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Flunarizine – Its Effect on Pentylenetetrazol-Kindled Seizures and on Related Cognitive Disturbances

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BECKER, A. AND G. GRECKSCH. *Flunarizine – Its effects on pentylenetetrazol-kindled seizures and on related cognitive disturbances.* PHARMACOL BIOCHEM BEHAV **52**(4) 765–769, 1995. – Epileptics are often faced with impaired intellectual processes. The basis of these impairments is still poorly understood. Kindling is an accepted model for the study of the convulsive component of epilepsy. Furthermore, it was demonstrated that pentylenetetrazol-kindled rats show diminished shuttle-box learning. Therefore, we used this model to study the influence of flunarizine, a calcium antagonist, on kindled seizures as well as related learning impairments. It was found, that acutely administered flunarizine significantly suppressed the expression of kindled seizures, but there was no effect on the developmental character of kindling. Moreover, the substance had an anticonvulsant action when administered after completion of kindling. The learning ability of kindled rats was significantly augmented when flunarizine was injected prior to each convulsive stimulation or when administered after completion of kindling. The results were explained in terms of interactions of a depressive effect on abnormal neuronal excitation, a protection against calcium-induced neurotoxicity and, finally, the vascular effect of flunarizine.

Epilepsy Kindling Pentylenetetrazol Learning Shuttle box Rats Flunarizine

EPILEPTIC patients are often faced with impaired learning and memory processes (8,9,13). Although many investigations have dealt with EEG-related changes, the secondary alterations linked with epilepsy, especially in the field of cognition, are still poorly understood.

For study of the convulsive component of epilepsy, kindling is a widely accepted model. Furthermore, it was demonstrated that rats after completion of pentylenetetrazol (PTZ)-kindling show a remarkable deficit in learning a two-way active avoidance task (1). It was speculated that PTZ-kindling can be regarded as a suitable model for studying the basic mechanisms of epileptogenesis and cognitive deficits associated with epileptogenesis.

The influx of calcium ions is involved in the genesis of epileptiform processes (11,18,21,24,25). Thus, it could be of interest to test calcium antagonists as a candidate for epilepsy treatment. However, in this respect, the literature is not unanimous. For instance, Popoli et al. (17) found an effect of flunarizine against PTZ-induced seizures, whereas Trommer and Pasternak (23) reported that this substance had little if any effect on amygdala kindling.

Therefore, in the present study, we tested the effect of flunarizine on the development of PTZ kindling. Moreover, these rats were tested for their learning capacity using a two-way active avoidance task in a shuttle box.

METHOD

Animals

Our experiments were performed with 8-week-old (at the beginning of kindling) male Wistar rats from our own breeding stock. 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 relative 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.

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Pentylenetetrazol Kindling

For PTZ kindling an initially subeffective dose of 45 mg/kg body weight PTZ 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: convulsive waves through the body; stage 3: myoclonic jerks, rearing; stage 4: turn over into side position; stage 5: turn over into back position, generalized clonic-tonic seizures.

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 for rats tested according to schedule B (see below).

Drug Testing

The effect of intraperitoneally administered flunarizine on the learning deficit was investigated in two different experiments. In series A, flunarizine (1 mg/kg or 5 mg/kg) or saline was given 1 h before each saline (controls) or PTZ (kindled rats) injection. The learning experiment was performed without any injection. In this way, the influence of flunarizine on the development of PTZ-kindled seizures as well as secondary injuries could be measured.

In series B, the animals were kindled until they reached the criterion as described above (Sect. 2). Saline or flunarizine (1 mg/kg or 5 mg/kg) was injected during the learning experiment 1 h before each daily training session. In this experiment, the effect of the substance on kindling-induced alterations was tested.

One of the characteristics of kindling is the lowered seizure threshold. To test the effect of flunarizine on kindling success, after completion of the shuttle-box experiments the rats prepared in series A received a challenge dose of 35 mg/kg PTZ 8 days after finishing the kindling procedure.

In a fourth experiment the effect of 1 mg/kg and 5 mg/kg flunarizine on the convulsive effect of a PTZ-dose of 45 mg/kg was tested in fully kindled rats. For this purpose, a separate group of rats was kindled (see Sect. 2). After reaching the criterion, rats were injected with saline or flunarizine and received the PTZ dose 1 h later. The resultant seizures were scored as described.

Flunarizine (Sigma) was dissolved in saline by adding a drop of Tween 80 and was administered in doses of 1 and 5 mg/kg body weight, respectively. Controls received saline with a drop of Tween 80 added. Injection volume was 10 ml/kg body weight.

Learning Procedure

Two-way active avoidance—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 (a 40 W bulb located on the central ceiling of each compartment) and a sound produced by a buzzer. The unconditioned stimulus was an electric foot shock of maximum 1 mA, delivered through stainless steel rods forming the floor. The conditioned stimuli-unconditioned stimulus interval lasted 4 s. One trial was limited to 20 s if the animal failed to respond before. The time interval between the trials was randomized and lasted for 20–30 s. 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.

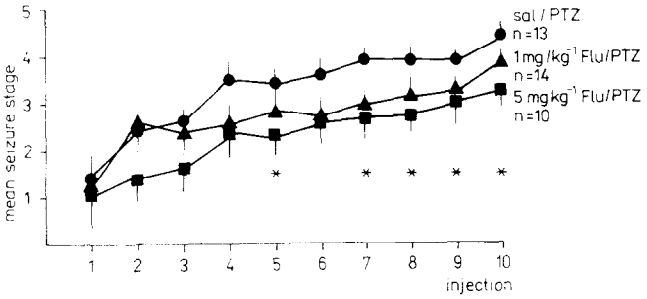

FIG. 1. Development of pentylenetetrazol-evoked kindling in rats pretreated 1 h before each kindling session with saline (●), 1 mg/kg (▲), and 5 mg/kg (■) flunarizine. Asterisks indicate significant differences ($p < 0.05$) sal/PTZ vs. 5 mg/kg flunarizine/PTZ. Data are mean seizure stage ± SEM.

The number of escapes (reaction time > 4 s) and the number of conditioned reactions (reaction time < 4 s) were measured for further analysis.

The learning experiment was started 24 h after the last kindling injection.

Statistics

Seizure scores were analyzed for significance by the two-tailed Kruskal-Wallis *H*-test and the Mann-Whitney *U*-test. Data obtained in the learning esxperiment were examined by analysis of variance (ANOVA), and a post hoc Duncan test was used to determine significant differences between the different groups. A probability level of 0.05 was considered as significant.

RESULTS

As shown in Fig. 1, flunarizine, in a dose of 5 mg/kg, suppressed significantly the expression of PTZ-kindled sei-

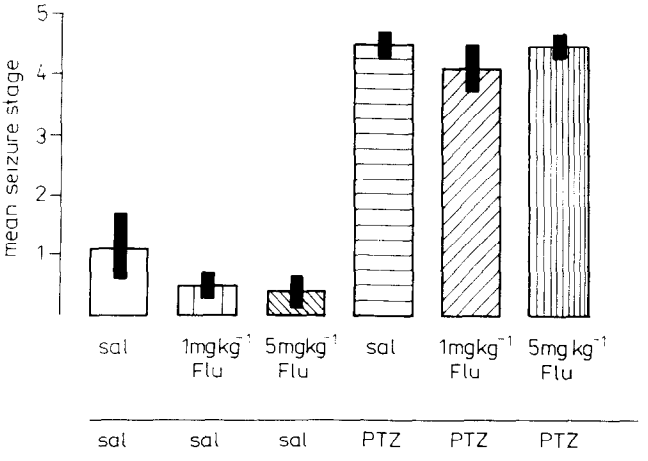

FIG. 2. Response of rats to a challenge dose of 35 mg/kg pentylenetetrazol 8 days after completion of kindling. Left three bars: rats were pretreated 1 h before each injection with saline, 1 mg/kg, or 5 mg/kg flunarizine (controls); right three bars: rats were pretreated 1 h before each kindling injection with saline, 1 mg/kg, or 5 mg/kg flunarizine (kindled animals). Data are mean seizure stage ± SEM.

zures. Although a tendency is also to be seen in the group that received 1 mg/kg flunarizine, the suppression was significant in the 5 mg/kg group, only.

After receiving the challenge dose of 35 mg/kg PTZ (rats from series A) the sal/PTZ group exhibited clonic-tonic seizures with a mean seizure stage of 4.5 (Fig. 2). The groups having received flunarizine prior to the convulsant reacted in the same manner. In contrast, the saline-injected controls showed only minor seizure activity. The differences between the groups that received sal/sal or flunarizine/sal and animals that received sal/PTZ or flunarizine/PTZ are significant at all points. That means that the convulsive element of kindling was acutely suppressed by flunarizine (Fig. 1), but the progression of the kindling process was not retarded. Furthermore, we tested the acute effect of flunarizine in fully kindled rats. As demonstrated in Fig. 4, doses of 1 and 5 mg/kg of the substance suppressed kindled seizures efficaciously in a dose-dependent manner.

In PTZ-kindled rats a remarkably impaired performance in the shuttle-box learning could be measured (Fig. 3). However, both groups of rats learned the instrumental reaction in an identical manner that demonstrated that the learning deficit is not due to motor impairments. Interestingly enough, this deficit was not detectable in the experimental group that was injected with 5 mg/kg flunarizine 1 h prior to the convulsant (series A, Fig. 3). Similarly to seizure suppression, a slight but not significant effect of 1 mg/kg was found.

In series B, rats were kindled until the criterion was reached. Twenty-four hours after the last stimulation they were tested for the learning performance in the shuttle box. As shown in Fig. 3 (series B), flunarizine in both doses significantly improved the learning performance in kindled rats. To provide more clarity in these pictures, we illustrated only the percent values of the conditioned responses without SEM.

DISCUSSION

Calcium ions function widely as intracellular messengers and regulators. Furthermore, it was found that a calcium inward current and calcium dependent membrane currents participate in the genesis of paroxysmal depolarization shifts (21). Because the influx of calcium probably depends upon the activation of voltage-operated channels, several studies have examined the effect of calcium channel antagonists on various animal models of epilepsy. The results provide a diffuse picture concerning the effect of these substances [compare (5–7,14,17,26)].

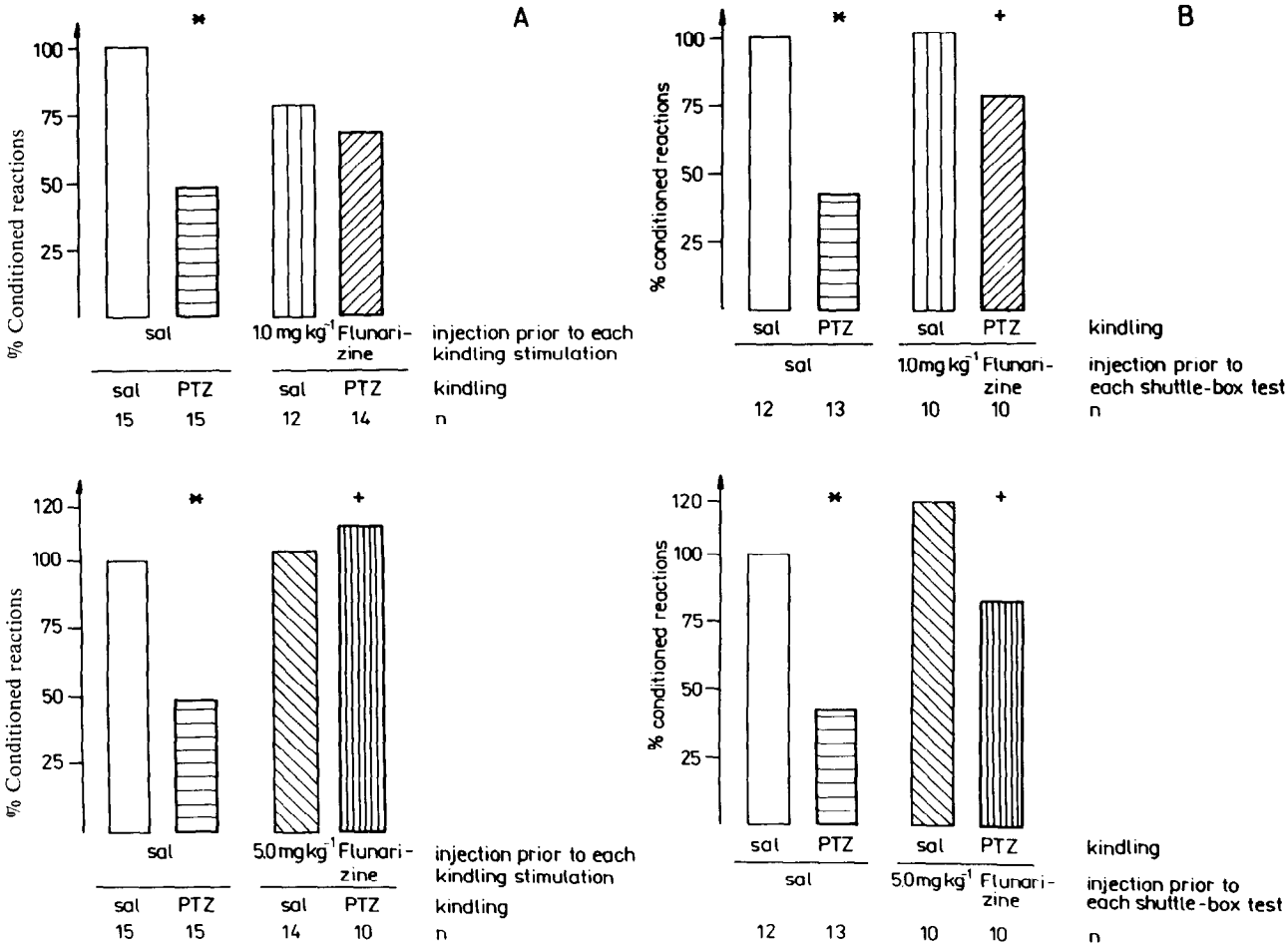

FIG. 3. Shuttle-box performance in control and PTZ-kindled rats on 4th day of training. (A) Shuttle-box performance in control (sal) and PTZ-kindled rats who were pretreated 1 h before each kindling session. (B) Performance in controls (sal) and PTZ-kindled rats who were pretreated with saline, 1 mg/kg or 5 mg/kg flunarizine 1 h before each shuttle-box session. Data are expressed in percentage of sal/sal-treated control groups. *$p < 0.05$ sal/sal vs. sal/PTZ; +$p < 0.05$ sal/PTZ vs. 1 or 5 mg/kg flunarizine/PTZ.

Using CA3 neurons of hippocampal slices of guinea pigs paroxysmal depolarization shifts elicited by PTZ or bicuculline were abolished by verapamil and flunarizine (2,3,25). These results may underline the depressive effect of calcium antagonists on neuronal excitation.

In our experiments, we found a dose-dependent effect of flunarizine against PTZ-kindled seizures (Fig. 1) when the substance was injected during kindling development. This finding is in accordance with other (7,17). It was explained by these authors in terms of interactions with the adenosine system. On the other hand, it was found that flunarizine does not delay the development of generalized seizures by amygdala kindling (23). Perhaps this difference might be based on different models for kindling induction. The amygdala-model shares characteristics of focal epilepsy, whereas in PTZ-kindling the stimulant acts in a more generalized way.

After receiving a challenge dose of 35 mg/kg, PTZ, in a test after completion of kindling induction without the acute influence of flunarizine the sal/sal- or flunarizine/sal-injected groups exhibited only negligible seizure activity (Fig. 2). In contrast, all the other groups reacted to this injection with severe convulsions irrespective of the pretreatment with flunarizine. We interpret these results together as a suppression of motor-kindled seizures by an acute effect of the substance (Fig. 1), but the process of kindling, i.e., the progressive intensification of the susceptibility to the stimulus, is not delayed. On the contrary, it was demonstrated that diazepam antagonized kindling development (20). After application of a challenge dose of PTZ (35 mg/kg) to these rats, the diazepam/PTZ- treated group showed decreased seizure activity compared to sal/PTZ-treated animals.

This finding may explain the diverse results concerning the effect of different drugs tested in the kindling model. Kindling includes both the motor expression of seizures and the developmental component. It seems likely that substances may interfere differently with both components of kindling (for example flunarizine and diazepam). Therefore, we might hypothesize that both aspects should be taken into consideration for characterizing substances concerning their antiepileptic action.

PTZ kindling resulted in a significantly diminished shuttlebox performance (Fig. 3). However, if flunarizine was injected prior to the convulsant, the learning capacity of these rats was improved markedly. We may speculate that flunarizine protects the brain against functional and/or structural neuronal damage after cerebral ischemia following generalized seizures.

This cerebrovascular effect of flunarizine could possess direct neuroprotective action. Flunarizine may block Ca^{2+} and Na^{+} channels (15). A decreased calcium influx could prevent further release of glutamate, and activation of NMDA receptors gated Ca^{2+} channels at physiological pH. Epileptic events are often associated with concomitant acidosis. The resulting unphysiological pH may enhance the prevention of Na^{+} influx and related cytotoxicity (15). In this context, a preventive role of Ca^{2+} entry blockers on the development of the neurodegeneration by overstimulation of various glutamate receptor subtypes was shown (16).

It remains at present uncertain whether flunarizine, despite being a potent calcium entry blocker, owes its anticonvulsant action to an effect on sodium channels (4). Furthermore, it is difficult to answer the question as to what extent this sodium component attributed to the learning improvement.

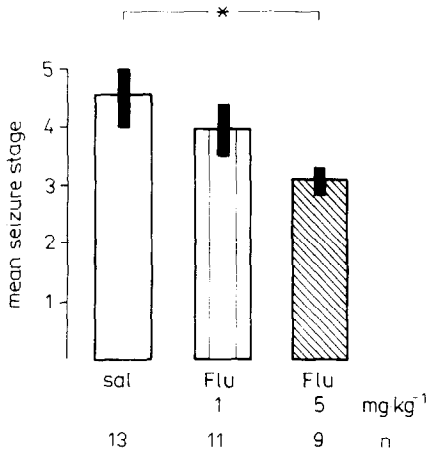

FIG. 4. Effect of a single injection of flunarizine on seizure activity in fully kindled rats pretreated 1 h before PTZ injection (45 mg/kg) with saline, 1 mg/kg, or 5 mg/kg flunarizine. Data are mean seizure stage ± SEM.

As another candidate mechanism, the substance preferentially relaxes vascular smooth muscles (10,19). The normalized cerebral flow could be the basis for improved glucose and oxygen supply, a prevention of neuron swelling, regulation of intracranial hypertension and, finally, a protection against neuronal cell death.

In this context, a beneficial effect of flunarizine on postischemic energy metabolism was found (22). In comparison to controls, restoration of flow was considerably faster, complete, and without reduction during the further course of reperfusion. Furthermore, a remarkable efficacy of calcium antagonists in preventing ischemia-reperfusion-induced damage to organs was reported (12), in which a role for free radicals has been postulated.

All together, i.e., effects on cerebral blood vessels, the protection against neurotoxicity of excitatory amino acids as well as the protection against Ca^{2+} influx may explain the protection by flunarizine administered during kindling.

As shown in Fig. 4, flunarizine has also an restorative effect when administered after completion of kindling. Perhaps this effect and the improved learning ability in controls could be a result of the vascular action of this substance.

In summary, the present data indicate a role of flunarizine in counteracting kindling-related neuronal and/or functional degeneration. This substance may emerge as an useful additive in epilepsy therapy.

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