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Chronic Effects of Clonazepam, Phenytoin, Ethosuximide, and Valproic Acid on Learning in Pigeons as Assayed by a Repeated Acquisition Procedure[1]

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POLING, A., E. BLAKELY, W. WHITE AND M. PICKER. *Chronic effects of clonazepam, phenytoin, ethosuximide, and valproic acid on learning in pigeons as assayed by a repeated acquisition procedure.* PHARMACOL BIOCHEM BEHAV **24**(6) 1583–1586, 1986.—The acute and chronic effects of the antiepileptic drugs clonazepam (0.06, 0.13, and 0.25 mg/kg), phenytoin (2.5, 5, and 7.5 mg/kg), ethosuximide (40, 80, and 120 mg/kg), and valproic acid (40, 80, and 120 mg/kg) were evaluated in pigeons responding under a repeated acquisition procedure. At certain doses, acute administrations of all drugs impaired learning (i.e., increased errors) and reduced rate of responding. Appreciable tolerance developed to these effects with chronic exposure, although the physiological mechanism responsible for this outcome is unknown.

Clonazepam Phenytoin Ethosuximide Valproic acid Anticonvulsant drugs
Repeated acquisition procedure Tolerance Pigeons

A number of recent investigations, reviewed elsewhere [4], have examined the acute effects of anticonvulsant drugs on the operant behavior of nonhumans. Findings reveal qualitative and quantitative differences in the acute behavioral effects of various anticonvulsant drugs. Moreover, whether a given drug and dose interferes with behavior appears to depend on how the response in question is maintained. Such findings may be of interest, but epileptic humans typically receive anticonvulsant medications chronically. Hence, if basic research with nonhumans is intended to further understanding of drug effects in humans, investigations of the chronic effects of anticonvulsants appear to be merited.

To date, however, few such studies have appeared. In one [5], complete or nearly complete tolerance developed to the accuracy-reducing effects of clonazepam and valproic acid when administered chronically to pigeons responding under a delayed-matching-to-sample procedure. Whether given acutely or chronically, neither phenytoin nor ethosuxmide affected accuracy. When given acutely, each of the four anticonvulsants typically reduced rate of responding to the sample stimulus. Tolerance did not develop to the rate-decreasing effects of any of the drugs tested.

The purpose of the present study was to explore further the chronic effects of selected anticonvulsant drugs by examining in pigeons responding under a repeated acquisition procedure the effects of repeated administrations of clonazepam, phenytoin, ethosuximide, and valproic acid. A previous study [3] found that, when given acutely, all of these drugs except ethosuximide strongly interfered with learning (i.e., increased errors) under the repeated acquisition procedure. However, the effects of chronic exposure were not determined.

METHOD

Subjects

Eight experimentally-naive White Carneaux pigeons, maintained at 80% of free-feeding weights, served as subjects. Each bird was individually housed with unlimited access to water and grit in a constantly-illuminated room.

Apparatus

Three Lehigh Valley Electronics (BRS/LVE, Lehigh Val-

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ley, PA) pigeon chambers, measuring 32 cm long, 36 cm high, and 35 cm wide, were employed. In each chamber, three response keys 2.5 cm in diameter were located 23 cm from the bottom of the front wall, approximately 5.5 cm apart. Each key could be illuminated in white, red, yellow, or blue-green. A minimum of 0.2 g pressure was required for key operation. An aperture centered horizontally on the front wall 7.5 cm above the floor allowed access to a hopper filled with mixed grain when the hopper was raised. When raised, the hopper was illuminated by a 7-W white bulb. A 7-W white bulb (houselight) centrally mounted 33 cm from the chamber floor provided ambient illumination and a fan provided masking noise and ventilaion. Scheduling of experimental events and data collection were accomplished through the use of a Digital Equipment Corporation (Maynard, MA) PDP/8 minicomputer using interfacing and software (SUPERSKED) supplied by State Systems, Inc. (Kalamazoo, MI).

Behavioral Procedure

Details of initial training, during which subjects learned to peck each key when illuminated in white, red, yellow, or blue-green, are described by Picker and Poling [3]. After initial training, pigeons received food (3 sec) dependent upon five successful completions of a four-response chain. Each component in the chain (response sequence) was associated with a different key color (yellow, blue-green, white, and red, from the first to fourth component, respectively), three response options were available during each component (i.e., left-key peck, center-key peck, and right-key peck), and the correct response for each component was defined by spatial locus. All correct responses resulted in a 0.5-sec flash of the magazine light (intended to serve as a conditioned reinforcer), followed immediately by presentation of the key color associated with the next component. Incorrect responses (e.g., pecking the right key when the left key was designated as correct) were followed by a 3-sec timeout, during which the keylights and houselight were darkened and responses had no programmed consequences. Incorrect responses (errors) did not reset the response chain; that is, the stimuli presented after the timeout (and the response designated as correct) were identical to those arranged at the time of the error.

The sequence of responses designated as correct changed on a daily basis and sequences were selected according to criteria outlined by Thompson [8]. On Monday, for example, the correct sequence might be peck left, peck right, peck left, peck center, whereas the sequence center, left, right, center might be designated as correct on Tuesday. Throughout the study, sessions ended after 1 hr or 70 food deliveries, whichever occurred first, and were conducted 6 days per week, at about the same time each day. During each session, response rate, total responses, percentage of total responses that were incorrect (errors), and number of errors made before the delivery of each reinforcer were recorded.

Pharmacological Procedure

After the percentage of errors per session for individual birds showed no obvious trend across sessions (40–80 sessions), the acute effects of clonazepam (0.06, 0.13, and 0.25 mg/kg), phenytoin (2.5, 5, and 7.5 mg/kg), ethosuximide (40, 80, and 120 mg/kg) and valproic acid (40, 80, and 120 mg/kg) were evaluated. Drug doses were selected on the basis of prior findings from our laboratory [3,5], and are somewhat higher than those typically used in the clinical management of epilepsy. Clonazepam (Hoffmann-La Roche, Nutley, NJ) was dissolved in a vehicle consisting of 4 parts propylene glycol, 1 part ethyl alcohol and 5 parts distilled water diluted with isotonic saline solution to the desired injeciton volume, which was 1 ml/kg for all drugs. Ethosuximide (Warner-Lambert, Ann Arbor, MI) and valproic acid (Saber Laboratories, St. Louis, MO) were dissolved in distilled water; sufficient sodium hydroxide to neutralize the drug to the sodium salt was added to the valproic acid preparation. Phenytoin was injected as a commercially prepared solution (Parke-Davis, Morris Plains, NJ) diluted with isotonic saline solution. Two birds received each drug. During acute dosing, each dose was given once, in an irregular sequence that varied across subjects. During this phase of the study, drugs were administered in a BCDBBCD design, where B represents baseline sessions; C, vehicle control sessions; and D, drug sessions. All injection were given intramuscularly 30 min prior to the experimental session.

Following acute dose-response determinations, subjects were given 1 baseline session with no injection followed by 5 vehicle control sessions, after which chronic dosing was begun. During chronic exposure, a subject received the lowest dose of the drug being evaluated prior to 10–12 consecutive sessions, then the middle dose prior to 10–12 consecutive session, and finally the highest dose prior to 30 consecutive sessions. Injections were given each day during the chronic regimen, but behavioral testing did not occur on Sundays.

RESULTS

The acute and chronic effects of each drug on percent errors and response rates of individual birds are shown in Fig. 1, which depicts data for control sessions, acute drug sessions, and the final session of chronic exposure to each drug dose. As in our earlier studies (e.g., [3]), percent errors for control sessions reflect performance during predrug sessions until a number of reinforcers equivalent to that obtained during drug sessions was earned. This was done since, in the absence of drug, the majority of errors occurred early in the session; with repeated exposure to the four-response sequence, the number of errors per reinforcer declined rapidly. Given this, if a drug slowed responding so that few reinforcers were obtained, it might appear that learning was impaired relative to control sessions in which more reinforcers were obtained. Comparing drug data with appropriate control data (i.e., data representing an equivalent number or reinforcers) avoids this potential confound.

During acute dose-response testing, percent errors by both subjects exposed to clonazepam feel outside the control range at two of the three doses. Phenytoin and valproic acid also increased errors beyond the control range in the two subjects exposed to each of these drugs. With both drugs, this occurred at the highest dose for one subject and at the middle dose for the second, and those subjects in which error rates were increased by the middle dose failed to respond when initially exposed to the high dose. When administered acutely, the highest dose of ethosuximide increased errors to beyond the control range in both subjects; lower doses failed to have such an effect. Appreciable tolerance developed to the accuracy-reducing effects of clonazepam, phenytoin, ethosuximide, and valproic acid. During the final session of exposure to the two lower doses of clonazepam, and to all doses of phenytoin, ethosuximide, and valproic acid, percent errors was not above the control range.

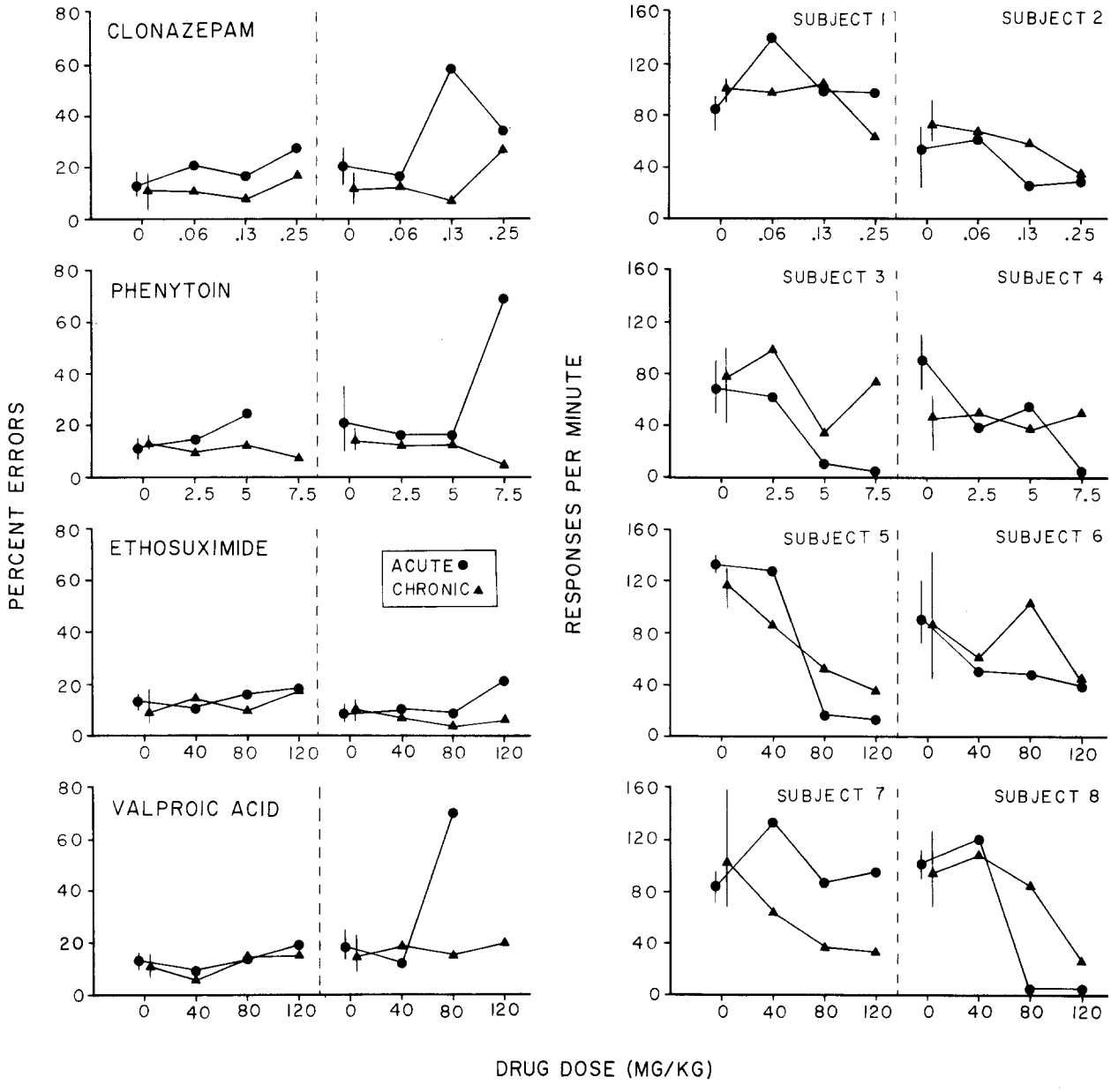

FIG. 1. Acute (circles) and chronic (triangles) effects of four anticonvulsant drugs on percent errors (left frames) and response rates (right frames) of individual pigeons. Two birds were exposed to each drug, and the reported data represent performance during control sessions (mean and range), acute drug sessions, and the final session of chronic exposure to each dose. Control data for the acute regimens reflect the three vehicle control sessions immediately prior to drug administration, whereas control data for the chronic regimens represent the six baseline sessions immediately prior to beginning chronic drug administration.

When acutely administered, phenytoin and ethosuximide reduced response rates in dose-dependent fashion. Partial tolerance (i.e., a diminished drug effect with repeated exposure, without complete return to non-drug response levels) developed to the rate-decreasing effects of these drugs when chronically administered. Both clonazepam and valproic acid (at the two higher doses) initially reduced response rates in one subject, and had little consistent effect on the response rate of the other subject. In the former subjects, partial tolerance appeared to develop to the rate-decreasing action of these drugs.

DISCUSSION

The rate-reducing effects of acute administrations of clonazepam, phenytoin, ethosuximide, and valproic acid evidenced in the present study are similar to those observed in a previous investigation [3], as are the accuracy-reducing actions of clonazepam, phenytoin, and valproic acid. Previous data [3] also indicate that, as in the present experiment, ethosuximide occasionally interfered with learning (i.e., increased errors) under the repeated acquisition procedure, although in the earlier study this drug disrupted learning rel-

atively little when compared to clonazepam, phenytoin, valproic acid, and phenobarbital.

The most interesting aspect of the current data concerns the chronic effects of the four anticonvulsants studied. A primary reason for examining the behavioral effects of antiepilepsy medications in nonhumans is to understand better the possible side effects of such drugs in humans [4]. Since epileptic humans receive drugs on a chronic basis [6,9], it appears that the value of research with nonhumans will increase if chronic as well as acute effects are determined. The present data indicate that, in pigeons exposed to a repeated acquisition procedure, a degree of tolerance developed to the rate- and accuracy-reducing effects of each of the drugs studied. Previous research [5] found that, in pigeons exposed to a delayed-matching-to-sample task, tolerance developed to the accuracy-decreasing effects of clonazepam and valproic acid (phenytoin and valproic acid had no effect on accuracy whether given acutely or chronically). Significant tolerance did not develop to the rate-decreasing effects of any of the four drugs under this procedure, although earlier studies with pigeons responding under operant schedules of food delivery (fixed-ratio and fixed-interval) found that partial tolerance developed to the rate-decreasing effects of phenytoin, ethosuximide, and valproic acid [1,4].

The factors that determine whether tolerance develops to the behavioral effects of anticonvulsant drugs presently are unclear. One factor that has been posited to influence the development of tolerance to the behavioral effects of various compounds is the consequences of drug-induced changes in behavior. The "reinforcement-loss hypothesis," formulated several years ago [7], underscores the potential importance of this variable: "Behavioral tolerance will develop in those aspects of the organism's behavioral repertoire where the action of the drug is such that it disrupts the organism's behavior in meeting the environmental requirements for reinforcement. Conversely, where the actions of the drug enhance, or do not affect, the organism's behavior in meeting reinforcement requirements, we do not expect the development of behavioral tolerance" (p. 181). Although some findings fail to support the reinforcement-loss hypothesis (see [2]), the observation that tolerance developed to the rate-decreasing effects of antiepileptic medications in pigeons responding under a repeated acquisition procedure (this study), but not under a delayed-matching-to-sample procedure [5], is consistent with such an analysis, for reductions in response rate appreciably reduced reinforcement rate under the former procedure, but not under the latter.

The physiological mechanisms whereby antiepileptic agents affect behavior, and reduce seizures, are only poorly understood [6], and the physiological basis for the development of tolerance to the behavioral effects of these agents is unknown. However, since little or no tolerance develops to the anticonvulsant actions of such medications, and the development of tolerance to their behavioral effects appears to be modulated by environmental factors (e.g., the procedure under which behavior is maintained), it is apparent that no simple physiological model of tolerance will suffice.

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