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BRIEF COMMUNICATION

Tolerance to the Anticonvulsant and Ataxic Effects of Pentobarbital: Effect of an Ascending-Dose Regimen

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KIM, C. K., L. E. KALYNCHUK, J. P. J. PINEL AND T. E. KIPPIN. *Tolerance to the anticonvulsant and ataxic effects of pentobarbital: Effect of an ascending-dose regimen.* PHARMACOL BIOCHEM BEHAV **52**(4) 825-829, 1995.—We assessed the effect of an ascending-dose regimen on the development of tolerance to the anticonvulsant and ataxic effects of pentobarbital in four groups of amygdala-kindled rats. Each rat received 20 bidaily (one every 48 h) trials in which an intraperitoneal (IP) pentobarbital or vehicle injection was delivered 1 h before a convulsive amygdala stimulation. On each trial, the rats in the three pentobarbital groups received either a high dose (50 mg/kg), a low dose (10mg/kg), or ascending doses of pentobarbital that began at 10 mg/kg and increased to as high as 26 mg/kg by 1 mg/kg increments as tolerance developed to its anticonvulsant effect; the rats in the vehicle group received saline. The rats in the ascending-dose condition displayed significantly more tolerance to the anticonvulsant effect of pentobarbital than did the other rats; in contrast, the high-dose rats displayed more tolerance to the ataxic effect of pentobarbital than did the other rats. These findings extend previous reports of the facilitatory effect of ascending-dose regimens on the development of tolerance to the anticonvulsant effect of benzodiazepines, and show that the facilitatory effect of ascending-dose regimens does not extend to all drug effects.

Drug tolerance Pentobarbital Anticonvulsant Ataxia Epilepsy Seizure Convulsion Kindling Amygdala Rat

DRUG tolerance can be a major problem in the treatment of epilepsy: A low to moderate dose of antiepileptic medication is initially prescribed, and the dose is gradually increased until the seizures are brought under control; however, as tolerance develops seizures begin to recur, and the dose is increased further. After several cycles of tolerance, resumption of seizures, and increasing the dose, the tolerance is often so great that the drug is ineffective even at prohibitively high doses [e.g., (2,6,8)]. Kalynchuk et al. (10) attempted to model this ascending-dose pattern of anticonvulsant drug prescription and found that tolerance to the anticonvulsant effect of diazepam on kindled convulsions developed to a substantially greater degree in rats that were exposed to bidaily (one every 48 h) diazepam injections of gradually increasing dosage (from 1.0 mg to 3.0 mg/kg) than it did in rats that consistently received either low (1.0 mg/kg) or high (10 mg/kg) bidaily injections. The purpose of the present experiment was to test the generality of the Kalynchuk et al. (10) finding in two ways. It assessed the effect of an ascending-dose regimen on the development of tolerance to the anticonvulsant effect of a different drug, pentobarbital, and a different drug effect, ataxia.

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METHOD

Subjects

Adult male 350–500 g, Long-Evans rats (Charles River, Montreal, Quebec, Canada) served as subjects (n = 52). They were individually housed in stainless-steel mesh cages in a colony room with an ambient temperature of about 21°C and a 12 L : 12 D cycle (lights on at 0800 h). Purina rat chow and water were available continuously in the home cages. All experimental manipulations were conducted in the colony room during the light phase of the light–dark cycle.

Drugs

Sodium pentobarbital was purchased from BDH Chemicals (Vancouver, BC, Canada). All doses of pentobarbital were delivered intraperitoneally (IP) in a 5-ml/kg volume of isotonic saline vehicle; the saline vehicle injection volume was also 5 ml/kg, IP.

Surgery

A single bipolar electrode (MS-303-2; Plastic Products Company, Roanoke, VA) was stereotaxically implanted in the left basolateral amygdala of each of the 52 subjects, under sodium pentobarbital anesthesia (65 mg/kg, IP). The electrode tip was aimed at a site 2.8 mm posterior, 5.0 mm left, and 8.5 mm ventral to the skull surface at bregma, with the incisor bar set at −3.3 mm [coordinates from Paxinos and Watson (14)]. The electrode was secured to the skull with stainless-steel screws and dental acrylic. Powdered tetracycline was sprinkled on the incision before suturing to reduce infection.

Kindling

Following a postsurgical recovery period of at least 5 days, each rat was stimulated (1 s, 60 Hz, 400 μA) three times per day, 5 days per week, for 3 weeks. There was a minimum of 2 h between consecutive stimulations. As is usual [see (17,19)], the initial stimulations produced no behavioral response other than a momentary behavioral arrest, but by the end of the 45 kindling stimulations, almost every stimulation elicited a clonic convulsion characterized by facial clonus, forelimb clonus, rearing, and loss of equilibrium. The measure of convulsion severity was the duration of forelimb clonus elicited by each stimulation; this measure has been shown to be particularly reliable and sensitive to a variety of pharmacologic manipulations [e.g., (12,15)]. Of the original 52 subjects, six failed to complete the kindling phase of the experiment: Five dislodged their electrode caps and one developed running fits.

Procedure

After the 45 kindling stimulations was the baseline phase, which was composed of three bidaily convulsive stimulations; the bidaily stimulation schedule was maintained until the end of the experiment. Following the baseline phase at 48-h intervals were the saline baseline test and then the drug baseline test. On the saline and the drug baseline tests, each rat was injected with saline vehicle or pentobarbital (20 mg/kg), respectively, followed 20 min after the injection by a righting test and 1 h after the injection by a convulsive stimulation. During the righting test, each rat was placed on its back on a surface of commercial bedding material, and the latency of the righting reflex (i.e., the amount of time for each rat to roll over onto all four paws) was recorded [see (20,25)]. The eight rats that displayed <20 s of forelimb clonus on the saline baseline test and the two rats that displayed >5 s of forelimb clonus on the drug baseline test were not studied further. The remaining 35 rats were divided into four groups so that the mean duration of forelimb clonus and the mean latency of the righting reflex on both the saline and drug baseline tests were approximately equal for each group.

The tolerance-development phase of the experiment consisted of 20 bidaily trials in which each rat received pentobarbital or saline 1 h before a convulsive stimulation. This bidaily injection schedule was employed to reduce the possibility of drug accumulation (23). On each trial, the saline group (n = 8) received the saline vehicle, the high-dose group (n = 9) received 50 mg/kg of pentobarbital, and the low-dose group (n = 9) received 10 mg/kg of pentobarbital. The ascending-dose group (n = 9) started at 10 mg/kg of pentobarbital, and then each rat received a 1 mg/kg dose increase each time it demonstrated at least 5 s of forelimb clonus, irrespective of the responses of the other rats in the group. The maximum doses reached by the ascending-dose rats during the tolerance-development phase ranged from 18–26 mg/kg. The tolerance test was identical to the drug baseline test: The test dose of pentobarbital was 20 mg/kg and tolerance to pentobarbital's anticonvulsant and ataxic effects was assessed. The measure of the ataxic effect was the percentage of rats that righted themselves within 1.5 s: All subjects righted themselves within 1.5 s on the saline baseline test.

Statistical Analyses

The statistical significance of the results was assessed nonparametrically (22) because of the heterogeneity of variance and nonnormal distribution of the data. Comparisons among the groups were assessed using Kruskal–Wallis one-way analysis of variance (ANOVA) by rank or χ^2 tests, followed by appropriate posthoc pairwise comparisons. The significance level for all comparisons was $p < 0.05$.

Histology

At the conclusion of behavioral testing, all rats were killed with CO_2 according to the Canada Council on Animal Care guidelines. Their brains were removed, preserved in formalin, frozen, sliced along the coronal plane, mounted on slides, and then stained with cresyl violet to confirm the location of the electrode tips.

RESULTS

Figure 1 illustrates the mean forelimb clonus durations for all four groups on the three critical trials: the saline baseline test, drug baseline test, and tolerance test. The groups did not differ significantly in their responsiveness to the convulsive stimulation on the saline baseline test or in their responsiveness to the anticonvulsant effect of pentobarbital on the drug baseline test. However, on the tolerance test, only the rats in the ascending-dose group displayed substantial tolerance to the anticonvulsant effect of pentobarbital. There was an overall significant difference among the four groups in the duration of forelimb clonus on the tolerance test (Kruskal–Wallis; $p < 0.0001$), and posthoc analyses revealed that the ascending-dose group displayed significantly more clonus than the high-dose, low-dose, and saline groups ($p < 0.05$), and that the latter three groups did not differ significantly among themselves ($p < 0.05$).

Figure 2 illustrates the percentage of rats in each group

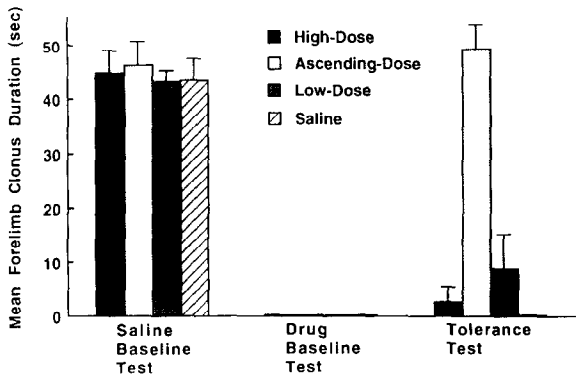

FIG. 1. Effect of different treatment doses on the development of tolerance to the anticonvulsant effect of pentobarbital. The mean forelimb clonus durations are shown for each group on the key test trials: saline baseline, drug baseline, and tolerance. The rats that received the ascending-dose regimen became significantly tolerant to the anticonvulsant effect of pentobarbital, whereas the rats that received the high-dose, low-dose, or saline regimens did not. The SEM for each group is indicated.

that righted themselves within the 1.5-s criterion on the three critical trials. On the saline baseline test, every rat righted itself within 1.5 s; however, only 38% of the rats righted themselves within 1.5 s on the drug baseline test. On the drug tolerance test, there was a significant overall difference in the proportion of rats in the four groups that righted themselves within 1.5 s (χ^2; $p < 0.02$). Posthoc tests revealed that a significantly greater proportion of the high-dose group responded within 1.5 s than that of the ascending-dose and saline groups ($p < 0.05$), and that other differences among the groups were not statistically significant ($p > 0.05$).

Histologic analysis confirmed that all of the electrode tips had been positioned in or on the margins of the amygdala complex.

DISCUSSION

The major finding of this experiment was that an ascending-dose regimen facilitated the development of tolerance to pentobarbital's anticonvulsant effect on kindled convulsions. Because both previous studies of the effects of ascending-dose regimens on tolerance development [i.e., (10,11)] researched the anticonvulsant effect of benzodiazepines, the present results constitute the first evidence that the facilitatory effect of ascending-dose regimens is not restricted to benzodiazepines. With the exception of the drug under investigation, the methods of the present experiment were similar to those of Kalynchuk et al. (10). Thus, although the two experiments, when considered together, establish the generality of the facilitatory effect of ascending-dose regimens on tolerance development over two different anticonvulsant drugs, they do little to establish its generality along other dimensions. For example, can ascending-dose regimens facilitate the development of tolerance to anticonvulsant drug effects: a) in species other than rats, b) when the drugs are administered on other than a bidaily schedule, or c) when the convulsions are not kindled? The observations of Killam et al. (11) indicate that the answer to all three questions is yes. During a study of the anticonvulsant effects of two benzodiazepines (diazepam and RO 5-4023) on photically elicited seizures in a strain of seizure-prone baboons, *Papio papio*, they found that chronic daily low doses of either drug, which initially blocked epileptic activity, became ineffective; and therefore, they increased the doses. But they found that the new doses provided only temporary relief, and they increased them again. Eventually, even doses that were high enough to produce prohibitive side effects would not block the seizures. In contrast, moderately high doses administered daily from the start remained effective against seizures for the duration of the study.

Why do ascending-dose protocols induce such high levels of tolerance to anticonvulsant drug effects? Given that ascending-dose protocols facilitate the development of tolerance to both benzodiazepines and barbiturates, the answer to this question is unlikely to be derived from a consideration of the pharmacokinetics of the particular drugs that have been used to demonstrate the effect. Instead, we believe that the answer lies in a consideration of the drugs' interactions with concurrent patterns of epileptic neural activity. The mechanism underlying tolerance development with the ascending-dose protocol may be similar to that proposed in demonstrations of contingent tolerance (3) as summarized by the drug-effect theory of functional drug tolerance (16,18). According to this theory, functional tolerance is an adaptation to the disruptive effects of drugs on concurrent patterns of neural activity, rather than to drug exposure per se. Thus, repeated "task performance" under the influence of the drug is an important factor in the development of tolerance to a particular drug effect [see reviews (4,7,24) for examples]. If a dose of drug is initially just enough to suppress the response, and the development of tolerance allows the response to reappear, each small increment of dosage provides the opportunity for task performance to add a further increment of tolerance. In contrast, if there were no task performance under the influence of the drug, there would be no stimulus for tolerance development. This condition can be achieved either with constant low doses that have become ineffectual after the development of a small amount of tolerance or with constant high doses that continue to prevent the seizure from occurring. In this way, we believe that drug tolerance to anticonvulsant drug effects is like other forms of neural adaptation [see (16)]; and thus, it is not unreasonable to expect that it would be facilitated by shaping: starting with slight perturbations of epileptic neural activity and

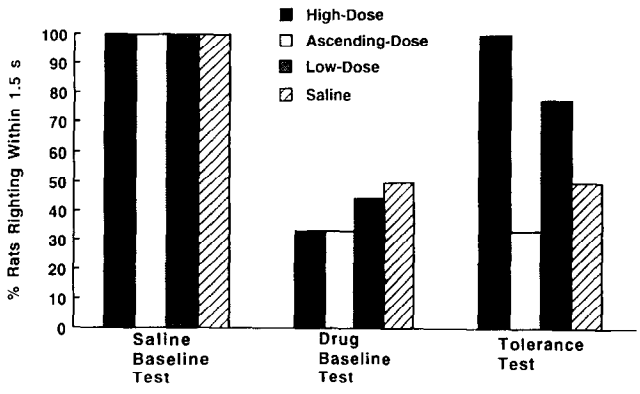

FIG. 2. Effect of different treatment doses on the development of tolerance to the ataxic effect of pentobarbital. The percentage of rats responding within 1.5 s are shown for each group on the key test trials: saline baseline, drug baseline, and tolerance. The rats that received the high-dose regimen displayed the greatest degree of tolerance to pentobarbital's ataxic effect.

progressively increasing the magnitude of perturbations, as would occur with an ascending-dose regimen.

The second important finding of this study was that the ascending-dose protocol did not facilitate the development of tolerance to the ataxic effect of pentobarbital. Tolerance to this effect was greatest in the high-dose group [cf. (1,9)]. Thus, there was a clear dissociation between the anticonvulsant and ataxic effects of pentobarbital within the same subject. Why did the ascending-dose regimen facilitate the development of tolerance to pentobarbital's anticonvulsant effect, whereas in the same subjects it did not facilitate the development of tolerance to its ataxic effect? Previous studies employing chronic drug administration have shown that tolerance to the ataxic effect of diazepam, clonazepam, clobazam (21), and abecarnil (13), and to the anesthetic effect of phenobarbital (5) developed independently from tolerance to their anticonvulsant effect. These dissociations in tolerance development have been assumed to occur via different mechanisms of action underlying the different drug effects (5). Given that this is the first report of such a dissociation using an ascending-dose regimen, it would be premature to offer a specific interpretation. However, the dissociation may be explained in terms of the drug-effect theory of tolerance. The anticonvulsant effect of pentobarbital was potentially expressed for all three drug conditions on each bidaily tolerance development trial, because the convulsive stimulation was delivered during the periods of drug exposure. However, because the task performance was optimally expressed with the ascending-dose condition, it produced the greatest tolerance to the anticonvulsant effect. The ataxic effect of pentobarbital, although assessed with the righting test only on the drug baseline and tolerance test trials, was potentially expressed for all three drug conditions on each bidaily tolerance development trial, because the ataxic effect is generally an inevitable consequence of drug exposure in freely moving subjects. However, the task performance was optimally expressed in the high-dose group, because the higher dose produced the greater ataxia, and so this group displayed the greatest tolerance to the ataxic effect. It is also possible that the dissociation could have resulted from the production of anticonvulsant and ataxic effects by different metabolites of pentobarbital, from some fundamental difference between the neural mechanisms underlying kindled convulsions and the righting reflex, or from an interaction of pharmacokinetic and neural factors.

The present results may ultimately have implications for the treatment of epilepsy. It is common clinical practice in the treatment of epilepsy to begin treating a patient with a low dose of anticonvulsant medication, and then to increase the dose incrementally each time seizures recur (8). However, the present results, in combination with those of Kalynchuk et al. (10) and Killam et al. (11), suggest that this procedure may facilitate the development of tolerance to the therapeutic action of the medication. Thus, in some cases, a better therapeutic strategy may be to commence treatment with a higher dose of medication, although side effects may be more problematic at the higher dose. Admittedly, there is a substantial difference between kindled convulsions in rats and epilepsy in humans, but the ascending-dose effect has already displayed generality across species (rats and baboons), types of seizures (kindled and photic), and drugs (diazepam, RO5-4023, and pentobarbital)—enough generality to warrant the attention of clinical researchers.

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