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BRIEF COMMUNICATION

Effects of Phenobarbital in Combination With Phenytoin or Valproic Acid on the Delayed-Matching-to-Sample Performance of Pigeons[1]

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KARAS, C. A., M. PICKER AND A. POLING. *Effects of phenobarbital in combination with phenytoin or valproic acid on the delayed-matching-to-sample performance of pigeons.* PHARMACOL BIOCHEM BEHAV **25**(4) 929–932, 1986.—The present study examined the effects of phenobarbital (5, 10, 20, and 40 mg/kg), phenytoin (2.5, 5, 7.5, and 15 mg/kg), and valproic acid (40, 60, 80, and 120 mg/kg), and those of phenobarbital (10 and 20 mg/kg) in combination with phenytoin (2.5, 5, and 7.5 mg/kg) or valproic acid (40, 60, and 80 mg/kg), on the delayed-matching-to-sample performance of pigeons. In general, high doses of each individual drug reduced accuracy. Drug combinations also reduced accuracy relative to control values. Reductions in accuracy produced by drug combinations were very similar in magnitude to those predicted by a response-addition model of drug interaction.

Phenobarbital Phenytoin Valproic acid Drug interactions Delayed-matching-to-sample Anticonvulsant drugs Pigeons

POLYPHARMACY is common in the clinical management of epilepsy although the widespread use of multiple-drug therapy has been severely criticized, in part because such treatment appears to increase the likelihood of deleterious side effects [10,11]. Phenobarbital and phenytoin, and phenobarbital and valproic acid, are two frequently used drug combinations [4]. Some information is available concerning the physiological basis of their interactions but, with the exception of sedation, the behavioral side effects of these combinations in epileptic patients are unclear [3–5].

In recent years, researchers have made an attempt to understand further the behavioral effects of antiepilepsy medications by examining their actions in nonhuman subjects [2,8]. A single investigation [6] has examined the effects of phenobarbital, in combination with phenytoin or valproic acid, on the operant behavior of nonhumans. In that study, the effects of drug combinations on the responding of rats maintained under fixed-ratio and interresponse-time-greater-than-t schedules of food delivery were very similar to those predicted by an effect-addition model of drug interaction, wherein the effects of individual drug doses are arithmetically summated to predict the effects of drug combinations [13].

The purpose of the present study was to examine the effects of phenobarbital, in combination with phenytoin or valproic acid, on pigeons' performance under a delayed-matching-to-sample (DMTS) procedure. This procedure is of some interest to behavioral pharmacologists because it provides a sensitive assay of the effects of drugs on complex conditional discriminations and on what might be referred to as "short-term memory." The DMTS procedure, which requires subjects to match or "remember" stimuli separated by short intervals of time, has provided a wealth of information regarding the effects of drugs [12]. Acute and chronic

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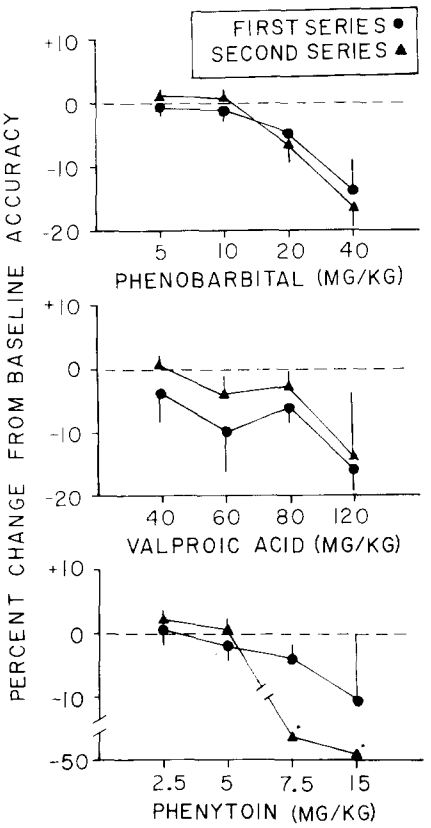

FIG. 1. Effects of acute administrations of individual drugs on the DMTS performance of pigeons. Data are summed across five delay values for three birds, and are expressed as mean change (+ or −1 S.E.) from control performance. Control performance, calculated by determining the mean percent correct responses across all vehicle control sessions, was 92% correct, with a range across subjects and sessions of 85 to 96%. Circles represent the initial dose-response determination, triangles show the post-combination dose-response determination. For the post-combination phenytoin dose-response determination, values at 7.5 and 15 mg/kg (asterisks) are −33% (S.E.=35) and −50 (S.E.=29), respectively.

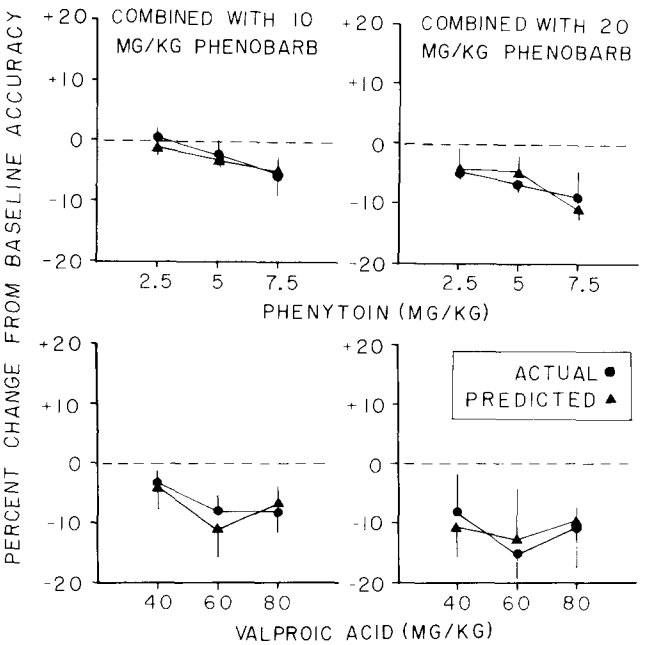

FIG. 2. Effects of acute administrations of drug combinations on the DMTS performance of pigeons. Data are summed across five delay values for three birds, and are expressed as mean change (+ or −1 S.E.) from control performance. Control performance, calculated as described in Fig. 1, was 92% correct, with a range across subjects and sessions of 86 to 95%. Circles indicate actual performance, triangles represent performance predicted by an effect-addition model of drug interaction.

effects of phenobarbital, phenytoin, and valproic acid under this procedure have been determined previously [7,9], but drug combinations have not been examined.

METHOD

Subjects

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

Apparatus

Three identical Lehigh Valley Electronics (BRS/LVE, Lehigh Valley, PA) operant conditioning chambers, measuring 32 cm long, 36 cm high, and 35 cm wide, were used. On the front wall of each, three response keys (2.5 cm diameter) were located 5.5 cm apart 23 cm from the chamber floor. Each key could be illuminated in red or blue-green. A minimum force of 0.2 g was required for key operation. A food hopper centered in the front wall 7.5 cm above the floor and illuminated by a 7-W white bulb allowed access to mixed grain. A 7-W white bulb (houselight) located on the front wall 33 cm from the floor provided ambient chamber illumination. Masking noise and ventilation was provided by an exhaust fan. A Digital Equipment Corp. (Maynard, MA) PDP8/A minicomputer using interfacing and software (SUPER-SKED) supplied by State Systems Inc. (Kalamazoo, MI) was used to control experimental events and to collect data.

Behavioral Procedure

All subjects initially were exposed to an autoshaping procedure as described elsewhere [7]. Once all birds reliably pecked all keys under the autoshaping procedure, they were exposed to the DMTS procedure. Under this procedure, discrete trials were programmed with a 10-sec intertrial interval (ITI). Each trial was preceded by a 0.25-sec darkening of the chamber, after which the center key was illuminated in either red or blue-green; illumination of the center key constituted presentation of the sample stimulus. A response to the center key extinguished the sample stimulus and initiated a delay interval of 0, 1, 2, 4, or 8 sec. During the delay period, the houselight remained illuminated, responses had no programmed consequences, and the keys were dark. Delays were selected at random with each programmed to occur equally often. At the end of the delay period, the two side keys were illuminated in 1 of the 2 possible configurations of color and position (i.e., red on left key and blue-green on right key, or red on right key and blue-green on left key). Illumination of the side keys constituted presentation of the sample stimulus. A response to the comparison stimulus that

matched the sample stimulus in color (i.e., a matching response) darkened both side keys and produced 3-sec access to grain, then initiated the ITI. Nonmatching responses (errors) also darkened the keys and initiated a 10-sec ITI. Such trials were repeated until the subject responded to the appropriate comparison stimulus. Repeating of trials in which errors were made was intended to prevent pigeons from developing position preferences.

When the percentage of correct responses ((matching responses/matching responses + nonmatching responses) × 100) for individual birds showed no visually evident trend over 5 consecutive 140-trial sessions, the response requirement for extinguishing the sample stimulus was increased to 5 (i.e., a fixed-ratio 5 schedule was arranged) and only every second correct response was followed by food delivery. Correct responses not followed by food delivery were followed by a 1-sec flash of the feeder light and then a 10-sec ITI. Red and blue-green sample stimuli were presented randomly, with equal probability of occurrence, and each of the five delay values appeared twice during each block of 10 trials. Trials terminated if the response requirement for center-key pecks (i.e., those directed to the sample stimulus) was not met within 35 sec of trial initiation, or if the subject failed to respond to one of the side keys within 35 sec of the onset of presentation of comparison stimuli. Such aborted trials were repeated after a 10-sec ITI, and were not recorded as incorrect responses. Total aborted trials under all experimental conditions were less than 5% of completed trials. During the experiment proper, sessions terminated after 140 trials or 60 minutes, whichever occurred first, and were usually conducted 6 days a week at about the same time each day.

Pharmacological Procedure

When each subject had completed 40 sessions under the DMTS procedure, the acute effects of phenobarbital (5, 10, 20, and 40 mg/kg), phenytoin (2.5, 5, 7.5, and 15 mg/kg), and valproic acid (40, 60, 80, and 120 mg/kg) were evaluated. These doses were selected on the basis of prior findings from our laboratory [7,9]. Phenobarbital (Sigma Chemical Co., St. Louis, MO) and valproic acid (Saber Laboratories, Morton Grove, IL) were dissolved in distilled water with sufficient sodium hydroxide added to neutralize the drug to the sodium salt. Phenytoin was injected as a commercially prepared solution (Parke-Davis, Morris Plains, NJ) diluted with isotonic saline solution. In all phases of the study: (1) Injections were given intramuscularly (IM) 30 min prior to the experimental session at an injection volume of 1 ml/kg. Prior findings (e.g., [8]) indicate that phenobarbital, phenytoin, and valproic acid are behaviorally active when given IM at this presession injection interval. (2) Drugs and doses were given in an irregular sequence, and active drug was given no more often than twice a week. (3) Drug sessions always were preceded by vehicle control sessions, in which IM injections of 1 ml/kg isotonic saline were given 30 min prior to behavioral testing. Each subject received each dose of an individual drug once during initial dose-response testing.

Following dose-response testing for individual drugs, the effects of drug combinations were evaluated. Twelve combinations of drugs and doses were evaluated: each subject received 10 and 20 mg/kg phenobarbital in combination with 2.5, 5, and 7.5 mg/kg phenytoin, and in combination with 40, 60, and 80 mg/kg valproic acid. Initial dose-response determinations and prior data from our laboratory [6, 7, 9] suggested that these combination doses would be behaviorally active, but would not suppress responding totally. When drug combinations were given, each drug was injected separately, and one injection was administered on each side of the breast. Control injections for drug combinations were given in the same fashion. After the drug combinations were evaluated, a second dose-response determination for individual drugs was completed in the same manner as the initial dose-response evaluation.

RESULTS AND DISCUSSION

Figure 1 shows the effects of individual drugs on accuracy (mean percent correct responses across the five delay values, expressed as percent change from baseline performance). In general, as in previous studies [7,9], acute administrations of the three lowest doses of phenobarbital, valproic acid, and phenytoin had little effect on accuracy, although reductions in accuracy were apparent at the highest dose of each drug. Initial and post-combination dose-response determinations yielded very similar data for phenobarbital and valproic acid. The two highest doses of pheytoin, however, produced greater reductions in mean accuracy during the second dose-response determination, largely because one subject responded very little, and made a very high percentage of errors, when exposed to these doses for the second time. Comparison of initial and post-combination dose-response curves for individual drugs indicates that tolerance did not develop during the course of the investigation.

Figure 2 shows the effects of drug combinations. The most interesting aspect of the data involves the relatively small decrement in accuracy produced by the drug combinations. In general, phenobarbital in combination with phenytoin or valproic acid slightly increased errors; the magnitude of this effect was greater at higher combination doses. Reductions in accuracy, although small, were very similar in magnitude to those predicted by a response-addition model of drug interaction [13], in which the effects of individual drugs and doses (initial dose-response determinations) were arithmetically summed to predict the effects of drug combinations. A sign test [1] indicated that greater than predicted changes in accuracy did not occur significantly more often ($p>0.05$) than smaller than predicted changes, that is, the effects were simply additive. This finding agrees with the results of a previous investigation in which the effects of these combinations on rats' schedule-controlled behavior were examined [6].

The use of polypharmacy in the management of epilepsy has been questioned in two regards (e.g., [10,11]). One concerns whether drug combinations are more effective in reducing seizures than are single medications, an issue upon which the present data do not bear. Another concerns whether drug combinations produce more deleterous side effects than individual medications. The present data surely do not resolve this issue for the drug combinations evaluated, but they do suggest that the acute effects of these combinations on DMTS performance, as on schedule-controlled responding [6], are not synergistic. Insofar as the DMTS procedure is a sensitive assay of drug effects [12], and is offered as an assay of "short-term" memory, which is in epileptic patients sometimes impaired by anticonvulsant medications, this outcome may be of some interest.

It is clearly established that phenytoin elimination kinetics are dose-dependent, and that repeated exposure to phenobarbital predictably induces phenytoin induction [4]. Such induction might be expected to result in infra-additive

behavioral effects when the drugs are administered together. Phenobarbital also acts as a competitive inhibitor with phenytoin as substrate, since both drugs undergo parahydroxylation and glucoronidation. These effects often balance out, such that interaction of the drugs is neither infra-additive nor synergistic [4], as was the case in the present study. Chronic drug exposure might, however, lead to stronger induction and infra-additive effects. When the drugs are given chronically, ". . . the coadministration of valproic acid and phenobarbital inhibits the biotransformation and hydroxylation of phenobarbital to hydroxyphenobarbital, increases its elimination half-life, and ultimately causes elevation of serum phenobarbital levels" ([5], p. 581). Such an interaction might well produce synergistic behavioral effects, but would not be operative under the conditions of the present study, in which drugs were given acutely and their effects assessed relatively soon after administration.

Like chronic exposure, higher combination doses might alter the nature of drug interactions due to dose-dependent kinetics. The DMTS procedure used in the present study appears, however, to be ill-suited for studying such combinations, since strong (i.e., >40%) reductions in response rates were observed with the highest combination doses we examined.

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