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Generalization Between Benzodiazepine- and Triazolopyridazine-Elicited Discriminative Cues

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McELROY, J. F. AND R. S. FELDMAN. *Generalization between benzodiazepine- and triazolopyridazine-elicited discriminative cues.* PHARMAC. BIOCHEM. BEHAV. 17(4) 709–713, 1982.—Using a milk reinforced two-lever operant procedure, rats were trained to discriminate 3 mg/kg chlordiazepoxide (CDP) from saline. Following this, generalization experiments were conducted with the triazolopyridazine CL 218,872, a synthetic non-benzodiazepine (BDZ) ligand for the BDZ receptor. CL 218,872 produced CDP lever selection in a dose related fashion and thus generalized to the standard CDP treatment. However, this generalization was antagonized by the concurrent administration of pentylenetetrazol or amphetamine, but not by strychnine or bicuculline. Also, there was evidence for cross tolerance for a sedative effect between CDP and CL 218,872.

Benzodiazepines Triazolopyridazines Chlordiazepoxide Drug discrimination

IT has been well established that drugs may produce discriminative stimuli that can be utilized in a variety of pharmacological experiments to study drug actions and interactions [1, 18, 21]. In most of these experiments, animals are trained to produce one response when treated with a drug and to produce an alternate response when administered the drug vehicle. Once response differentiation is reliably established, stimulus generalization and antagonism experiments are often employed in an attempt to establish the biochemical mechanisms underlying elicitation of the discriminative stimulus. Chlordiazepoxide (CDP), a prototype benzodiazepine (BDZ), is able to produce such a discriminative stimulus in rats [3, 4, 17]. It was found that the potencies at which other BDZs generalize to the CDP cue correlate well with the potencies of their ataxic and anticonvulsant effects [5], suggesting that the BDZ cue is relevant to the clinical effects of this class of drugs.

It has been reported that CL 218,872 (3-methyl-6-[3-trifluoromethylphenyl]-1,2,4-triazolo[4,3-b]pyridazine), a synthetic non-BDZ compound in the triazolopyridazine (TPZ) class selectively displaces ^{3}H- diazepam from its binding sites with a potency intermediate between that of diazepam and CDP [9]. Similar to the BDZs, CL 218,872 increases punished responding in a conflict situation and protects against pentylenetetrazol-induced convulsions [10], pharmacological properties which are highly predictive of anxiolytic activity. However, unlike the BDZs, CL 218,872 was relatively devoid of depressant side effects and was very weak in its ability to inhibit the convulsions produced by bicuculline and strychnine [11].

The purpose of the present study was to further compare these structurally distinct, yet pharmacologically similar compounds. Specifically, the aim of this experiment was to train rats to discriminate between CDP and saline in a 2-lever operant task, and to investigate the ability of CL 218,872 to produce a CDP-like cue. Lastly, convulsant and non-convulsant central nervous system (CNS) stimulants were tested for possible antagonism of any CDP stimulus generalization to CL 218,872.

It was previously reported that pentylenetetrazol failed to antagonize the discriminative cue produced by a 5 mg/kg dose of CDP [4]. Utilizing a modified drug discrimination procedure originally demonstrated by Overton [17], we recently showed (manuscript in preparation) that in rats initially trained to discriminate 5 mg/kg CDP from saline and retrained to discriminate 3 mg/kg CDP, pentylenetetrazol successfully blocked the CDP cue. Thus, in order to increase the sensitivity of this behavioral test paradigm, all generalization tests in the present experiment were conducted following retraining using this reduced dosage of training drug.

METHOD

Subjects

The subjects used in this experiment were 46 male albino rats obtained from the Holtzman Co., Madison, WI. Each rat weighed between 250 and 350 g at the start of the experiment and was singly housed in a temperature controlled room with a reversed 12 hr light/12 hr dark cycle. Throughout the study, all rats had free access to tap water, while availability to

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standard Purina Laboratory Chow was restricted to a daily 4 hr period following testing. All training and testing was done during the dark cycle, 1 hr prior to daily feeding, Monday through Friday of each week.

Apparatus

The behavioral apparatus consisted of standard Skinner boxes housed in light-proof, sound attenuated, and fan ventilated chambers. Each Skinner box was equipped with two levers, one on either side of a centrally placed dipper, which delivered 0.1 ml of a liquid reinforcement for three seconds. The reinforcement mixture was 25% Liquid Similac, 25% condensed milk, and 50% tap water. Standard electromechanical programming and cumulative counters were used to record and control behavior.

Drugs

Chlordiazepoxide hydrochloride (Roche), strychnine sulfate (Sigma), pentylenetetrazol (Sigma), and D-amphetamine sulfate (Sigma) were dissolved in physiological saline, whereas (+) bicuculline (Sigma) and CL 218,872 (Lederele) were suspended in a 20% propylene glycol in water solution. All injections were given intraperitoneally in a volume of 2 ml/kg body weight.

Discrimination Training

Following habituation to the experimental chamber and preliminary shaping, the animals were trained to alternate between response levers on a CRF schedule of reinforcement (only one lever was reinforced for 5 min, then the other, for a total of 30 min). Once lever pressing was well established, the reinforcement contingency was increased incrementally to an FR 10 schedule of reinforcement, while maintaining the lever alternation.

Next, the animals were trained to discriminate between 5 mg/kg CDP and physiological saline. Half of the rats were randomly assigned the left lever as "CDP-correct" and the right lever as "saline-correct," while the lever assignment was reversed for the remaining animals. Every tenth response on the "CDP-correct" lever led to reinforcement on days when animals were 30 min pretreated with CDP, whereas the opposite lever was reinforced following saline injections. Daily saline or CDP treatments were given according to the following two weekly alternating sequences: CDP-saline-saline-CDP-CDP, and saline-CDP-CDP-saline-saline. Discrimination sessions, 10 min in duration, were continued until each animal reached the performance criterion of no more than three incorrect responses before the first reinforcement on 9 out of 10 consecutive sessions.

After each animal attained this initial training criterion, the dosage of CDP was reduced to 3 mg/kg and discrimination training was resumed until each animal again attained the performance criterion of three or fewer errors prior to the initial reinforcement on 9 out of 10 consecutive days.

Stimulus Generalization and Antagonism Experiments

Following retraining using 3 mg/kg CDP, a test session was conducted on Friday of each week. On Monday through Thursday, training sessions in the order of saline-CDP-CDP-saline were continued for the purpose of providing appropriate baseline data and to ensure that discrimination was intact. If any animal failed to demonstrate reliable discrimination (each day with three or fewer errors), testing with that animal was postponed and discrimination training continued until this performance criterion was attained. For the stimulus generalization experiments, the training injection was replaced by an injection of CL 218,872 or by a different dose of the training drug. In the stimulus antagonism tests, a CNS stimulant was administered concurrently with a dose of CL 218,872 or by a different dose of the training drug. In the stimulus antagonism tests, a CNS stimulant was administered concurrently with a dose of CL 218,872. During test sessions, 10 min in duration, the lever on which the rat first totaled 10 responses was reinforced and subsequent FR 10 reinforcement was made contigent upon pressing this "selected" lever. The accuracy of lever selection for each animal was computed as the percentage of responses on the CDP-correct lever at the occurrence of the first reinforcement. For example, if a rat responded three times to the saline lever before making 10 responses on the CDP lever, its cue detection score was 10/13 or 77%; if a rat made one response on the CDP lever and 10 responses on the saline lever the score would be 1/11 or 9.1%. Thus, high scores indicate preference for the CDP lever and low scores indicate preference for the saline lever. For each drug treatment, 7–10 rats randomly selected from the pool of animals available for testing were assigned to each dose group. Thus, for each drug treatment, no animal participated in more than one dose group. The Wilcoxon test was employed to compare the rate of responding on test days to the response rate on the previous saline control session.

To assess the effects of CDP pretreatment on the response rate following administration of CL 218,872, 10 drug naive rats were trained to lever press on an FR 10 schedule of reinforcement. Once a steady baseline of lever responding was attained, each rat received an acute 30 min preinjection of CL 218,872 (5 mg/kg), and the total response output for a 10 min interval was compared to the output under saline conditions.

RESULTS

Base-Line Data

All rats reliably learned to discriminate 5 mg/kg CDP from saline, requiring a median number of 27 training sessions (including the 10 criterion sessions) in order to meet the performance criterion of fewer than three errors per session on 9 out of 10 consecutive sessions. When the training dosage of CDP was reduced to 3 mg/kg, discrimination was easily maintained, with a median number of 11 additional training sessions (including the 10 criterion sessions) required to reattain performance criterion.

On the standard saline and CDP discrimination sessions conducted Monday through Thursday of each week following the retraining period, all animals reliably selected the injection appropriate lever after either saline or CDP treatment. Incorrect lever selection occurred rarely and each rat reached a highly significant level (one-tailed; $p<0.001$; Binomial-test) of correct lever selection. The median percent-correct lever selection value was 100% for each animal following either drug or saline administration.

Stimulus Generalization and Antagonism Experiments

The data from the stimulus generalization experiments with CDP and CL 218,872 are summarized in Fig. 1. The training dose of CDP (3 mg/kg) generalized to lower doses of

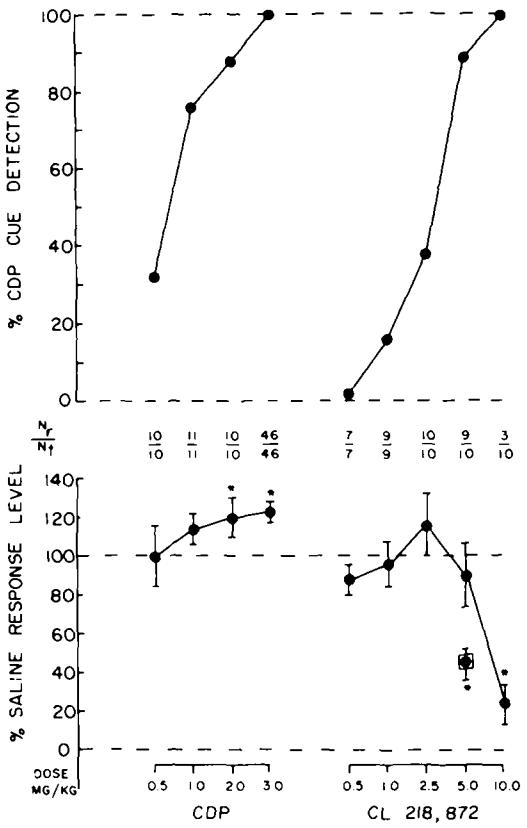

FIG. 1. Stimulus generalization between chlordiazepoxide (CDP) and CL 218,872 in rats trained to discriminate CDP (3 mg/kg) from saline. Percent CDP cue detection is expressed as the mean and refers to the percentage of responses emitted on the CDP lever at the occurrence of the first reinforcement. Nr/Nt represents the number of rats responding sufficiently to demonstrate lever selection, out of the total number of rats tested. The response level indicates the mean (+1 SEM) number of responses for 10 minutes, and is expressed as a percentage of the previous control performance. The squared data point indicates the mean number of responses for 10 drug-naive rats. The asterisks denote values that differ significantly (two-tailed; $p < 0.05$; Wilcoxon test) from the corresponding saline values.

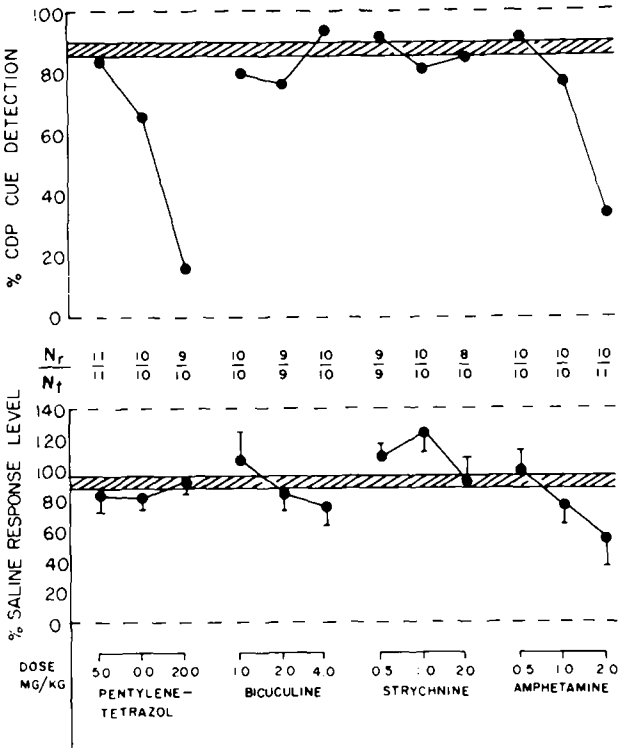

FIG. 2. Effects of pentylenetetrazol, bicuculine, strychnine and amphetamine on the generalization of CL 218,872 (5 mg/kg) to the CDP discriminative stimulus. The ordinates and Nr/Nt are similar to those of Fig. 1. The dashed areas represent the cue detection and response values in test sessions with 5 mg/kg CL 218,872 alone.

CDP (0.5 to 2.0 mg/kg) in a dose dependent fashion. CDP produced a dose related increase in responding, with the response rate following 2.0 and 3.0 mg/kg significantly above saline levels. CL 218,872 (0.5 to 10.0 mg/kg) produced CDP lever selection in a dose related manner and therefore generalized to the standard (3 mg/kg) CDP treatment. However, only three of the 10 rats tested with 10 mg/kg CL 218,872 responded sufficiently (10 times to either lever) to indicate a lever preference. The rest were mostly inactive during the test session. Whereas CL 218,872 (0.5 to 5.0 mg/kg) failed to significantly alter the total amount of responding, the highest dose tested (10 mg/kg) nearly suppressed responding completely. In marked contrast to its effect on responding in CDP trained animals (90.1% of control levels), 5 mg/kg CL 218,872 significantly depressed responding (46.9% of control) in drug naive rats.

The results of the stimulus antagonism experiments are shown in Fig. 2. Following concomitant treatment with CL 218,872 (5 mg/kg) and either bicuculline (1.0 to 4.0 mg/kg) or strychnine (0.5 to 2.0 mg/kg), the animals continued to select the CDP lever, thus demonstrating that the generalization of CL 218,872 to the standard CDP treatment had not been antagonized. However, the concurrent administration of CL 218,872 (5.0 mg/kg) and either pentylenetetrazol (5.0 to 20.0 mg/kg) or amphetamine (0.5 to 2.0 mg/kg) caused the rats to select the saline lever in a dose dependent fashion, therefore indicating that the perception of the CDP cue otherwise evident after a 5 mg/kg injection of CL 218,872 had been antagonized. When administered alone, each of these stimulants reliably induced saline selection (one-tailed; $p < 0.005$; Binomial-test). The response rate following the acute administration of CL 218,872 (5.0 mg/kg) was not significantly altered by the addition of any of these CNS stimulants.

DISCUSSION

The results of this study demonstrate that rats previously trained to discriminate 5 mg/kg CDP from saline can easily discriminate a dose as low as 3 mg/kg. We have also clearly shown that CL 218,872, a synthetic non-BDZ ligand for the BDZ receptor, produces CDP lever selection in a dose related manner and thus generalizes to the standard CDP treatment. It is further shown that cue detection following injection of CL 218,872 is independent of the rate of responding since generalization occurs at a dose (5 mg/kg) that does not significantly alter the rate of lever pressing. Our demonstration that 10 mg/kg CL 218,872 virtually suppresses re-

sponding completely is at odds with a previous report that a 200 mg/kg dose of CL 218,872 is required to reduce locomotor activity by 50% [11]. This discrepancy may be due to methodological differences as their drug to test interval was 60 min while out interval was only 30 min. Secondly, motoric activity as estimated by lever pressing for a milk reinforcement may not be equivalent to locomotor activity as measured by an activity meter (Animex®).

It is well known that BDZs produce an initial general depression or sedation, usually shown by a decrease in unpublished responding or exploratory behavior, an effect which disappears after a few BDZ treatments [14]. In the present experiment CL 218,872 (5 mg/kg) significantly depressed responding in drug-naive rats (shown by the squared data point in Fig. 1), where the same dose did not affect responding in animals that had repeated prior CDP treatments. This data may suggest that a cross tolerance to the initial sedative effect occurred between CDP and CL 218,872. There is the assumption here that the rats with prior CDP treatment had become tolerant to the depressant effect of CDP by the time they had learned the discrimination task. However, clarification of this issue will require a more complete investigation.

CL 218,872 potently antagonizes the convulsions produced by pentylenetetrazol, but unlike the BDZs is very weak in its ability to inhibit the convulsions induced by bicuculline or strychnine [10]. Consistent with this selectivity, our data reveal that the generalization of the CDP discriminative stimulus to CL 218,872 is antagonized by pentylenetetrazol, but not by bicuculline or strychnine. This suggests that stimulus generalization is not associated with a general anticonvulsant activity but rather with a specific antipentylenetetrazol effect. This mutual antagonism between pentylenetetrazol and CL 218,872 may indicate that the same neuronal mechanism subserves both the antipentylenetetrazol and discriminative stimulus properties of the BDZs.

The presence of saturable, high-affinity binding sites of BDZs in the mammalian CNS has been demonstrated [2,16]. There exists a strong correlation between the ability of BDZs to inhibit ^{3}H-diazepam binding and their potency in both the rat conflict test [12] and in protecting against pentylenetetrazol-induced seizures [11]. That the antipentylenetetrazol effect of the BDZs in vivo is mediated through ^{3}H-diazepam bindings sites is further supported by the recent reports that pentylenetetrazol treatment increases the number of BDZ receptors [23]. These data suggest that pentylenetetrazol is a competitive antagonist of central BDZ receptors, and is consistent with the findings that CDP and pentylenetetrazol mutually antagonize their respective discriminative stimuli ([22]; manuscript in preparation).

In has been proposed that two biochemically and pharmacologically distinct types exist among BDZ receptors [9]. Type I receptors are GABA-independent, display a high affinity for both BDZs and TPZs, and mediate the anxiolytic and antipentylenetetrazol actions of these drugs. Type II receptors are GABA-dependent, show a high affinity for BDZs but a low affinity for TPZs, and mediate the depressant side effects associated with the BDZs. In the interest of establishing whether the BDZ discriminative cue can be traced to once receptor type or the other, our data reveal that CL 218,872 produces a discriminative cue without sedation, at doses similar to those that produce anticonflict and antipentylenetetrazol effects [10]. Since generalization occurs without sedation (at 5.0 mg/kg), it may be that the discriminative stimulus properties of BDZs are mediated via stimulation of type I receptors. That stimulus generalization is antagonized by pentylenetetrazol but not by the GABA receptor blocker bincuculline, further suggests the involvement of type I receptors. It may be that the anxiolytic, antipentylenetetrazol, and discriminative stimulus properties of BDZs are mediated via activation of type I BDZ receptors.

Since several neurotransmitter systems have been postulated to be associated with the pharmacological actions of the BDZs (for a review see [6]), it is difficult to establish which neurotransmitter is responsible for the discriminative stimulus properties of this class of drugs. However, the antagonism of the stimulus generalization of CDP to CL 218,872 by the catecholamine agonist amphetamine, but not by strychnine or bicuculline, receptor blockers for glycine and GABA respectively [7,24], may suggest involvement of either norepinephrine or dopamine. The ability of pentylenetetrazol to antagonize stimulus generalization does little to clarify this issue as acetylcholine [20], norepinephrine [15] and GABA [8] have been implicated in the convulsant action of pentylenetetrazol. Resolution of this issue will require additional research.

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