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Discriminated Taste Aversion With Chlordiazepoxide

FRED WOUDENBERG AND THEO H. HIJZEN[1]

Netherlands Institute for Drugs and Doping Research, Faculty of Pharmacy, University of Utrecht Sorbonnelaan 16 3584 CA Utrecht, The Netherlands

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WOUDENBERG, F. AND T. H. HIJZEN. *Discriminated taste aversion with chlordiazepoxide*. PHARMACOL BIOCHEM BEHAV **39**(4) 859–863, 1991.—Discriminative stimulus effects have been studied extensively with the two-response, food-reinforced operant procedure and more recently also with discriminated taste aversion (DTA) procedures. DTA procedures have the advantage of a more rapid discrimination training. However, the test phase, i.e., drug substitution, of the DTA procedure is more time consuming (1 test per 4 days) than the test phase of the two-response procedure (2 tests per 5 days). The present study investigated whether a DTA procedure with 2 tests per 5 days could be implemented. In addition, the specificity of the DTA procedure was investigated. Rats were trained to discriminate chlordiazepoxide (CDP, 20 mg/kg, IP) from vehicle using a discriminated taste aversion procedure. Selective suppression of saccharin consumption after CDP injections was maximal after seven CDP-LiCl pairings. In subsequent substitution tests, with 2 tests per 5 days, CDP-mimicking effects were found only for another benzodiazepine, diazepam, and for a barbiturate, pentobarbital. The results indicate that rats can be rapidly trained to discriminate CDP from vehicle in the discriminated taste aversion procedure and that the CDP-cue so produced has the same specificity as in a two-response, food-reinforced operant procedure. However, the DTA procedure has a number of drawbacks that make its advantage over the two-response procedure questionable.

Discriminated taste aversion Drug discrimination Conditioned taste aversion Benzodiazepine Chlordiazepoxide Rat

IN drug discrimination, an animal learns to emit alternative responses under different injection conditions, i.e., push a left lever in a Skinner Box while being drugged and push a right lever after injection with drug vehicle. After learning has been established, subsequent tests with other drugs can be performed to characterize the stimulus properties of the learned discrimination. In most instances, this characterization entails classification of training and test drugs. In a drug versus vehicle discrimination, only test drugs similar to the training drug will lead the animal to emit the training drug response.

Recently, discriminated taste aversion (DTA) was introduced as an alternative for the two-lever operant discrimination procedure. During DTA training, injection of a drug is followed by a conditioned taste aversion trial in which consumption of a flavoured solution is followed by a sickness-inducing injection of a toxin. Injection of vehicle is followed by a safe trial, in which consumption of the same flavoured solution is followed by a second vehicle injection. Rats learn to discriminate both trials after a few drug-toxin pairings, that is, consumption of the flavoured solution is decreased strongly after drug injections and remains normal after vehicle injections. When these effects are compared to the unconditioned effects of the drug on fluid consumption in rats not subjected to drug-toxin pairings, discriminative stimulus effects of the drug are singled out and can be studied selectively.

By comparison, drug discrimination is more rapidly acquired in the taste-aversion design than in the two-response design. The time needed to learn a two-lever operant discrimination (including classical conditioning and nondiscriminant operant training in the beginning) varies from 24 to 65 (1, 3, 4, 6). With the DTA procedure significant discrimination is found after 3 to 6 conditioning trials, that is, after 7 to 20 sessions (7–10, 12). Furthermore, the opiate antagonist naloxone could be discriminated from saline in the DTA procedure, but not in the classical drug-discrimination paradigms (7), suggesting a greater sensitivity for drug cues within the DTA procedure.

Taken together, these findings suggest that DTA can be a valuable alternative for the two-response procedure. However, little is known about the specificity of the stimulus effects within the DTA procedure. In contrast, the specificity of drug cues, especially the specificity of the benzodiazepine (BDZ) cue, has been investigated extensively within the two-response paradigm and shown to be very robust (1,5). Few compounds not binding to the benzodiazepine receptor substitute for benzodiazepines. Reliably, this is only found for the barbiturates (2), a class of compounds with behavioural effects resembling those of the benzodiazepines and binding at a site allosterically coupled to the benzodiazepine receptor in the same GABA receptor complex (10).

It was the main objective of the present study to investigate the specificity of the BDZ cue within the DTA procedure. Hereto, rats were trained to discriminate the prototypical benzo-

[1]Requests for reprints should be addressed to T. H. Hijzen.

diazepine chlordiazepoxide (CDP, 20 mg/kg) from vehicle in a DTA design. After discrimination was established, substitution tests were conducted with drugs from several pharmacological classes in order to delineate the specificity of the CDP cue. These compounds were: CDP and diazepam (benzodiazepines), pentobarbital (barbiturates), clonidine (alpha-adrenergic agonist sedatives), haloperidol (neuroleptics), morphine (opiates), buspirone (5-HT_{1a}-agonist anxiolytics), and amphetamine (psychomotorstimulants). It has previously been found (3) that an antagonist of the central benzodiazepine receptor, flumazenil, can, depending on procedural variables, antagonise as well as partially substitute for a benzodiazepine. Therefore, substitution as well as antagonism tests with flumazenil were performed.

In addition to the specificity of the CDP cue, it was investigated whether the test phase of the DTA procedure could be shortened (2 tests per 5 days) as compared to previous studies (1 test per 4 days).

METHOD

Animals

Sixteen male rats of an outbred Wistar strain (CPB:WU, CPB-TNO, Zeist, The Netherlands), weighing approximately 250 g at the beginning of the experiment, were individually housed under a nonreversed 12-h light-dark cycle and a room temperature of 20–22°C. Food was freely available.

Procedure

The training procedure was basically derived from the one described by Mastropaolo et al. (10). During the whole experiment, training and test sessions were conducted five days a week. No sessions were conducted during the weekends. Rats had free access to tap water from Friday afternoon until 24 h before the Monday session. During the rest of the week, fluid access was restricted to training and test sessions. The sessions took place in a separate room to ensure that rats would not expect a free-drinking period.

Phase I: Restricted drinking and matching. After habituation to the laboratory conditions for 1 week, rats were water deprived for 24 h. For the next two weeeks, rats had access to water for 30 min each session only. From the first day of the third week (session 11) water was replaced by a 0.1% w/v saccharin solution. For three days, an intraperitoneal (IP) 0.9% physiological saline (NaCl, 2 ml/kg) injection was given 15 min prior and immediately after the drinking period (S trials). Rats were assigned to two groups of 8 animals each on the basis of their fluid intake during the three S-trials.

Phase II: Acquisition. On the fourth day of the third week (session 14, the first D-trial), rats received an IP injection of CDP (20 mg/kg, 2 ml/kg) 15 min prior to the drinking period, which was followed by an IP injection of 1.5 mEq, 0.15 M lithium chloride (LiCl, 63.6 mg/kg, 10 ml/kg) for one group of rats (N = 8, LiCl group) and by an equivolume IP NaCl (10 ml/kg) injection for the other group of rats (N = 8, NaCl group). Session 15 was again an S-trial. Subsequently, S- and D-trials were given according to a 2-weekly alternating sequence: D-S-S-D-S, S-D-S-S-D.

Phase III: Testing. The test phase started when, during two consecutive days, the mean volume consumed by the LiCl group was less than 30% of the mean volume consumed by the NaCl group. For a test trial (T-trial), another dose of CDP or a dose of another drug was given before the 30-min access period to the saccharin solution, immediately followed by an IP NaCl (2 ml/kg) injection. At test sessions, all animals received the same drug dose. With few exceptions (in case additional drug doses became necessary), drug administration was systematically varied across dose substitution tests. The alternating weekly sequence of trials was changed to S-T-D-S-T. After fluid intake on the Monday S-trial appeared to be identical to fluid intake during S-trials on other days, a T-S-D-S-T sequence was used during the remainder of the experiment. T-trials immediately before and after D-trials, in which fluid intake in the LiCl group was more than 30% of the amount consumed by the NaCl group, were discarded and repeated after rats had been retrained to criterion. During the weeks in which antagonism tests with flumazenil were conducted, rats received an extra IP injection of flumazenil vehicle 10 min before the NaCl (S-trial) or CDP (D-trial) injection.

Drugs

D-Amphetamine sulphate (1–2 mg/kg), buspirone hydrochloride (3–6 mg/kg; Bristol-Myers, Evansville, IN), chlordiazepoxide hydrochloride (5–20 mg/kg; Hoffmann-La Roche, Basel, Switzerland), clonidine hydrochloride (0.01–0.1 mg/kg; Brunschwig, Amsterdam, The Netherlands), morphine hydrochloride (3–9 mg/kg), and sodium pentobarbital (10–30 mg/kg; OPG, Utrecht, The Netherlands), were dissolved in physiological (0.9%) saline. Diazepam (2.5–5 mg/kg; OPG, Utrecht, The Netherlands) was dissolved in a vehicle containing 1.5% benzyl alcohol, 8.5% ethyl alcohol, 41.5% propylene glycol and 48.5% distilled water. Flumazenil (7.5–60 mg/kg; Hoffmann-La Roche, Basel, Switzerland) was suspended in a vehicle containing distilled water to which Tween 80 (2 drops/10 ml) was added. Haloperidol (Haldol, 0.125–0.5 mg/kg; Janssen, Beerse, Belgium) was obtained from commercially available ampoules and diluted with distilled water. Similar dose ranges have been used in the more traditional drug discrimination literature. Doses refer to the forms indicated. Solutions and suspensions were freshly prepared each day. Drugs were administered IP in an injection volume of 2 ml/kg, except for diazepam, which was injected in a volume of 0.4 ml/kg. Drugs were given 15 min prior to testing, with the following exceptions. Diazepam was given 20 min and amphetamine and haloperidol 30 min prior to testing. In the antagonism tests, flumazenil was given 10 min prior to CDP and 25 min prior to testing.

Data Analysis

Phase I: Restricted drinking. The data for the 2-week period, in which rats were given access to water for only 30 min each session, were analysed with a multivariate analysis of variance (MANOVA) having Days as a within factor with 5 levels and Week as a within factor having 2 levels.

Phase II: Acquisition. Data during acquisition were analysed with a MANOVA having Group as a between factor having two levels (LiCl and NaCl), Trial as a within factor having two levels (D and S), and Pairings as a second within factor having 7 levels (i.e., the number of CDP-LiCl pairings needed to reach the discrimination criterion). For each individual rat, the mean of all S-trials preceding a D-trial was taken as one single S-trial.

Phase III: Testing. Data of test compounds were analysed with a MANOVA having Group as a between factor with two levels (LiCl and NaCl), and Dose as a within factor. If a significant Group × Dose effect was found, the analysis was contin-

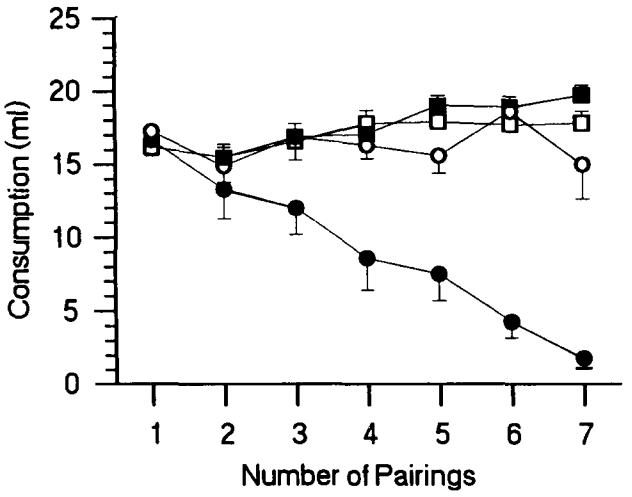

FIG. 1. Fluid intake for the NaCl group after administration of CDP (○) and NaCl (□) and for the LiCl group after administration of CDP (●) and NaCl (■), for 7 consecutive CDP-LiCl pairings, showing acquisition of discriminated taste aversion with CDP (20 mg/kg) as the discriminative stimulus. Vertical bars indicate the standard errors of the mean.

ued in the following way. For each dose of the test drug, fluid intake by the LiCl group was compared with fluid intake by the NaCl group on the same test day and with fluid intake by the LiCl group on the nearest D-trial. For the D-value of the LiCl group, fluid intake during the D-trial performed in the same week as the T-trial was taken.

Substitution occurred if fluid intake after administering the dose of the test drug to the LiCl group was significantly lower than for the NaCl group and not significantly higher than the D-value. Unconditioned effects of doses of test drugs were determined by comparing fluid intake of the NaCl group in a T-trial with the intake in the S-trial immediately preceding or following the T-trial. All comparisons were made by means of Student's *t*-test. For all tests a significance level of 5% was chosen.

RESULTS

Phase I: Restricted Drinking

There was no difference in fluid intake beween weeks or day of the week for the period in which animals were given access to water for only half an hour each day.

Phase II: Acquisition

For the data during acquisition, a significant decrease in fluid intake was found for the LiCl group, $F(1,14)=15.3$, $p<0.01$, and in D-trials, $F(1,14)=35.3$, $p<0.01$ (Fig. 1). A clear indication of learning the discrimination is reflected by the significant Group × Trial × Pairings interaction, $F(6,9)=17.3$, $p<0.01$, and by the observation that only the D-trial curve of the LiCl group gradually declined (Fig. 1). The D-trial of the LiCl group

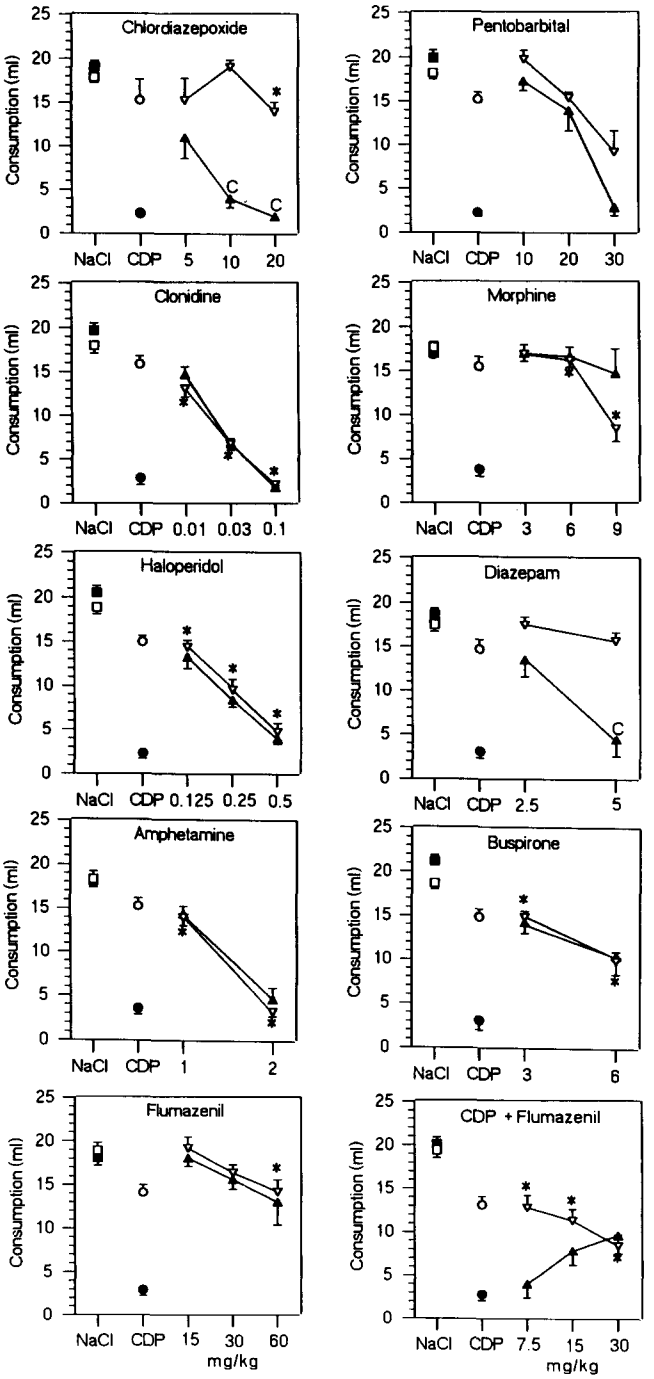

FIG. 2. Effects of test compounds on fluid intake by rats trained to discriminate 20 mg/kg CDP from vehicle (LiCl group, ▲), and by a control group of rats not trained to discriminate CDP from vehicle (NaCl group, ▽). Substitution for the LiCl group is indicated by the letter c (c = complete substitution, partial substitution was not found). Asterisks indicate suppression of fluid intake for the NaCl group (*$p<0.05$). Vertical bars indicate standard errors of the mean. NaCl: data from the nearest NaCl session; NaCl group = □, LiCl group = ■. CDP: data from the nearest CDP 20 mg/kg session; NaCl group = ○, LiCl group = ●.

differed significantly from the D-trial of the NaCl group and the previous S-trial of the LiCl group at the third drug session, $F(1,13)=3.9$, 5.7, respectively; $p<0.05$.

At the end of the acquisition period the animals of the NaCl group consumed less after CDP administration than after saline administration (Fig. 1; $p<0.05$). Moreover, the lower consumption after CDP persisted during the test phase (Fig. 2), indicating that the sedative effects of 20 mg/kg CDP did not tolerate in the present setting, and may have overruled a dipsogenic effect of CDP.

Phase III: Testing

Significant differences between the LiCl and NaCl group were found for CDP, $F(1,14)=41.4$, $p<0.01$, diazepam, $F(1,14)=22.6$, $p<0.01$, and pentobarbital, $F(1,14)=5.1$, $p<0.05$. Significant Dose × Group effects were found for CDP, $F(2,13)=7.4$, $p<0.01$, diazepam, $F(1,14)=6.3$, $p<0.05$. Subsequent analyses (see the Method section) showed that substitution was induced by the 10 and 20 mg/kg doses of CDP and the 5 mg/kg dose of diazepam (Fig. 2).

A significant Dose × Group effect was also found for the combination of flumazenil + CDP, $F(2,13)=9.2$, $p<0.01$, indicating antagonism of CDP by flumazenil.

Unconditioned effects of doses of test drugs on fluid intake are indicated by asterisks in Fig. 2.

From the beginning of LiCl administration, an increasing difference between the weights of the rats in the LiCl and NaCl group developed. At the end of the study the LiCl group weighed 27.4 g less than the NaCl group. The differences in body weight between both groups averaged over Monday trials was 17.6 g, $F(1,14)=5.1$, $p<0.05$.

DISCUSSION

The present results show that rats can be trained to discriminate CDP from vehicle using a DTA procedure. Since the animals almost immediately adapt to the restricted water regime, acquisition is substantially faster in the DTA procedure than in the two-way response procedure. In previous studies, this advantage was offset by a longer test phase (7, 8, 10, 12). In the present study, the test phase was performed as fast as in the two-response procedure, with two tests per five days.

The substitution and antagonism test results suggest that the discriminative stimulus properties of CDP in the present procedure had the same high degree of specificity as in the two-response, food-reinforced operant procedure. Substitution was not induced by prototypical members of pharmacological classes of drugs which did not substitute for benzodiazepines in the two-response procedure, e.g., clonidine (13), haloperidol (1), morphine (4), buspirone (6), and amphetamine (4). In these studies, CDP was not always the training drug. Nevertheless, the present results may be compared with results obtained with other benzodiazepines, since the discriminative stimulus properties of the different benzodiazepines are highly similar, especially at high training doses (14,15).

Substituation was found for the two benzodiazepines, CDP and diazepam. Although expected, a significant Dose × Group interaction was not found for pentobarbital. Furthermore, in a post hoc analysis, complete substitution was found for the 30 mg/kg dose of pentobarbital only. These results suggest that pentobarbital did not induce dose-dependent substitution for CDP. Complete substitution for CDP was tentatively found at a dose that had a strong unconditioned suppressive effect on fluid intake (cf.12). In the two-response procedure, doses of pentobarbital substituting completely for a high dose of CDP have also been found to suppress responding strongly (2). The antagonism of CDP by flumazenil indicates that the discriminative stimulus effects of CDP were mediated by the central benzodiazepine receptor. Similar results were found with the two-response procedure [cf. (3)]. Flumazenil did not substitute for CDP. This finding is in agreement with studies reporting that flumazenil partially substitutes for a low, but not for a high dose of CDP (3).

In the present study, an injection schedule different from that in previous experiments (8,10) was used. In the procedures used by Mastropaolo et al. (10) and Lucki (8) there is 1 test day every 4 sessions. In the present experiment there were 5 sessions a week with 2 test days. A problem with this schedule is that in a 5-day sequence, test days cannot be preceded by both a D- and an S-trial. This may have contributed to the loss of discrimination found twice after administration of drugs substituting for the training drug.

The present results show that, in the DTA procedure, rats can be rapidly trained to discriminate a benzodiazepine from vehicle. The discriminative stimulus properties of CDP in the DTA procedure are highly similar to those obtained in the two-response procedure. Nevertheless, the DTA procedure has a number of drawbacks.

Firstly, the number of injections is greater in the DTA procedure than in the two-response procedure. Animals are injected twice a day and during antagonism testing three times a day. Further, although postinjections of saline may be omitted at control days, the great number of injections may affect the health of the rats. Secondly, only LiCl rats receive sickness inducing LiCl injections. These could have a variety of effects which might interfere with test results in no way related to the discrimination. One example is the effect on weight found in the present experiment. This type of problem can be prevented by injecting control animals also with LiCl, though not paired with the drug stimulus (9).

Finally, trials themselves are time consuming. Animals have to be injected twice. Also, rats cannot serve as their own controls for unconditioned effects of drugs on fluid intake and, therefore, one extra group of rats is required.

In summary, the data of the present study indicate that discriminative stimulus properties of benzodiazepines in the DTA procedure are highly similar to those obtained with the two-response procedure. The present results also show that the DTA procedure can be shortened to five-day cycles with two test sessions a week. Some negative side-effects of the DTA procedure were discussed.

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