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Ethanol-Chlordiazepoxide Interactions in the Rat

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SCHECHTER, M. D. AND D. M. LOVANO. *Ethanol-chlordiazepoxide interactions in the rat.* PHARMACOL BIOCHEM BEHAV 23(6) 927–930, 1985.—One group of rats (n=9) was trained to discriminate between the effects induced by 600 mg/kg ethanol and saline, whereas another group (n=5) had to discriminate between 5.0 mg/kg chlordiazepoxide (CDZ) and saline, administered intraperitoneally (IP) 15 min prior to the training sessions. Once trained, decreasing doses of ethanol in ethanol-trained rats produced decreased discriminative performance; the ED_{50} was 239.4 mg/kg. Likewise, decreasing doses of CDZ in CDZ-trained rats produced decreased discrimination, with an ED_{50} = 1.5 mg/kg. Substitution of 1.25–10.0 mg/kg CDZ in ethanol-trained rats produced a transfer of the ethanol-induced interoceptive cue in a dose-responsive manner, whereas ethanol did not substitute for CDZ in CDZ-trained rats. Analysis of dose-response curves suggested that CDZ is acting by a similar mechanism/site in both groups of rats but by a site different than ethanol. Co-administration of the ED_{50}'s for ethanol and CDZ in ethanol-trained rats did not produce additive effects. The observed one-way asymmetrical generalization of drug effects, as well as the lack of additive effects of co-administration, are discussed.

Drug discrimination Chlordiazepoxide Ethanol Generalization Drug combination Rats

CHLORDIAZEPOXIDE (CDZ) is effective against a variety of symptoms of ethanol withdrawal including anxiety, agitation, tremor and delirium tremens [14]. The mechanism of action for this efficacy of CDZ has not been fully elucidated and it has been presumed that modulation of the gamma-aminobutyric acid (GABA) receptor function is an important determinant. Thus, drugs which mimic the effects of GABA, such as baclophen and gamma-hydroxybutyric acid, abolish the locomotor stimulation produced by ethanol in mice [3], whereas the GABAergic antagonist picrotoxin enhances the effects of ethanol [9]. In addition, CDZ may possess properties of other commonly-used drugs for treating withdrawal by virtue of cross-tolerance with ethanol due to common mechanisms of action [8], although definitive data regarding this phenomenon are lacking [2, 5, 11].

The drug discrimination procedure has proven to be a sensitive, specific and stable method to allow determination as to whether subjective cues generated by one drug are similar or dissimilar to those produced by other drugs [4]. Within this behavioral paradigm, which is essentially a drug detection procedure, rats are trained to discriminate between a drug and a non-drug (saline or vehicle) state using operant techniques. Thus, food-deprived rats are trained to press one of two levers for food reinforcement, under a drug state whereas, on different days, the same animals are trained to press the opposite lever in the non-drug state. Rats learn to associate one lever with drug and the opposite lever with saline, so that the drug becomes the stimulus that enables the animal to choose the correct lever that will deliver reinforcement on a given day. The usefulness of this procedure in determining the stimulus properties of drug action is contingent upon the animal subjects' learning and retaining the acquired discriminative stimulus, i.e,, the interoceptive cue produced as a consequence of drug administration. Once the discrimination is attained and maintained, studies can subsequently be conducted to ascertain the ability of the animals to discriminate other drugs.

The purpose of the present study was to train a group of rats to discriminate between the effects of CDZ and saline and a second group of rats to discriminate between ethanol and saline, and to investigate the generalization of these discriminations to the other drug. The aim of these experiments was to further elucidate the possibility of a common mechanism of action between a benzodiazepine compound and ethanol, as well as to investigate the effect of co-administration of these drugs.

METHOD

Subjects

Fourteen experimentally-naive male ARS/Sprague-Dawley rats weighing 360–460 g at the beginning of experimentation were used. They were housed in individual living cages and their weights were adjusted, by daily rationing of commercial rat chow, to approximately 80 to 85% of their free-feeding weights as determined by daily weighing of two control free-feeding rats purchased at the same time as experimental animals from the supplier (Zivic-Miller, Allison Park, PA). Water was continuously available in the home cages which were kept at a constant temperature (20–22°C) and maintained on a 12-hour light/12-hour dark daily cycle.

Apparatus

The experimental space consisted of four identical stand-

TABLE 1

DOSE-RESPONSE AND GENERALIZATION TO ETHANOL IN CDZ-TRAINED RATS

Treatment	Dose (No. trials) mg/kg	Quantal	Quantitative (±SD)
Saline	– (24)	4.0	25.7 (4.2)
CDZ	10.0 (4)	100.0	96.2 (0.6)
	5.0 (24)	95.0	89.2 (3.1)
	2.5 (4)	86.7	81.3 (11.0)
	1.25 (4)	40.0	47.0 (4.0)
	0.63 (4)	13.3	29.6 (6.2)
Ethanol	1200 (2)	28.6*	37.4 (20.2)
	900 (2)	37.5	39.8 (6.5)
	600 (2)	37.5	34.5 (10.2)
	300 (2)	0.0	15.3 (0.0)

n=5.
*Seven of 10 sessions produced delays.

TABLE 2

DOSE-RESPONSE, GENERALIZATION AND INTERACTION RESULTS IN ETHANOL-TRAINED RATS

Treatment	Dose (No. trials) mg/kg	Dose Quantal	Quantitative (±SD)
Saline	– (20)	5.6	17.7 (7.2)
Ethanol	600 (20)	96.8	82.8 (6.6)
	450 (2)	62.5	54.2 (8.4)
	300 (2)	50.0	52.3 (2.3)
	150 (2)	36.1	42.4 (14.2)
CDZ	10.0 (2)	84.2	69.4 (6.3)
	5.0 (2)	77.8	66.4 (2.9)
	2.5 (2)	61.1	63.2 (1.4)
	1.25 (2)	48.1	51.7 (4.2)
Ethanol and Saline	240 (4)	55.6	54.1 (1.8)
Ethanol and CDZ	240 (4) 1.5	61.1	56.8 (11.8)

n=9.

ard rodent operant chambers (Lafayette Instruments Corp., Lafayette, IN) each equipped with two operant levers located 7 cm apart and 7 cm above the grid floor. A food pellet receptacle was mounted 2 cm above the grid floor at an equal distance between the two levers. The test cage was housed in a sound-attenuating cubicle equipped with an exhaust fan and 9 W house-light. Solid-state programming equipment (LVB Corp., Lehigh Valley, PA) was used to control and record the sessions and was located in an adjacent room.

Discriminative Training

Drug discrimination training was based upon procedures described in detail elsewhere [13]. There were two training phases. In the first phase, the food-deprived rats learned to press the lever indicating saline administration and received a food reward (45 mg Noyes pellet) for each correct response, on a fixed ratio 1 (FR1) schedule. This schedule was made progressively more difficult, in daily 15 min sessions, over 10 days until an FR10 schedule was achieved, i.e., the rats had to press the lever 10 times to receive reinforcement. Throughout lever press training, all rats received daily intraperitoneal (IP) injections of saline (0.9% sodium chloride, 1 ml/kg body weight) 15 min prior to being placed into the two-lever operant box. Immediately following the attainment of the FR10 schedule after saline administration, the opposite lever was activated and rats received a food reward for each correct response (FR1 schedule), after the IP administration of an equal volume of saline containing either ethanol (10% v/v; 600 mg/kg; n=9) or chlordiazepoxide hydrochloride (5.0 mg/kg; n=5). Daily sessions, of 15 min duration, with drug administration were conducted until an FR10 schedule was attained. In order to minimize effects due to any possible position preference, the rats in each group were divided into two unequal subgroups. For one subgroup, responding on the left lever was reinforced by delivery of food pellets in every session following drug injection, whereas the other group was reinforced with food after responding on the right lever following drug injection. Responses on the opposite lever were reinforced with food pellets after saline injection.

Phase II discrimination training then began. Subjects were trained 5 days per week with reinforcement in a pseudo-random sequence. Thus, in each two week period, there were five days with drug lever (D) and five days with saline lever (S) correct. The pattern was D,S,S,D,D; S,D,D,S,S. The rats had to respond on the appropriate lever to receive food reinforcement. Which lever was correct was dependent upon whether the training drug or saline had been administered prior to the start of the session. Responses upon the inappropriate lever were recorded but they had no programmed consequences. The training criterion was reached when the animal selected the appropriate lever, according to the drug state imposed at the onset of each training session, on eight of ten consecutive sessions.

Dose-Response Relationships

After the rats attained the discriminative training criterion with each of the two agents, testing and training sessions of 15 min duration with alternating administrations of either 600 mg/kg ethanol and saline (ethanol-trained group) or 5.0 mg/kg CDZ and saline (CDZ-trained group) were continued on Mondays, Wednesdays and Fridays. It was intended that if a rat was observed to make more than two incorrect lever selections in any of 10 consecutive maintenance sessions, the data on that rat's performance would be deleted from the results. This, however, did not occur. On Tuesday and Thursdays, the rats of each group were injected IP with one of several different doses of ethanol or CDZ other than used for initial training and, 15 min later, they were placed into the experimental chamber. They were allowed to lever press, without receiving reinforcement, until ten presses were made on either lever. To preclude training at a drug dose different than that employed to train the animals, the rats were immediately removed from the experimental chamber

once the total responses on one lever reached 10 presses. Each of the test doses of drugs was tested in each animal on two or four occasions with each test preceded by both a drug and a saline maintenance session.

Transfer of Discrimination

After the dose-response experiments, each group of trained rats was administered various doses of the other drug to test transfer or generalization to the other agent. Thus, CDZ-trained rats received 300, 600, 900 and 1200 mg/kg ethanol (IP) on two trials, each preceded by a CDZ and saline maintenance trial, whereas the ethanol-trained rats were administered (IP) 1.25, 2.5, 5,0, and 10.0 mg/kg CDZ. Upon pressing one lever ten times after administration of the test drug/dose, the rats were immediately removed from the test chamber without reinforcement in order to preclude training with a drug different from that used in discrimination training.

Co-Administration of CDZ and Ethanol

As the ethanol-trained rats transferred to the effects of decreasing doses of CDZ and, thus, a dose-response relationship could be generated for both drugs, the ED_{50} of ethanol together with either saline or the ED_{50} of CDZ were co-administered on 4 occasions each. As before, each trial was preceded by one maintenance session with each of ethanol and saline and the rats were removed upon making 10 responses on either lever.

Measurements

The first lever that was pressed 10 times was designated as the "selected" lever. The percentage of rats selecting the lever appropriate for the training drug was the quantal measurement of discrimination. In addition, the total number of lever presses on both levers made before completion of ten presses on either lever were counted constitutes the quantitative measurement, i.e., the number of responses on the drug-correct lever divided by total responses made (including the ten on the drug-correct lever) times 100. The advantage in using both measurements has been discussed by Stolerman and D'Mello [15]. The quantal data for the dose-response experiments were analyzed by the method of Litchfield and Wilcoxon [10] which employs probit vs. log-dose effects and generates ED_{50}'s and tests for parallelism. Verification of analysis was made on a TRS-80 computer using published computer programs [16].

RESULTS

The CDZ-trained rats selected the CDZ-appropriate lever in 95% of trials with the training dose of 5.0 mg/kg, whereas they chose this lever on 4% of trials (and, thus, selected the saline-correct lever on 96% of trials) after saline (Table 1). Administration of 10 mg/kg CDZ produced 100% correct responses and administration of decreasing doses of CDZ was observed to produce decreased discriminative performance in terms of both quantal and quantitative measurements. Analysis of the dose-response curve for CDZ [10] indicated an ED_{50} of 1.5 mg/kg. Administration of 300 mg/kg ethanol produced saline-appropriate responses and doses of 600 and 900 mg/kg ethanol elicited 37.5% of selections upon the CDZ-lever. The highest dose of 1200 mg/kg ethanol led to extensive behavioral disruption in 7 of 10 trials. Maintenance trials with CDZ interspersed throughout these experiments indicated that discrimination to CDZ did not decrease over time. This would suggest that there was no tolerance to either CDZ or its active metabolites in its effect upon discrimination performance.

Results of dose-response effects, and CDZ generalization trials, in ethanol-trained rats appear in Table 2. The training dose of 600 mg/kg ethanol produced 96.8% first choice responses on the ethanol-correct lever, whereas saline administration resulted in 5.6% selections on this lever (or 94.4% on the saline-correct lever). Decreasing doses of ethanol produced decreased discriminative performance in terms of both quantal and quantitative measurements and generated a calculated [10] ethanol ED_{50} = 239.4 mg/kg for the quantal measurement. Administration of 10 mg/kg CDZ produced 84.2% responses upon the ethanol-correct lever and decreasing CDZ doses produced decreased discriminative responding. The CDZ ED_{50} in ethanol-trained rats was calculated to be 1.36 mg/kg. Saline injection prior to the administration of the approximate ED_{50} for ethanol resulted in 55.6% response upon the ethanol-lever, whereas co-administration, in separate syringes, of the ED_{50}'s for both ethanol and CDZ in these rats produced 61.1% responses on the ethanol-correct lever.

Further analysis using the method of Litchfield and Wilcoxon [10] indicated that the slopes of the ethanol and CDZ dose-response curves in the ethanol-trained rats were significantly different (calculated $t=3.03 >$ critical $t=2.98$), whereas the CDZ dose-response curve in CDZ-trained rats was parallel to this curve in ethanol-trained rats (calculated $t=0.89 <$ critical $t=2.57$).

DISCUSSION

Rats trained to discriminate between the presence and absence of effects produced by intraperitoneally administered 5.0 mg/kg CDZ did not generalize to ethanol in doses from 300–1200 mg/kg. In contrast, rats trained to discriminate between intraperitoneally administered ethanol at 600 mg/kg selected the ethanol-correct lever when tested by substitution with the highest (10 mg/kg) dose of CDZ. Furthermore, decreasing doses of CDZ produced ethanol-appropriate responding in a dose-responsive manner.

Analysis of the dose-response curves [10] in ethanol-trained rats indicates an ED_{50} for ethanol of 239.4 mg/kg and an ED_{50} for the substituted CDZ of 1.36 mg/kg. Further analysis of these curves indicates that they are not parallel within statistical limits. This observation confirms previous work from this laboratory [13] that suggests that ethanol and chlordiazepoxide do not share a common site/mechanism of action. Analysis of the dose-response curve generated by various doses of CDZ in the CDZ-trained rats indicates an ED_{50} of 1.5 mg/kg. Comparison of the slopes of the CDZ dose-response curves in the chlordiazepoxide- and ethanol-trained rats indicates that they are parallel within statistical limits [10]. This would suggest [8] that chlordiazepoxide acts upon a similar site or set of receptors in both chlordiazepoxide- and ethanol-trained rats.

The one-way, asymmetrical transfer of drug effects as seen here has been previously reported to occur. Overton [12] first reported that rats trained with pentobarbital transfer to CDZ, whereas rats trained with CDZ do not readily transfer to pentobarbital, and Barry and Krimmer [1] reported that rats trained with ethanol transfer to pentobarbital but rats trained to discriminate pentobarbital do not transfer to ethanol. Lastly, Järbe and McMillan [7] have recently

reported that pigeons trained to discriminate diazepam do not transfer to ethanol, whereas 80% of the birds trained with ethanol transfer to diazepam. Indeed, York [17 has postulated that asymmetrical generalization may occur as animals trained in the discriminative procedure learn to focus on a particular pharmacological effect of one drug not shared by a second drug, whereas animals trained with the second drug focus upon a second pharmacological effect, which is shared by both drugs. To extend this hypothesis to the results of the present study, chlordiazepoxide may produce interoceptive cues involving both anti-anxiety effects mediated by GABAergic receptors and central depressant effects, whereas ethanol may be producing solely central depressant effects. This reasoning can also be used to explain why the co-administration of the ED_{50}'s for ethanol and chlordiazepoxide in ethanol-trained rats (Table 2) was not addictive. If the cueing properties of ethanol are substantially based upon its central depressant activity, the addition of the 1.5 mg/kg dose of chlordiazepoxide, with its primarily anti-anxiety cue property, would not produce significantly greater discriminative "value."

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