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Tolerance to the Anticonvulsant Effects of Carbamazepine, Diazepam, and Sodium Valproate in Kindled Rats[1]

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MANA, M. J., C. K. KIM AND J. P. J. PINEL. *Tolerance to the anticonvulsant effects of carbamazepine, diazepam, and sodium valproate in kindled rats.* PHARMACOL BIOCHEM BEHAV **41**(1) 109–113, 1992.—We assessed the development of tolerance to the anticonvulsant effects of carbamazepine (CBZ), diazepam (DZP), and sodium valproate (VPA) on convulsions elicited by amygdala stimulation in kindled rats in three similar experiments. In each experiment, amygdala-kindled rats were assigned to a drug group or to a corresponding vehicle control group. The rats in the three drug groups received a total of 10 bidaily (one every 48 h) IP injections of CBZ (70 mg/kg), DZP (2 mg/kg) or VPA (250 mg/kg) at a dose that initially blocked the forelimb clonus elicited by an amygdala stimulation (400 μA, 60 Hz, 1 s) administered 1 h after the injection. The rats in the three vehicle control groups were similarly treated except that they received injections of the saline vehicle. The drug tolerance test occurred 48 h after the final tolerance-development trial; the rats from each drug group and the corresponding vehicle control group received an injection of the appropriate drug followed 1 h later by the administration of a convulsive stimulation. The drug tolerance test revealed almost total tolerance in each of the three drug groups but no tolerance in any of the three vehicle control groups. Such large tolerance effects are inconsistent with the less dramatic effects reported in previous studies; possible reasons for this inconsistency were considered.

Carbamazepine Diazepam Sodium valproate Tolerance Anticonvulsant Kindling Convulsion
Rat Amygdala Seizure Valproic acid

PERIODIC electrical stimulation of the brain at an intensity that is initially incapable of eliciting a convulsion can lead to the development and progressive intensification of elicited convulsions. For example, daily amygdala stimulations (e.g., 400 μA, 1 s, 60 Hz) initially elicit no convulsive responses in rats, but after approximately 15 such stimulations, each stimulation reliably elicits a generalized clonic convulsion. This phenomenon was first studied by Goddard and his colleagues, who referred to it as kindling (7,8). It has subsequently been shown that kindling can be elicited by both chemical and electrical stimulation to a wide variety of brain sites in many different species [for reviews, see (16, 26, 27)], although the majority of kindling experiments have involved electrical stimulation of the amygdala in rats.

The kindling model has emerged as a useful tool for assessing anticonvulsant drug effects [e.g., (1–3, 14)]. Many therapeutically effective antiepileptic drugs reduce both the intensity and duration of kindled convulsions and of the underlying afterdischarges [see Racine and Burnham (27) for a review]. Recently, the kindling paradigm has been used to study the development of tolerance to anticonvulsant drug effects [e.g., (10, 15, 19, 32)]. Kindled convulsions have some important advantages over the experimental convulsions elicited by electroconvulsive shock or pentylenetetrazol, which have traditionally been employed to assess the development of tolerance to anticonvulsant drugs. Electroconvulsive shock and pentylenetetrazol-induced convulsions are variable in form and duration, are difficult to measure, and are often associated with subject injury or fatality [e.g., (31)]. This latter problem is particularly troublesome in those studies of tolerance in which anticonvulsant effects are repeatedly assessed in the same subjects, because any systematic change in the apparent anticonvulsant action of a drug is confounded by the progressive debilitation and attrition of those subjects experiencing the most severe convulsions. In contrast, kindled rats generally remain healthy and easy to handle for the duration of an experiment, and kindled convulsions are reliable, stereotyped, and easy to score (23).

We have used the kindling paradigm to study the development of tolerance to ethanol's anticonvulsant effect [e.g., (19–22)]. In the present three experiments, we used the same procedures to assess the development of tolerance to the anticonvulsant effects of carbamazepine (CBZ), diazepam (DZP), and sodium valproate (VPA). These drugs were chosen for three reasons. First, each of the three drugs is effective against generalized

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tonic-clonic epileptic seizures in humans [e.g., (9, 13, 28)] which are closely modelled by generalized kindled convulsions [see (27)]. Second, each has been shown to exert a reliable anticonvulsant effect on kindled convulsions in rats [e.g., (1,2)]. And third, each belongs to a different family of antiepileptic drugs with a different putative mechanism of action.

METHOD

Because the three experiments reported in this paper were conducted in a similar fashion, they are described here as one.

Subjects

The subjects were 74 male Long-Evans rats (Charles River, Canada), weighing between 350 and 400 g at the time of surgery and between 550 and 650 g at the completion of the experiment. The rats were individually housed in wire-mesh cages with continuous access to Purina Laboratory Chow and water. All experimental procedures were conducted during the light phase of the 12:12-h light:dark cycle (lights on at 8:00 a.m.).

Surgical Procedure

A single bipolar electrode (Plastic Products, MS-303-2) was implanted in the left basolateral amygdala of each rat [1.2 mm posterior, 5 mm lateral, and 10 mm ventral to the skull surface at bregma, with the incisor bar set at +5.0; coordinates from (18)] under sodium pentobarbital anesthesia (65 mg/kg, IP). The electrode was fixed in place with stainless steel screws and dental acrylic. Tetracycline was sprinkled on the incision before suturing, and it was added to the drinking water for 7 days after surgery.

Drugs

All drugs were administered intraperitoneally in a 2% Tween 80 (J. T. Baker Chemical) isotonic saline vehicle at a volume of 4 ml/kg. The DZP (2 mg/kg; Hoffmann-LaRoche) was injected in solution; both the CBZ (70 mg/kg; Geigy) and the VPA (250 mg/kg; Abbott) were injected as suspensions. These drug doses were selected on the basis of previous studies [e.g., (1,3)] and our own pilot observation that they were effective at suppressing forelimb clonus in amygdala-kindled rats.

Kindling Phase

The kindling phase of the experiment began at least 7 days after surgery. Each rat was stimulated (1 s, 60 Hz, 400 μA) three times per day, 5 days per week, for 3 weeks, with at least 2 h between consecutive stimulations. The rats' response to the first stimulation was limited to a brief period of behavioral arrest, but by the end of the kindling phase each stimulation elicited a stereotypical generalized-clonic convulsion characterized by facial clonus, forelimb clonus, rearing, and a loss of equilibrium in almost every rat [see (24,25)]. All rats progressed to the no-drug baseline phase.

No-Drug Baseline Phase

The no-drug baseline phase began 48 h after the completion of the 45-stimulation kindling phase. Each rat received four amygdala stimulations, one every 48 h (±2 h); this bidaily stimulation schedule was maintained for the remainder of the experiment. The duration of forelimb clonus was the measure of convulsion severity; it is highly correlated with other indices of motor seizure severity (e.g., motor seizure class), it is particularly reliable, and it has been shown to be sensitive to a variety of pharmacological manipulations [see (20,21)]. Electrographic activity was not recorded. Rats that did not demonstrate at least 20 s of forelimb clonus on the no-drug baseline test (i.e., on the fourth no-drug baseline trial) were not studied further (n = 9).

Drug Baseline Test

The initial anticonvulsant effect of each drug was assessed on the drug baseline test, which occurred 48 h after the no-drug baseline test. In each of the three experiments, every rat received the drug (CBZ, DZP, or VPA) 1 h before the scheduled convulsive stimulation. Rats not displaying at least an 80% decrease in forelimb clonus duration on the drug baseline test relative to the duration of their forelimb clonus on the no-drug baseline test were not studied further. One rat receiving CBZ, two rats receiving DZP, and two rats receiving VPA did not meet this criterion for inclusion. All injections were administered 1 h prior to convulsive stimulation because pilot observations indicated that each drug was effective at suppressing forelimb clonus at this interval. The remaining rats in each experiment were then assigned to a drug group and a vehicle control group in such a way that the mean duration of forelimb clonus on both the no-drug baseline test and the drug baseline test were approximately equal for both groups.

Tolerance-Development Phase

The tolerance-development trials began 48 h after the drug baseline test. During each of the 10 tolerance-development trials, each rat was removed from its home cage, weighed, and the appropriate dose of drug (CBZ, n = 12; DZP, n = 12; VPA, n = 12) or vehicle (CBZ Control, n = 8; DZP Control, n = 8; VPA Control, n = 8) was administered 1 h before the scheduled convulsive stimulation was delivered.

Drug Tolerance Test

The drug tolerance test, which was identical to the drug baseline test, occurred 48 h after the last tolerance-development trial; 1 h before the convulsive stimulation, the rats in each drug group and corresponding vehicle control group received the drug that they had received on the drug baseline test. Thus, it was possible to assess the development of tolerance in each rat by comparing the duration of its forelimb clonus on the drug tolerance test with the durations of its forelimb clonus on the no-drug baseline test and the drug baseline test.

Histology

All subjects were sacrificed in a CO_2 chamber, and their brains were removed and sectioned to permit histological verification of the stimulation sites.

Statistical Analyses

Nonparametric statistical techniques were used for all analyses because the data violated the parametric assumptions of homogeneity of variance and normality of distribution. Wilcoxon Signed-Ranks tests (30) were used to assess the significance of within-subject differences ($p<0.05$) and Wilcoxon-Mann-Whitney tests for large samples (30) were used to assess the significance of between-group differences ($p<0.05$).

RESULTS

Tolerance developed to the anticonvulsant effects of CBZ, DZP, and VPA in the three drug groups but not in the corresponding vehicle control groups (see Fig. 1). The potent anticonvulsant effects of CBZ (panel A), DZP (panel B), and VPA (panel C), which were observed on the drug baseline test, were almost totally absent in the three drug groups on the drug tolerance test, but there was no reduction in their magnitude in the three vehicle control groups.

The statistical analyses established the statistical significance of these results. Wilcoxon Signed-Ranks tests revealed a significant increase in the durations of forelimb clonus between the drug baseline test and drug tolerance test for each of the three drug groups ($p<0.01$). In contrast, there were no significant differences between the durations of forelimb clonus observed on the drug baseline test and drug tolerance test for any of the three vehicle control groups ($p>0.05$). Accordingly, although there were no significant differences between each drug treatment group and its corresponding control group on either the no-drug baseline test or the drug baseline test ($p>0.05$), the rats in each drug treatment group displayed significantly longer forelimb clonus than the rats from the corresponding vehicle control group on the drug tolerance test ($p<0.01$). The duration of forelimb clonus elicited in each of the three drug groups on the drug tolerance test was not significantly different from that elicited from the same rats on their no-drug baseline test ($p>0.05$). This pattern of results was confirmed by an analysis of motor seizure class.

Histological analysis revealed that all of the electrode tips were in or near the amygdala, with the majority lying within the basolateral nucleus.

DISCUSSION

The present demonstrations of tolerance to the anticonvulsant effects of CBZ, DZP, and VPA on kindled convulsions in the rat are important for two reasons. First, they confirm earlier reports of tolerance to the anticonvulsant effects of DZP [e.g., (4,15)] and CBZ [e.g., (5,10)] and provide the first clear evidence of tolerance to VPA's anticonvulsant effect [but see (12)]. Second, the magnitude and consistency of the tolerance observed in the present study illustrate the utility of the kindling model in the study of tolerance to anticonvulsant drug effects (21,29).

There are two reasons why it is not possible to compare the rate of tolerance development to CBZ, DZP, and VPA on the basis of the present data. One is that each drug was studied in a separate experiment; they were reported here as one for the sake of brevity. The other is that there is no evidence that the single doses of each drug were equipotential. Be that as it may, there is a suggestion that tolerance develops to the anticonvulsant effects of VPA more slowly than it does to CBZ and DZP. This may explain why tolerance to the anticonvulsant effect of VPA has not previously been convincingly demonstrated.

It is not clear from the present experiment whether metabolic or functional changes were responsible for the development of tolerance to the anticonvulsant effects of CBZ, DZP, and VPA on kindled convulsions. Acceleration of metabolism by the induction of hepatic enzymes has been reported following CBZ exposure [e.g., (5)], thus raising the possibility that metabolic changes could have contributed to its loss of efficacy. However, neither VPA exposure (13) nor DZP exposure (6) appear to produce metabolic changes. A resolution to this question was beyond the scope of this experiment; however, the results of Mana, Kim, Pinel and Jones (17) support the notion that a functional change is primarily responsible for the tolerance that developed to all three drugs in the present experiments.

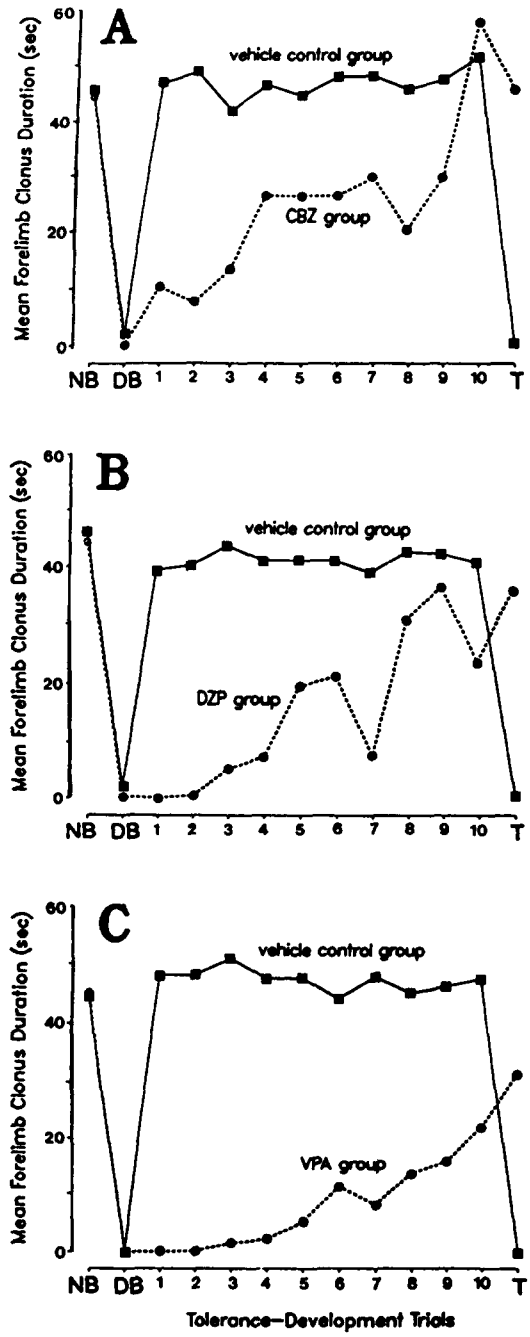

FIG. 1. Tolerance to the anticonvulsant effects of carbamazepine (panel A), diazepam (panel B), and sodium valproate (panel C.) On the no-drug baseline test (NB), each stimulation elicited about 45 s of forelimb clonus; on the drug baseline test (DB), each of the three drugs exerted a potent anticonvulsant effect in every rat; and on the drug tolerance test (T), the rats in each of the three drug groups displayed substantial tolerance to the anticonvulsant effects of the respective drugs, but there was no evidence of tolerance in any of the three vehicle control groups.

The magnitude of the tolerance effects that were observed in the present experiments warrants special comment; on the drug tolerance test, the suppressive effects of CBZ, DZP, and VPA

were 100%, 81%, and 71% less, respectively, than they had been on the drug baseline test. These effects are substantially greater than those observed in previous experiments. For example, Löscher and Schwark (15) reported tolerance to the anticonvulsant effect of DZP on the duration of kindled motor seizures that was substantially less (i.e., about 37%) than that observed in the present experiment, even though their DZP injections were larger (5 mg/kg, IP), more frequent (every 8 h), and more numerous (30 injections). Similarly, Hönack and Löscher (10) found the development of tolerance to the anticonvulsant effect of CBZ (30 mg/kg, IP, every 8 h for 10 days) on kindled seizures to be inconsistent; it waxed and waned throughout their experiment. Also, Young et al. (32) found no evidence of tolerance to VPA's (200 mg/kg, IP, every 12 h for 14 days) anticonvulsant effect on kindled convulsions in rats.

There are at least three plausible explanations for the differences between the magnitude of the effects observed in the present experiments and those observed by Löscher and Schwark, Hönack and Löscher, and Young et al. The first explanation involves differences in the drug-administration regimens employed in the respective experiments. In the present experiments, 10 injections were administered, one every 48 h; in the experiments of Löscher and Schwark, Hönack and Löscher, and Young et al., the injections were administered every 8 or 12 h. Although frequent injections are typically thought to facilitate the development of tolerance, it is possible that they may lead to an accumulation of the drug or its active metabolites that could obscure the detection of tolerance [see (11)].

A second possible explanation for the discrepancy between the present results and those reported by Löscher and Schwark, Hönack and Löscher, and Young et al. focuses on the differences in the kindling procedure. In the present experiment, every subject had demonstrated at least 30 fully generalized [i.e., class 5 or greater; see (24,25)] convulsions before the tolerance-development phase of the experiment began. In contrast, Löscher and Schwark and Hönack and Löscher began the tolerance-development phase after their subjects had demonstrated 10 class 5 convulsions. The Young et al. report is not clear on this point, although the authors state that they consider a rat that has displayed two consecutive class 5 convulsions to be fully kindled. Accordingly, it is possible that the physiological changes underlying the kindling process were more firmly established in the rats from our experiments than in those from the aforementioned studies. As a result, it may have been easier for the physiological changes underlying the development of tolerance to the anticonvulsant effects of CBZ, DZP, and VPA to express themselves in the rats used in the present experiments.

A third possible explanation for the differences between the results of the present experiment and those reported by Löscher and Schwark, Hönack and Löscher, and Young et al. is based upon our earlier observation that the development of tolerance to ethanol's anticonvulsant effect on kindled convulsions can be facilitated by the administration of convulsive stimulation during periods of ethanol exposure [e.g., (19–22)]. In the present experiment, each drug injection was followed 1 h later by a convulsive stimulation; in contrast, this condition was present on only half of the drug treatment trials in the experiments reported by Löscher and Schwark, Hönack and Löscher, and Young et al. Thus the differences between the present results and those reported earlier by these other investigators may reflect the fact that the relation between drug exposure and convulsive stimulation plays an important role in the development of tolerance to the anticonvulsant effects of CBZ, DZP, and VPA. Support for this latter interpretation is provided by the experiments of Mana et al. (17).

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