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Effects of Diazepam and Diphenylhydantoin on Elicited and Spontaneous Seizures in Kindled Rats: A Double Dissociation

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PINEL, J. P. J. *Effects of diazepam and diphenylhydantoin on elicited and spontaneous seizures in kindled rats: A double dissociation.* PHARMACOL BIOCHEM BEHAV **18**(1) 61–63, 1983.—Diazepam (1 mg/kg) was more effective than diphenylhydantoin (100 mg/kg) in suppressing motor seizures elicited in kindled rats by amygdaloid stimulation; however, the effect of these drugs on the incidence of spontaneous motor seizures in rats kindled by amygdaloid stimulation was just the opposite. At the same doses, diphenylhydantoin effectively suppressed spontaneous motor seizures, but diazepam did not. This double dissociation suggests the need for caution in drawing inferences concerning spontaneously recurring seizures from studies of elicited seizures.

Epilepsy Kindling Spontaneous seizures Diazepam Diphenylhydantoin Amygdala Double dissociation Rat

WHEN a rat is periodically stimulated through an implanted amygdaloid electrode, there is a development and progressive intensification of motor seizures elicited by the stimulation, i.e., kindling [5]. Because generalized motor seizures can be reliably elicited in kindled rats, many investigators have assessed the effects of drugs on such elicited seizures in an attempt to identify the chemical basis of epilepsy. However, despite the fact that epilepsy in its usual form is a self-sustained, spontaneously recurring dysfunction [3]—the reflex epilepsies are the obvious exception—, the effects of pharmacological agents on the spontaneous motor seizures that are prevalent in rats following long-term amygdaloid kindling [2, 3, 4] had never been investigated. Accordingly, the purpose of the present experiments was to assess the effects of the anticonvulsants, diazepam (Valium, Roche) and diphenylhydantoin (Dilantin, Parke Davis) on spontaneous motor seizures in kindled rats and to compare these effects with those on elicited kindled seizures.

Diazepam and diphenylhydantoin were the anticonvulsants selected for investigation because their effects at nontoxic doses on motor seizures elicited by amygdaloid stimulation in kindled rats had been studied previously. Both Babington and Wedeking [1] and Racine, Livingston, and Joaquin [6] had reported that diazepam was more effective than diphenylhydantoin in suppressing amygdaloid motor seizures elicited in kindled rats at a wide range of nontoxic doses.

EXPERIMENT 1

METHOD

Thirteen rats (Canadian Breeding Farm and Laboratories, St. Constant, Quebec) were stimulated (1 sec, 60 Hz, 400 μA rms) three times per day at intervals of no less than 2 hr for 2 weeks (5 days per week), and then once a day for the next 10 days. On the next 11 days, each subject received a daily test stimulation (1 sec, 60 Hz, 400 μA rms) 30 min following an IP injection of diphenylhydantoin, diazepam, or saline. Each rat received either 100 mg/kg of diphenylhydantoin or 1 mg/kg of diazepam both in aqueous solution, or a comparable volume of saline. These doses were selected because both Babington and Wedeking [1] and Racine *et al.* [6] had found that they suppressed kindled seizures without producing toxic side effects. The subjects were divided randomly into two subgroups of 6 and 7 subjects each prior to the first of the 11 test days. Although all subjects received saline on the odd-numbered test days, on even-numbered test days the members of one subgroup received diphenylhydantoin, while the members of the other received diazepam. The exact sequence of testing for the two subgroups is reflected in Fig. 1.

Each motor seizure was rated according to a version of Racine's widely employed 5-class scale of kindled seizures extended to 8 classes by Pinel and Rovner [3] to encompass

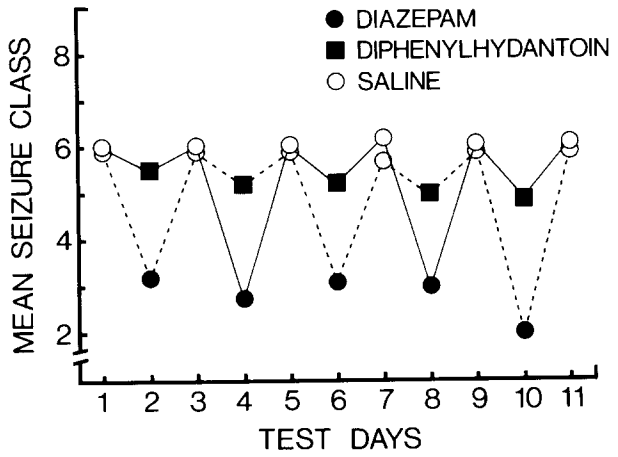

FIG. 1. Mean class of seizures elicited in the two subgroups of kindled subjects following injections of diazepam, diphenylhydantoin, or saline. There were six subjects in the subgroup represented by the solid line and seven in the subgroup represented by the broken line.

the entire course of long-term amygdaloid kindling. Accordingly, each motor seizure was assigned to one of the following classes: (1) facial movements only; (2) facial movements and head nodding; (3) facial movements, head nodding, and forelimb clonus; (4) facial movements, head nodding, forelimb clonus, and rearing; (5) facial movements, head nodding, forelimb clonus, rearing, and falling; (6) a class 5 pattern terminating with multiple rearing and falling episodes; (7) a running fit; (8) a seizure with periods of tonus.

RESULTS

The results summarized in Figure 1 confirm Babington and Wedeking [1] and Racine *et al.* [6] by showing that 1 mg/kg of diazepam is more effective than 100 mg/kg of diphenylhydantoin in blocking motor seizures elicited by amygdaloid stimulation in kindled rats. The statistical significance of this effect was confirmed by subjecting the mean seizure class of each subject under each of the three drug conditions to a repeated measures analysis of variance, $F(2,24)=43.34$, $p<0.001$, followed by a Tukey multiple comparisons test. Although both diazepam ($p<0.001$) and diphenylhydantoin ($p<0.05$) significantly reduced the mean motor seizure class below that observed on saline days, the effect of the diazepam was significantly greater ($p<0.001$). Neither drug produced obvious motor impairment at the doses employed.

EXPERIMENT 2

The purpose of Experiment 2 was to determine whether or not the superiority of diazepam over diphenylhydantoin in blocking seizures elicited by amygdaloid stimulation in kindled rats would be reflected in the effects of the same doses of these two drugs on the incidence of spontaneous motor seizures observed in rats following long-term amygdaloid kindling. The effects of drugs on spontaneous seizures in kindled subjects had never been assessed.

METHOD

The method of inducing spontaneously recurring motor seizures in rats by amygdaloid kindling was that of Pinel and Rovner [3,4]. Four rats received amygdaloid stimulation (1 sec, 60 Hz, 400 μA rms) through an implanted bipolar electrode about 15 times per week at intervals of no less than 2 hr and no greater than 48 hr. Each subject progressed routinely through the aforementioned eight classes of motor seizure development [3] before displaying its first spontaneous motor seizure (after a mean of 322 stimulations). Once a spontaneous motor seizure involving forelimb clonus (i.e., one of class 3 or greater) was observed in a subject, that subject was kept under observation for 1 hr per day, 5 days per week until at least one spontaneous motor seizure involving forelimb clonus had been observed during four consecutive observation sessions. Once this criterion had been met by a subject, that subject received no further stimulations and was observed for 3 hr per day thereafter until the end of the experiment. Each subject received either diphenylhydantoin (100 mg/kg), diazepam (1 mg/kg), or saline 30 min before the beginning of each test. The exact sequence of drug administration for each of the four subjects is reflected in Fig. 2.

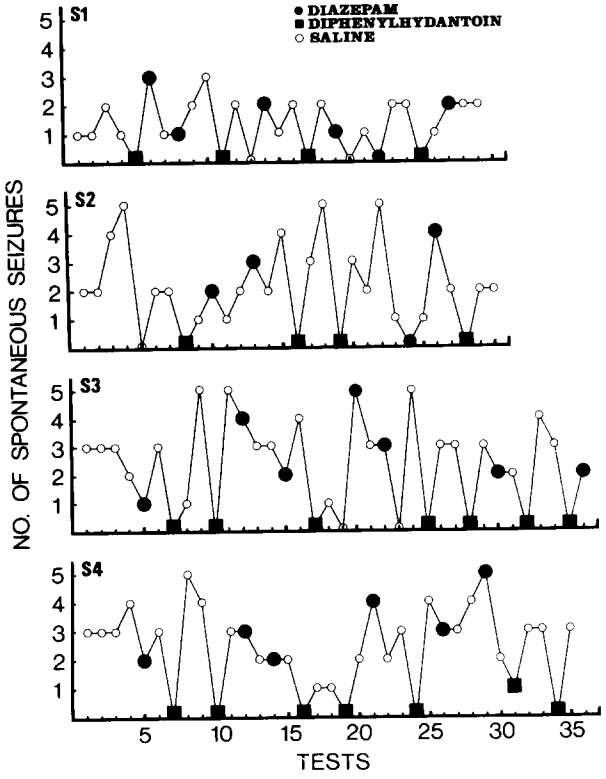

FIG. 2. Number of spontaneous seizures involving forelimb clonus observed during each test in each of the four subjects. Injections of saline, diazepam, or diphenylhydantoin were administered 30 min before each test.

RESULTS

The number of spontaneous motor seizures of class 3 or greater observed in each subject during each test is presented in Fig. 2. It is readily apparent from Fig. 2 that at the doses employed diphenylhydantoin reduced the incidence of spontaneous motor seizures below that seen on the saline days, whereas the diazepam had little, if any, effect,

$F(2,6)=52.01$, $p<0.001$. Accordingly, Tukey multiple comparisons revealed that there were significantly fewer spontaneous motor seizures observed following diphenylhydantoin administration than in either the saline ($p<0.01$) or diazepam ($p<0.01$) conditions.

GENERAL DISCUSSION

The results of the present experiments suggest that elicited and spontaneous kindled seizures may be differentially affected by anticonvulsant drugs. Whereas diazepam was significantly more effective than diphenylhydantoin in reducing the severity of kindled seizures elicited by amygdaloid stimulation (Experiment 1), at the same doses diphenylhydantoin was the more potent inhibitor of spontaneous seizures observed following long-term amygdaloid kindling (Experiment 2). In fact, although 1 mg/kg of diazepam was clearly effective against elicited kindled seizures (see Fig. 1), it had no detectable effect on comparable seizures that occurred spontaneously in kindled subjects (see Fig. 2).

Ideally, the comparison of the effects of diazepam and diphenylhydantoin on elicited and spontaneous kindled seizures requires the generation of dose-response curves for both drugs in both test situations. However, such an approach in the present situation was impractical because of the difficulty in generating and maintaining sufficiently large numbers of spontaneously convulsing subjects [2, 3, 4] to adequately conduct such tests. Instead a double dissociation was demonstrated between the effects of single doses whose effects on elicited kindled seizures had already been well documented [1,6]. The double dissociation has traditionally been the technique of choice for demonstrating localization of function in the brain, and it is no less useful as a control procedure for establishing the selective activity of drugs, particularly during the early stages of analysis. In the present instance, for example, it is clear that the doses employed were neither so low as to be ineffective or so high as to be totally debilitating; each dose almost totally disrupted the seizures in one test but had little effect in the other. Conversely, it is difficult to argue that one or both of the two tests was insensitive because each test successfully discriminated between the two drugs.

One of the difficulties inherent in any comparison between the effects of drugs on elicited and spontaneous motor seizures is that the very nature of spontaneous seizures precludes the possibility of precisely controlling the injection-seizure interval. Thus, in Experiment 1 it was possible to assess the effects of diazepam and diphenylhydantoin on seizures elicited exactly 30 min following injection; whereas, in Experiment 2 the effects of these drugs were assessed over a 3-hr interval. Did this temporal difference contribute substantially to the double dissociation observed in the present studies? Although it is not possible to completely rule out such a possibility, careful examination of the time course of effects in Experiment 2 suggested that is was unlikely. The diphenylhydantoin was as effective in suppressing spontaneous seizures between 0.5 and 1 hr following the injection as it was between 2.5 and 3 hr; in all but a single instance, seizures were totally suppressed during the entire test period (see Fig. 2). Conversely, there was no suggestion in the data that diazepam was any more effective early in the 3-hr test than it was during the final minutes.

The fact that diazepam and diphenylhydantoin differentially suppress elicited and spontaneous kindled seizures suggests there may be important differences in the mechanisms underlying the generation of these two types of seizures. Just such a point was made in a recent study of neocortical kindling [2]. Despite the fact epileptic discharges elicited at the electtrode tip during the course of neocortical kindling were presumably responsible for initiating the electrographic events that resulted in motor seizures, when spontaneous motor seizures were eventually observed in these same animals, they occurred in the complete absence of discharges from the kindling site.

While isomorphism between the test response and epilepsy is not a necessary feature of effective screening tests for anticonvulsant agents, it seems that attempts to understand the mechanisms underlying the development of spontaneous motor seizures must be based at least in part on the study of models in which the seizures recur spontaneously, even though spontaneous seizures are inherently less amenable to experimental manipulation and control than are elicited seizures. Thus, present results suggest that it would be profitable to expand the focus of epilepsy research to include forms of experimental epilepsy in which the motor seizures recur spontaneously.

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