kindling. This effect is independent of the anticonvulsive efficacy.

Different parameters of AED medication have been shown to affect cognitive functions. A comparative study (30) showed carbamazepine to be a drug with relatively few undesirable effects, whereas PB exhibited worsening. This is consistent with reports by Nichols et al. (21) that this substance appears to have more effects than other AEDs. However, other investigations in patients with epilepsy have failed to reveal clear effects, with a few exceptions (31).

Until now, there is no clear picture of AED therapy impact on cognition. Therefore, it seems to be important to test clinically used and potential new AEDs for such effects.

Chemical kindling is a model of human epilepsy. It reflects the convulsive component of this disease as well as secondary alterations in the field of cognition (2). Considering the fact that the basic mechanisms underlying kindling are not completely understood it is difficult to speculate about basic mechanisms causing such alterations.

It seems unlikely that (motor) seizures may be considered the very reason for cognitive impairments occurring after kindling because AED doses administered in our experiments have been proven effective in suppressing such seizures (Fig. 1). Recently, we reported that high-dosed diazepam showed similar effects (3). Although serious convulsions did not occur, the learning performance in kindled rats was poor. An electrophysiological analysis of PTZ kindling induced potenti-

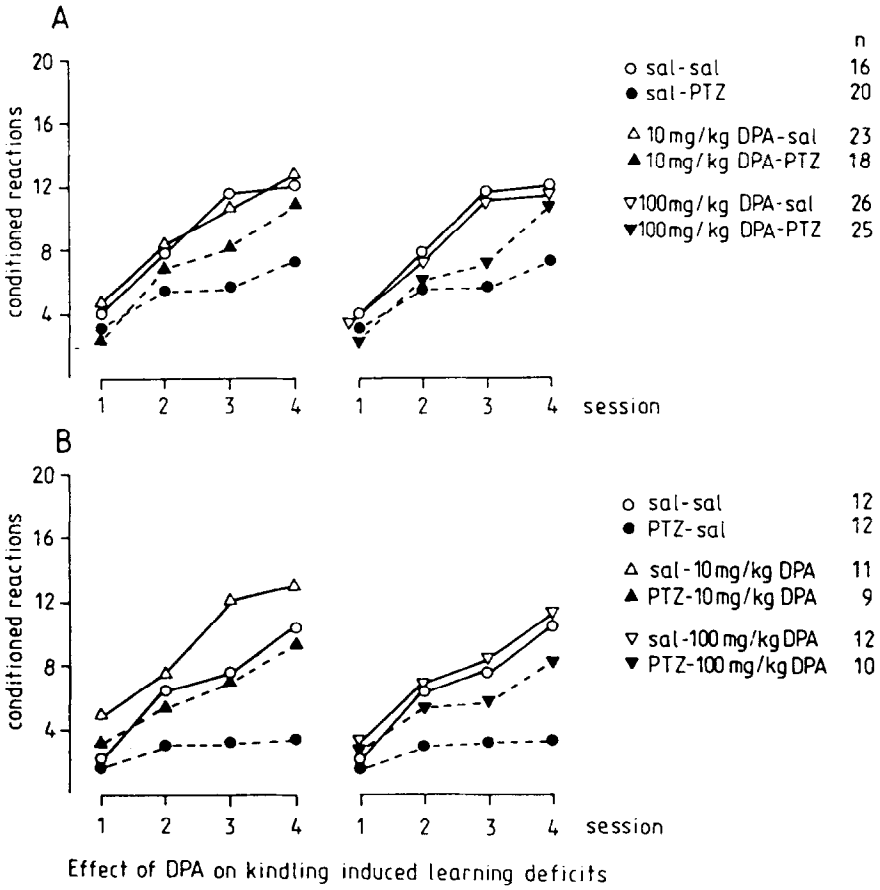

FIG. 3. Shuttle-box performance, number of conditioned reactions in saline-injected controls (means). Dipropylacetate (DPA) was administered in doses of 10 mg/kg or100 mg/kg. Series A: substance was administered prior to the kindling stimulation. Series B: substance was administered prior to the shuttle-box session.

ation effects that also failed to demonstrate a suppressive effect of diazepam (26,27). This kindling-induced potentiation effect occurred regardless of the DZP pretreatment. This result suggests that DZP actually suppresses the release of convulsions but is not capable of preventing all components of the kindling process and related secondary effects. It might be safely hypothesized that the drugs used in our experiments could have similar effects.

Concerning the target of AED action, there seems to be some linkage to the effect on learning. DPA administered prior to each kindling stimulation resulted in a slight increase in the number of conditioned reactions, whereas PB-treated kindled rats showed a similar number of conditioned reactions to controls (Figs. 3 and 4, series A). Similarly, when both substances were injected prior to each shuttle-box test, the learning deficit in kindled rats was eliminated (Figs. 3 and 4, series B). It is commonly accepted that DPA and PB exert anticonvulsive activity via GABA-mediated mechanisms (9,23). Whereas PB appears to inhibit synaptic neurotransmission by facilitation of Cl^- entry into neurons, DPA excerts its action by inhibiting the degradation of GABA. Regarding DPA beside GABAergic mechanisms, this substance seems to block cell firing induced by NMDA-type glutamate receptors (16). On the other hand, DPA was shown to facilitate acquisition of a conditioned reaction (19) and to improve retention performance in a brightness discrimination task (14). An increase was found of correct responses in a brightness discrimination in rats, posttraining injected intraventricularly with 100 μg GABA (15). A facilitaion of the acquisition of a conditioned reaction was found using DPA and 2-propyl-2-pentenoic acid that increased the GABA level in the brain, too (19,20). These data were interpreted in such a way that stimulation of GABAergic neurons would play an important role in acquisition and memory consolidation. Such interferences with GABAergic neurotransmitter systems might explain the effects of DPA and PB when administered prior to each kindling stimulation or to kindled rats prior to each learning session in terms of normalization in learning performance.

PTZ kindling is associated with several alterations in glutamatergic systems (28,29). This transmission system is thought to be involved in processes of learning and memory formation. Because DPA (16) and PB (23) have been reported to modulate excitatory actions of this amino acid, such a component in memory restoration in kindled rats cannot be answered in the negative. Possibly, a special balance between GABAergic and glutamatergic mechanisms might be responsible for shuttle-box learning improvement.

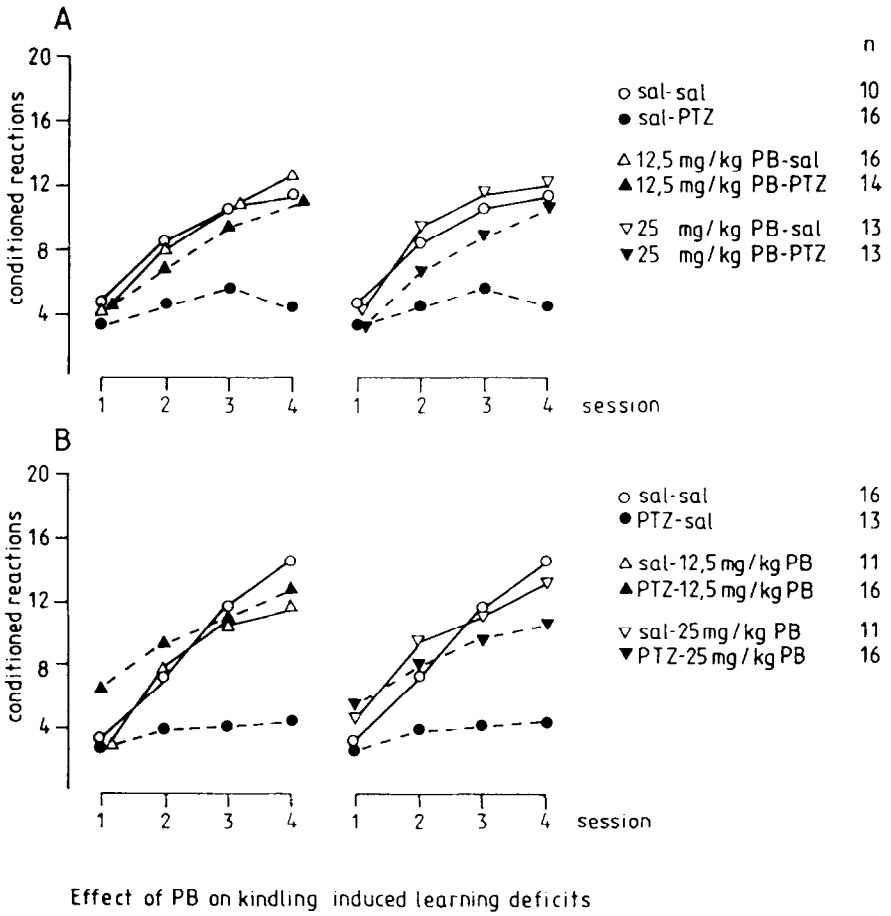

FIG. 4. Shuttle-box performance, number of conditioned reactions in saline-injected controls (means). Phenobarbital (PB) was administered in doses of 12.5 mg/kg or 25 mg/kg. Series A: substance was administered prior to the kindling stimulation. Series B: substance was administered prior to the shuttle-box session.

ETH is commonly used in the treatment of human primary generalized seizures of the absence type (11). Present evidence suggests that ETH does not significantly effect GABA-mediated inhibitory processes (11). The suggested dopaminergic mechanism of action (10) was not supported by Pohl et al. (22). It was found that ETH produces up to 40% reduction in the amplitude of the T-type Ca^{2+} current in thalamic neurons (7), which could explain the antiabsence efficacy (25). However, this substance injected prior to the kindling stimulation contributed to an improvement of learning performance in rats. It remains unclear whether this effect is due to dopaminergic effects or due to Ca^{2+} reduction.

The data presented show that the model used is potential in detecting side effects of AEDs on functions in the field of cognition. The aim should be to find substances that are effective in epileptic seizure suppression without detrimental side effects and, additionally, which are capable of counteract epilepsy-induced secondary alterations. Therefore, our model might be useful in the search for new strategies in the treatment of epilepsy.

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