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Discriminative Stimulus Properties of Phenytoin in the Pigeon: Determination Via a Cumulative Dosing Procedure[1]

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CLARK, R., H. SCHLINGER AND A. POLING. *Discriminative stimulus properties of phenytoin in the pigeon: Determination via a cumulative dosing procedure*. PHARMACOL BIOCHEM BEHAV **35**(3) 537–541, 1990.—Pigeons were trained in a cumulative dosing procedure to emit one response in the presence of 15 mg/kg phenytoin and another response in the absence of phenytoin. After reliable discrimination was established, generalization tests with other anticonvulsant drugs were conducted. Ethosuximide (20–120 mg/kg) and methsuximide (25–100 mg/kg) engendered very little phenytoin-appropriate responding. Clonazepam (0.125–1 mg/kg) engendered more phenytoin-appropriate responding, but less than the training dose of phenytoin. Similar results were obtained when these drugs were administered in conventional single dose per session generalization tests. When tested in that fashion, mephenytoin (80 and 160 mg/kg) engendered some phenytoin-appropriate responding, but less than the training dose of phenytoin. Administering 10 mg/kg pentylenetetrazol in combination with 5, 10, 15, and 20 mg/kg cumulative doses of phenytoin reduced phenytoin-appropriate responding relative to levels obtained with these doses of phenytoin alone.

Phenytoin Drug discrimination Cumulative dosing Anticonvulsant drugs Pigeons

CUMULATIVE dosing procedures have been used for many years in pharmacology [e.g., (13,14)]. Such procedures, which involve repeatedly administering a drug and measuring its effects over the course of a relatively long session, yield a great deal of data in a short time. In 1966, Boren (2) suggested that the technique could be profitably employed to examine the behavioral effects of drugs, and this has proven true [e.g., (3, 12, 15)]. For example, cumulative dosing has been used effectively to examine the discriminative stimulus properties of drugs [e.g., (1,6)].

The purpose of the present study was to examine further the discriminative stimulus properties of the anticonvulsant drug phenytoin by using a cumulative dosing procedure. A prior study has shown that pigeons trained under a conventional two-key drug discrimination procedure learned to discriminate 5 mg/kg phenytoin from saline injections (8), but over 50 training sessions were required for the discrimination to be acquired. The first phase of the present study involved discrimination training followed by cross-drug generalization testing with cumulative dosing. A previous study has demonstrated that chlorpromazine, *d*-amphetamine, diazepam, and phenobarbital failed to produce phenytoin-like patterns of responding in pigeons trained to discriminate phenytoin from saline injections (8). In the present study, clonazepam, ethosuximide, and methsuximide, all drugs with anticonvulsant actions, were examined in generalization tests. The ability of pentylenetetrazol to antagonize the discriminative cue of phenytoin was also examined under a cumulative dosing procedure. The second phase partially replicated generalization tests under conventional single dose per session procedures and also examined the extent to which mephenytoin would engender phenytoin-like patterns of responding. Some data suggest that cumulative and noncumulative dosing procedures produce similar results in generalization tests (1), but the generality of this finding is uncertain.

METHOD

Subjects

Six experimentally naive adult female White Carneau pigeons, maintained at 80% of their free-feeding weights (400–490 g), served as subjects. Each bird was individually housed with unlimited access to grit and water in a constantly illuminated and temperature-controlled room.

Apparatus

Three operant conditioning chambers were used. Each was 38 cm high, 30 cm wide, and 40 cm long and was equipped with three response keys located symmetrically on the front wall 24 cm above the floor. Only the left and right keys were used. An opening centered horizontally below the keys permitted access to a hopper filled with mixed grain when the hopper was raised. A clear 7-W

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white bulb located in the ceiling provided ambient illumination. Ventilation and masking noise were supplied by an externally mounted exhaust fan. Scheduling of experimental events and data collection were accomplished through the use of a Digital Equipment Corp. (Maynard, MA) PDP/8E minicomputer using interface and software (SUPERSKED) provided by State Systems (Kalamazoo, MI).

Behavioral Training Procedure

Birds were first trained to peck each key under a fixed-ratio 1 (FR 1) schedule, in which each peck produced 3-sec access to food. Over several sessions the FR value was gradually increased to 20. Each key was lighted in white during keypeck training; only one key was operative and lighted in an initial training session.

As soon as each bird responded reliably under the FR 20 schedule, discrimination training began. The training procedure employed was similar to that described elsewhere (1). Training sessions began with a 15-min timeout (TO) component. During this component the chamber was dark and food was never delivered. The TO component was followed by a component in which the chamber and both keys were lighted and an FR 20 schedule was in effect on one key and extinction on the other. The key on which the FR 20 schedule was arranged depended on whether phenytoin had been administered. For three birds, the left key was designated as phenytoin appropriate. The right key was phenytoin appropriate for the other three. The FR 20 component ended after 15 min. At that time, a second TO component began. TO and FR components then alternated at 15-min intervals until five of each had been completed, when the session ended. The FR 20 reset at the beginning of each FR component. Sessions were conducted five or six days per week at about the same time each day.

At the beginning of each TO component, the bird was removed from the chamber and handled as if an IM injection were being administered. Handling and injection at the start of each TO were a necessary part of the cumulative dosing procedure (below), hence they were arranged from the start of discrimination training. An IM injection of isotonic saline solution was administered prior to every fourth TO component, on average. Control injections were not given prior to each TO component to prevent the tissue damage that would result from giving 5 injections per session. During each training session, 15 mg/kg phenytoin was administered IM at the beginning of one of the TO components, selected at random. Phenytoin-appropriate responses were reinforced in all subsequent FR components.

Training continued until percent stimulus-appropriate responses prior to the first food delivery in each component was 80% or higher for each bird during 10 consecutive sessions. This measure was determined by dividing the number of stimulus-appropriate responses in an FR component prior to the first delivery of food in that component by the total number of responses emitted in this period.

Cumulative Dosing

Cumulative dose-response data were obtained for 5, 10, 15, and 20 mg/kg phenytoin. This was accomplished by injecting isotonic saline at the beginning of the first TO component and 5 mg/kg phenytoin at the beginning of each of the other four TO components. Cumulative dose-response data were obtained in similar fashion for ethosuximide (20, 40, 80, and 120 mg/kg), clonazepam (0, 13, 0.25, 0.5, and 1 mg/kg), and methsuximide (25, 50, 75, and 100 mg/kg). During generalization tests (i.e., in sessions when drugs other than phenytoin were given), in each FR component food was delivered following the twentieth response on either key. After food delivery, the chamber was darkened and both keys were rendered inoperative for the balance of the 15-min FR component.

In the last phase of the study, pentylenetetrazol (10 mg/kg) was administered in an attempt to determine if it would alter the stimulus control exercised by 5, 10, 15, and 20 mg/kg cumulative doses of phenytoin. Pentylenetetrazol was injected on one side of the breast muscle at the beginning of the first TO component and 5 mg/kg injections of phenytoin were given on the other side at the beginning of each TO component after the first. Pentylenetetrazol (10 mg/kg) was also tested alone. When this was done, pentylenetetrazol was injected at the beginning of the first TO component and saline injections were given at the beginning of each of the following four TO components.

Conventional Training and Testing

Following cumulative dose-response determinations, all subjects were exposed to a conventional two-key drug discrimination training procedure, described in detail elsewhere (4). Under this procedure, an injection of 15 mg/kg phenytoin or isotonic saline preceded each session by 15 min. Responding on one key was reinforced under an FR 20 schedule; the key on which reinforcement was available depended on whether phenytoin or saline was administered. Sessions were 15 min in length and data were recorded as described above. Training continued for 15 sessions. During the last 5 of these sessions, percent stimulus-appropriate responses prior to the first food delivery in each component was 80% or higher for each bird. Following training, generalization tests were conducted with 40 mg/kg ethosuximide, 0.13 mg/kg clonazepam, and 25 mg/kg methsuximide. Each of these drugs was given 15 min prior to the test session. Test sessions ended as soon as 20 responses were emitted on one or the other key. Food was not delivered during test sessions. Generalization tests were also conducted with mephenytoin (80 and 160 mg/kg), which was administered 8 hr prior to the test session. All drug doses and presession injection times were based on prior findings from our laboratory (5,11). The long presession injection interval required with mephenytoin made it impossible to test this drug under the cumulative dosing procedure.

Drugs

All drugs and isotonic saline were administered IM at a volume of 1 ml/kg. Phenytoin was injected as a commercially prepared solution (Parke-Davis, Morris Plains, NJ) diluted with distilled water. Methsuximide (Warner Lambert, Ann Arbor, MI) and clonazepam (Hoffmann-LaRoche, Nutley, NJ) were dissolved in a vehicle containing 80% propylene glycol and 20% ethyl alcohol. Mephenytoin (Sandoz, East Hanover, NJ) was dissolved in dimethyl sulfoxide. Isotonic saline solution was the vehicle for ethosuximide and pentylenetetrazol. Drugs used in generalization tests were selected because they represent three different chemical classes (ethosuximide and methsuximide are succinimides, clonazepam is a benzodiazepine, and mephenytoin, like phenytoin, is a hydantoin). Moreover, previous research in our laboratory [e.g., (11)] has revealed appropriate doses and presession injection intervals for examining the effects of these agents in pigeons.

RESULTS

Figures 1 and 2 present data for drugs that were administered via both the cumulative dosing and conventional dosing procedures. Data for mephenytoin, which was only administered via the latter procedure, appear in Table 1. Table 2 portrays the results of

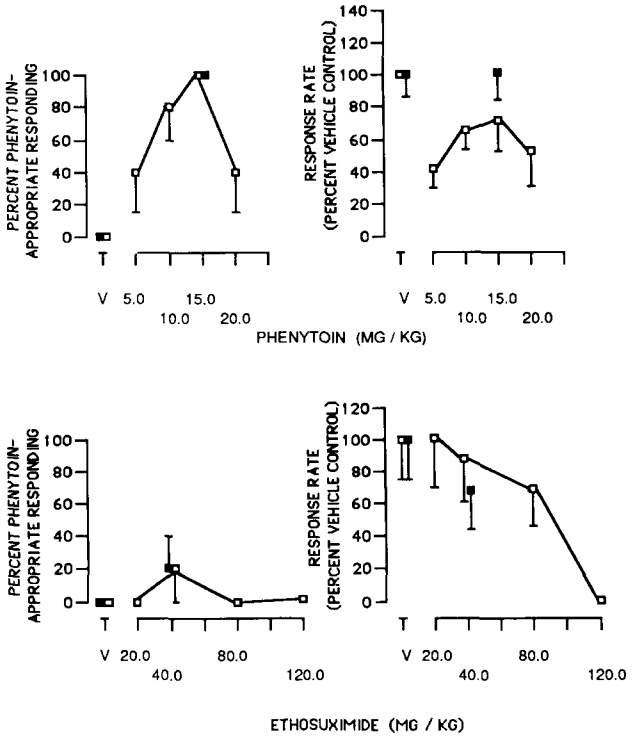

FIG. 1. Dose-effect relations for phenytoin and ethosuximide administered via cumulative (open squares) and conventional, single dose per session (closed squares) procedures. Data at C indicate performance during vehicle control sessions. The measure of variability is one standard error.

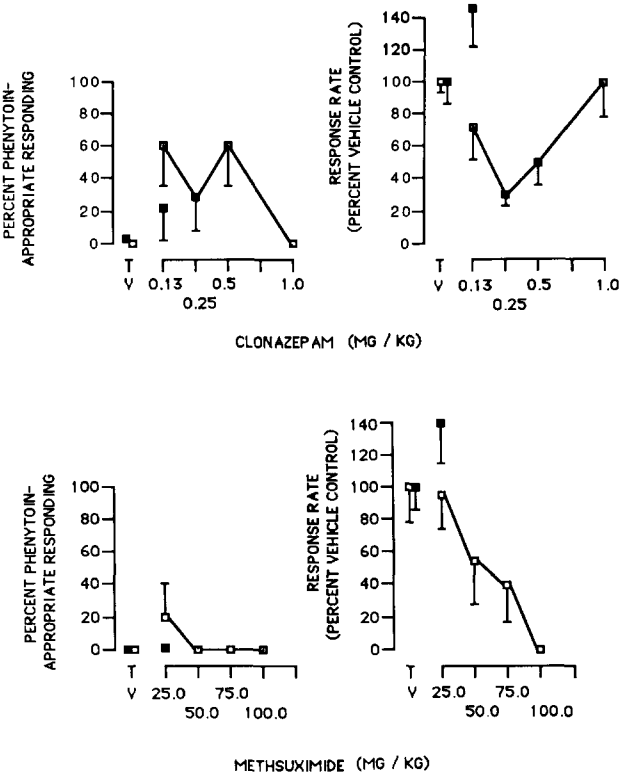

FIG. 2. Dose-effect relations for clonazepam and methsuximide administered via cumulative (open squares) and conventional, single dose per session (closed squares) procedures. Data at C indicate performance during vehicle control sessions. The measure of variability is one standard error.

administering pentylenetetrazol alone and in combination with phenytoin.

The behavior of all birds eventually came under the discriminative control of 15 mg/kg phenytoin injections. On average, 57 sessions were required to meet the initial training criterion, with a range across birds of 44 to 67 sessions. Percent phenytoin-appropriate responses increased with cumulative dose from 5 to 15 mg/kg, but decreased at 20 mg/kg. At 10 and 15 mg/kg cumulative doses of phenytoin, 80 and 100% of responses were phenytoin appropriate, but only 40% of responses were phenytoin appropriate at 20 mg/kg. All cumulative doses of phenytoin reduced response rates relative to saline control, although the magnitude of this effect was not obviously dose related.

None of the drugs tested for generalization produced levels of phenytoin-appropriate responding equal to the criterion established for discrimination (80%). All cumulative doses of ethosuximide and methsuximide were associated with low (20% or less) levels of phenytoin-appropriate responding. The 0.13 and 0.5 doses of clonazepam produced 60% phenytoin-appropriate responses; 0.25 and 1.0 mg/kg doses of clonazepam yielded less phenytoin-appropriate responding. All test drugs reduced response rates relative to control levels at all cumulative doses.

Results of single-dose testing did not differ systematically from those of cumulative dosing with respect to percent phenytoin-appropriate responses. Rate data did, however, differ. With all drugs, higher rates were obtained with single-dose testing than with cumulative dosing. Mephenytoin, which was not tested with the cumulative dosing procedure, produced 60% phenytoin-appropriate responding when administered at doses of 80 and 160 mg/kg 8 hours prior to testing. These doses reduced response rate relative to those obtained in saline control sessions.

Pentylenetetrazol alone (10 mg/kg) reduced response rate relative to saline control and did not engender phenytoin-appropriate responding. Cumulative doses of 5, 10, 15, and 20 mg/kg phenytoin administered in combination with 10 mg/kg pentylenetetrazol produced slightly less phenytoin-appropriate responding than these same doses administered alone. Correlated t-tests revealed, however, that mean percent phenytoin-appropriate responding at all phenytoin doses did not differ significantly ($p>0.05$) as a function of the presence or absence of pentylenetetrazol. In all cases, response rates were much lower when

TABLE 1

RESULTS OF GENERALIZATION TESTS WITH MEPHENYTOIN

Drug	Dose (mg/kg)	Percent Phenytoin-Appropriate Responses*	Response Rate (Percent Vehicle Control)*
Mephenytoin	0†	0 (0)	100 (0)
Mephenytoin	80†	60 (25)	80 (15)
Mephenytoin	160†	60 (25)	71 (19)

*Numbers outside parentheses represent the group mean, numbers inside parentheses represent one standard error. Rate data are expressed as percent vehicle control.

†Mephenytoin vehicle administered in one injection 8 hr prior to testing.

TABLE 2

RESULTS OF ANTAGONISM TESTS WITH PENTYLENETETRAZOL

Drug	Dose (mg/kg)	Percent Phenytoin-Appropriate Responses*	Response Rate (Percent Vehicle Control)*
Pentylenetetrazol	10†	6 (6)	43 (18)
Pentylenetetrazol + Phenytoin	10 5	20 (20)	29 (14)
Pentylenetetrazol + Phenytoin	10 10	60 (25)	29 (14)
Pentylenetetrazol + Phenytoin	10 15	60 (25)	17 (8)
Pentylenetetrazol + Phenytoin	10 20	60 (25)	16 (8)

*Numbers outside parentheses represent the group mean, numbers inside parentheses represent one standard error. Rate data are expressed as percent vehicle control.

†Administered in one injection 15 min prior to testing.

pentylenetetrazol was combined with phenytoin than when phenytoin was administered alone.

DISCUSSION

All pigeons in the present study eventually learned to discriminate phenytoin from control injections. On average, 57 sessions was required to meet the initial discrimination criterion in the present study. In a prior investigation from our lab in which noncumulative dosing was employed, an average of 61 sessions was required to bring the behavior of pigeons under the discriminative stimulus control of 5 mg/kg phenytoin (8). Although the cumulative dosing procedure clearly reduced training time in a prior study in which codeine, ketamine, and methohexital were established as discriminative stimuli for monkey's lever pressing (1), no clear decrease in training time was evident with phenytoin.

In some situations, results with cumulative dosing may differ from those obtained with conventional procedures. This was demonstrated in a study investigating the effects of cumulative and noncumulative administration of phencyclidine, pentobarbital, and *d*-amphetamine on the behavior of pigeons under a repeated acquisition procedure (12). Results with phencyclidine and pentobarbital indicated that the rate-decreasing and error-increasing effects were greater with noncumulative dosing. In contrast, cumulative dosing produced larger effects with *d*-amphetamine.

The present study revealed no systematic difference in the results of cumulative and noncumulative dosing with respect to percent phenytoin-appropriate responses. This agrees with the results of an earlier cumulative dosing study (1), which when compared to those of single dose per session studies revealed no major qualitative differences in the pattern of drug generalizations obtained when test drugs were administered to monkeys trained with codeine, methohexital, or ketamine as a discriminative stimulus. In contrast to discrimination data (i.e., percent phenytoin-appropriate responses), rate data in the present study did differ according to whether a given dose was administered cumulatively or as a single injection. Rate data are characteristically of secondary importance in drug discrimination studies.

Earlier studies have demonstrated that phenytoin can be established as a discriminative stimulus in pigeons (8) and in rats (9). Generalization tests with pigeons revealed that chlorpromazine, *d*-amphetamine, diazepam, and phenobarbital failed to produce phenytoin-like patterns of responding (8). In the present study, the anticonvulsant drugs ethosuximide, methsuximide, clonazepam, and mephenytoin also failed to engender phenytoin-like patterns of responding. Previous investigations have found that not all anticonvulsant drugs have similar discriminable properties (4, 7, 10, 11), and the results of the present generalization tests are in agreement with this finding. It is, of course, possible that anticonvulsants not tested in the present study (e.g., carbamazepine, primadone, ethotoin) would have produced phenytoin-like patterns of responding.

Phenytoin affects many different neuropharmacological systems and mechanisms, therefore it is not possible to account simply for its anticonvulsant and other actions (16). Phenytoin does not block seizures induced by pentylenetetrazol or other convulsants, or by electrical stimulation of the brain (16). Although pretreatment with pentylenetetrazol reduced phenytoin-appropriate responding in the present study, it did so only when responding was reduced to a very low rate. This suggests that the drug interaction involved something other than simple pharmacological antagonism.

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