Pharmacology Biochemistry and Behavior, Vol. 42, pp. 487–495, 1992
Printed in the U.S.A.

0091-3057/92 $5.00 + .00

Assessment of the Stimulus Properties of Anxiolytic Drugs by Means of the Conditioned Taste Aversion Procedure

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Received 20 December 1991

VAN HEST, A., T. H. HIJZEN, J. L. SLANGEN AND B. OLIVIER. *Assessment of the stimulus properties of anxiolytic drugs by means of the conditioned taste aversion procedure.* PHARMACOL BIOCHEM BEHAV 42(3) 487–495, 1992.—The conditioned taste aversion (CTA) procedure has recently been described as a more rapid alternative to two-lever operant procedures in drug discrimination research. We trained different groups of rats to discriminate the benzodiazepine chlordiazepoxide (CDP, 20 mg/kg) or the 5-hydroxytryptamine$_{1A}$ (5-HT$_{1A}$) agonist 8-hydroxy-2-(di-*n*-propylamino)tetralin (8-OH-DPAT) (0.4 mg/kg) from saline by means of the CTA procedure. The results were in agreement with findings from two-lever operant drug discrimination procedures. However, discrimination training took 40 sessions in the case of CDP and 72 sessions for 8-OH-DPAT, which is comparable to results obtained with two-lever operant procedures. Dose-response curves were determined and generalization tests were performed for different benzodiazepine and nonbenzodiazepine anxiolytics. Baseline behavior deteriorated in the course of generalization and substitution testing, thus preventing further generalization testing. Our experience is that the use of the CTA procedure in drug discrimination research does not have sufficient advantages over traditionally used procedures to replace the latter.

Conditioned taste aversion Drug discrimination Anxiolytics CDP 8-OH-DPAT

CONDITIONED taste aversion (CTA) refers to the reduced intake of a preferred solution due to a previous pairing of a novel taste with drug (LiCl)-induced sickness. The CTA procedure was originally described by Garcia and coworkers (9). Recently, a number of investigators reported the use of the CTA paradigm in drug discrimination learning. Water-deprived rats are injected with a drug and are subsequently exposed to a saccharin solution. Immediately following saccharin access, subjects are injected with LiCl. On recovery days, rats are injected with vehicle and given access to the saccharin solution, but on these occasions access to the saccharin solution is followed by injections with physiological saline (NaCl). In this procedure, therefore, the presence of a drug signals the pairing of saccharin with LiCl-induced sickness, whereas the absence of the drug signals the pairing of saccharin with saline. Subjects rapidly acquire the discrimination, avoiding saccharin consumption following drug administration and consuming saccharin following vehicle. In addition, it has been shown that rats will also learn to avoid saccharin following vehicle if vehicle has been given prior to saccharin–LiCl pairings (12,18,19). The aversion can thus be established independently of whether the presence or absence of the drug signals LiCl toxicosis.

Conditioning to the discriminative stimulus properties of drugs by means of CTA methodology has been demonstrated for various compounds, including phencyclidine (19), naloxone (13), pentobarbital (12,22), fentanyl (12), morphine (18), and several 5-hydroxytryptamine$_1$ (5-HT$_1$) agonists (14–16). In general, the results from these studies paralleled those from studies using lever pressing as the behavioral response, both during acquisition as well as during substitution tests. Consumption of saccharin water following drug injections gradually decreased in the course of training for subjects that received drug–saccharin–LiCl pairings. A dose-related suppression of saccharin intake was observed when dose-substitution sessions were interspersed between training sessions. In addition, it was reported that stimulus cues generalized to drugs from classes similar to the training drug but not to drugs from classes different from the training drug. On the basis of these results, the CTA procedure seems a valid method for the assessment of stimulus properties of drugs. However, the use of CTA in these kinds of studies is relatively new, and its efficacy and potency must be further assessed.

In recent years, a new class of nonbenzodiazepine anxiolytic drugs has been developed. Buspirone, which is clinically effective in alleviating anxiety, and the structurally related

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compounds gepirone and ipsapirone do not interact with benzodiazepine $GABA_A$ receptors, but preferentially bind to 5-HT_{1A} receptor sites localized in the hippocampus and other limbic structures that may be involved in anxiety (26). In animal studies, buspirone has been shown to increase punished responding in rats (8,30) and pigeons (1). These effects cannot be blocked by the benzodiazepine receptor antagonist Ro 15-1788 (flumazenil), nor by interference with the dopaminergic system (1,28). The results from drug discrimination studies have provided further evidence for a serotonergic mechanism of action by showing that buspirone and ipsapirone share stimulus properties with substances with high affinity for the 5-HT_{1A} receptor, such as 8-hydroxy-2-(di-*n*-propylamino)tetralin (8-OH-DPAT) and 5-methoxy-*N*-*N*-dimethyltryptamine (5-MeODMT), but do not generalize to benzodiazepine receptor agonists such as oxazepam, diazepam, or midazolam (11, 17,24).

The present experiments were designed to evaluate the use of CTA procedures in drug discrimination research. We chose to study benzodiazepine and nonbenzodiazepine anxiolytics because the stimulus properties of drugs from these classes are among the most well documented in the drug discrimination literature (3,6,7,29). Thirty-two rats were trained to discriminate the benzodiazepine receptor agonist chlordiazepoxide (CDP, 20 mg/kg) from saline. Another group of rats learned to discriminate the 5-HT_{1A} agonist 8-OH-DPAT (0.4 mg/kg) from saline. LiCl was given in doses up to 1.2 mEq (48.0 mg/kg), the precise doses depending upon the amount of fluid consumed. This manipulation was instated for different reasons. First, from a theoretical point of view it may be undesirable to impose the same aversive contingencies following two opposing responses (i.e., drinking and no drinking). Second, lowering the total amount of LiCl diminishes possible hazardous effects of prolonged LiCl treatment on the subjects' health. Dose-substitution tests were performed when subjects had achieved stable baseline performance. Generalization gradients were obtained for the benzodiazepine diazepam, the 5-HT_{1A}agonists buspirone and ipsapirone, and the 5-HT_{1B} agonist 1-(3-trifluoromethylphenyl)piperazine (TFMPP).

EXPERIMENT 1

METHOD

Subjects

Thirty-two male Wistar rats were obtained from Harlan (Zeist, The Netherlands) when they were approximately 5 weeks old. Upon arrival in the laboratory, subjects were individually housed under a reversed light–dark cycle (lights on 7:00 p.m.–7:00 a.m.). All tests were performed during the dark portion of the light–dark cycle. Food was always available.

Drugs

All drugs were freshly prepared before use. CDP (OPG, Utrecht, The Netherlands) was dissolved in distilled water containing 0.5% gelatine and 5% manitol [gelatine/manitol (GM)]. Diazepam (OPG) and buspirone (Sigma Chemical Co., St. Louis, MO) were dissolved in distilled water. All drugs were administered IP in a volume of 2 ml/kg. LiCl (OPG) was dissolved in distilled water and injected IP in a volume of 8 ml/kg. Saccharin was obtained from Sigma.

Adaptation

First, all subjects were adapted to a restricted drinking schedule. Water bottles were removed from the cages. Following 23.5 h of water deprivation, all subjects were given access to tapwater in the home cage once a day between 9:00–9:30 a.m. for 2 consecutive days. Water spouts were inserted on top of the cage. After 2 days of tapwater access, subjects were given daily access to a 0.1% w/v saccharin solution for 3 consecutive days. Saccharin was available between 9:30–10:00 a.m. The amount of fluid consumed was measured. Subjects were matched on saccharin consumption following the drinking period on day 5 and were assigned to one of four groups (n = 8 per group). Subjects drank an average of 20.1 (±3.11) ml saccharin water during the last day of adaptation.

TABLE 1

SACCHARIN WATER CONSUMPTION AND CORRESPONDING DOSES OF LiCL

Consumption (ml)	LiCl (8 ml/kg)
0–5.5	0.3 mEq
5.6–10.5	0.6 mEq
10.6–15.5	0.9 mEq
>15.6	1.2 mEq

Acquisition

The experiment started when subjects were 7 weeks old and weighed an average of 226.8 (±13.1) g. Throughout conditioning, subjects received an IP injection with either 20.0 mg/kg CDP (groups E_d and C_d) or vehicle (GM, groups E_v and C_v) 15 min prior to saccharin exposure. Immediately following the 30-min drinking period, subjects received an injection with either LiCl (groups E_d and E_v) or physiological saline (0.9% NaCl in distilled water, groups C_d and C_v). LiCl was given in doses up to 1.2 mEq (48.0 mg/kg), the precise doses depending upon the amount of saccharin consumed, according to the scheme presented in Table 1.

On recovery days, subjects in groups E_d and C_d received vehicle injections prior to saccharin exposure, while subjects in groups E_v and C_v received CDP. All groups received NaCl injections following saccharin exposure on these days. Drug and vehicle sessions were given in a quasirandom order. Table 2 presents an overview of the conditioning procedure during acquisition.

The complete cycle (days 1–10) was repeated until all subjects had acquired the discrimination and achieved stable baseline performance.

Substitution

The procedure during this phase was identical to the conditioning procedure during acquisition with the exception that on days 1, 5, and 8 of the cycle subjects received an injection with the test drug prior to saccharin water exposure. Saccharin exposure during substitution test sessions was never followed by LiCl or NaCl injections.

Acquisition and substitution sessions were conducted 5 days a week (Monday–Friday) and always between 9:00–11:00 a.m. During weekends, subjects were given free access to tapwater from 4:00 p.m. Friday until 9:00 a.m. Sunday.

RESULTS

Acquisition

Figure 1, session blocks 1–5, shows the mean saccharin consumption for subjects exposed to CDP–LiCl (group E_d,

TABLE 2
OVERVIEW OF THE CONDITIONING PROCEDURE

	Group			
Day	E_d	C_d	E_v	C_v
1	Vehi-NaCl	Vehi-NaCl	Drug-NaCl	Drug-NaCl
2	Vehi-NaCl	Vehi-NaCl	Drug-NaCl	Drug-NaCl
3	Drug-LiCl	Drug-NaCl	Vehi-LiCl	Vehi-NaCl
4	Vehi-NaCl	Vehi-NaCl	Drug-NaCl	Drug-NaCl
5	Drug-LiCl	Drug-NaCl	Vehi-LiCl	Vehi-NaCl
6	Drug-LiCl	Drug-NaCl	Vehi-LiCl	Vehi-NaCl
7	Vehi-NaCl	Vehi-NaCl	Drug-NaCl	Drug-NaCl
8	Drug-LiCl	Drug-NaCl	Vehi-LiCl	Vehi-NaCl
9	Vehi-NaCl	Vehi-NaCl	Drug-NaCl	Drug-NaCl
10	Drug-LiCl	Drug-NaCl	Vehi-LiCl	Vehi-NaCl

Vehi, vehicle; NaCl, natrium chloride; LiCl, Lithium chloride.

left panel) and vehicle-LiCl pairings (group E_v, right panel) and their appropriate controls in blocks of four sessions. Open symbols show mean saccharin consumption in NaCl sessions, that is, those sessions in which subjects in both the experimental (E_d and E_v) and control groups (C_d and C_v) received NaCl injections following saccharin consumption. Filled symbols depict consumption on those sessions during which subjects in experimental groups received LiCl injections following consumption while subjects in control groups received NaCl injections. The latter sessions will be referred to as LiCl sessions. Saccharin consumption during NaCl and LiCl sessions was subjected to analysis of variance (ANOVA). All differences between groups or sessions that are reported in the Results section adhere to a significance level of $p < 0.01$. Saccharin consumption during successive NaCl or LiCl sessions were analyzed separately. Mean saccharin consumption during the fifth block of four sessions did not differ from consumption during the immediately preceding block of four sessions ($p < 0.01$). From this analysis, it was concluded that all subjects had achieved stable baseline performance after 4 training cycles of 10 sessions each. Subjects in groups E_d and E_v consumed less than 5 ml saccharin water during LiCl sessions at the end of the acquisition phase.

Differences between groups E_d and C_d during NaCl sessions were not observed, but subjects in E_d consumed less than subjects in group C_d when subjects were exposed to CDP-LiCl (group E_d) or CDP-NaCl pairings [group C_d, $F(1, 14) = 29.39$]. The analysis revealed an overall effect of CDP treatment, $F(1, 7) = 13.39$, showing that the reduction in saccharin intake during LiCl sessions was not solely due to discrimination learning but that nonspecific drug effects might also have played a role. It was estimated that CDP alone, irrespective of whether CDP was followed by LiCl or NaCl treatment, accounted for a 2-ml reduction of saccharin consumption. Differences between consumption in NaCl and LiCl sessions in group E_d were then compared to the corresponding consumption means for subjects in group C_d to account for nonspecific CDP effects. This analysis showed that saccharin

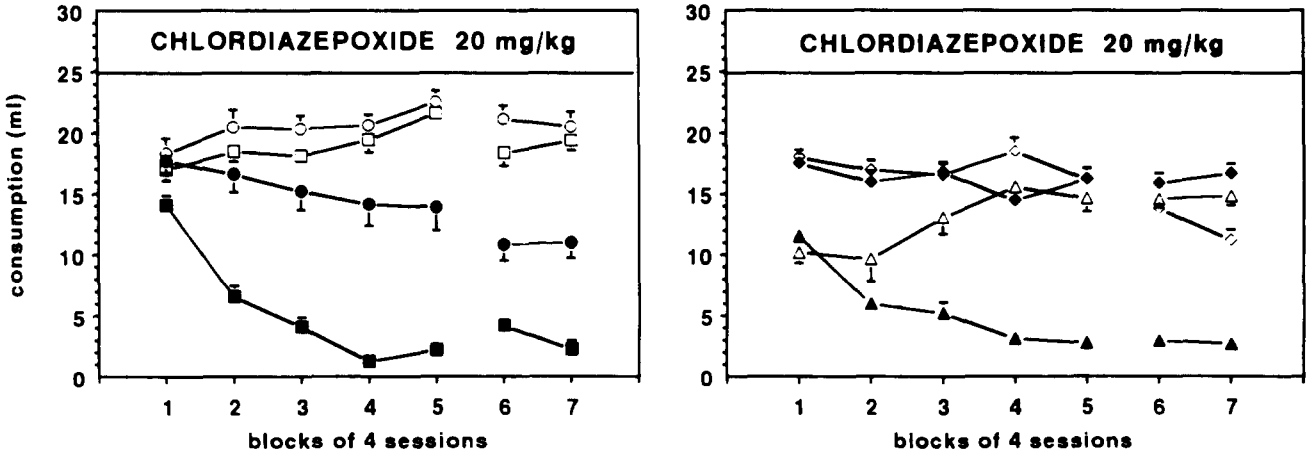

FIG. 1. Mean (±SEM) saccharin consumption for subjects trained to discriminate CDP from vehicle. Left: Consumption on days when both the experimental (□) and control (○) groups received NaCl following saccharin exposure and on days when experimental groups (■) received LiCl while control groups (●) received NaCl following saccharin consumption. Right: Consumption on days when both the experimental (△) and control (◇) groups received NaCl following saccharin consumption and on days when experimental groups (▲) received LiCl while control groups (◆) received NaCl following saccharin consumption.

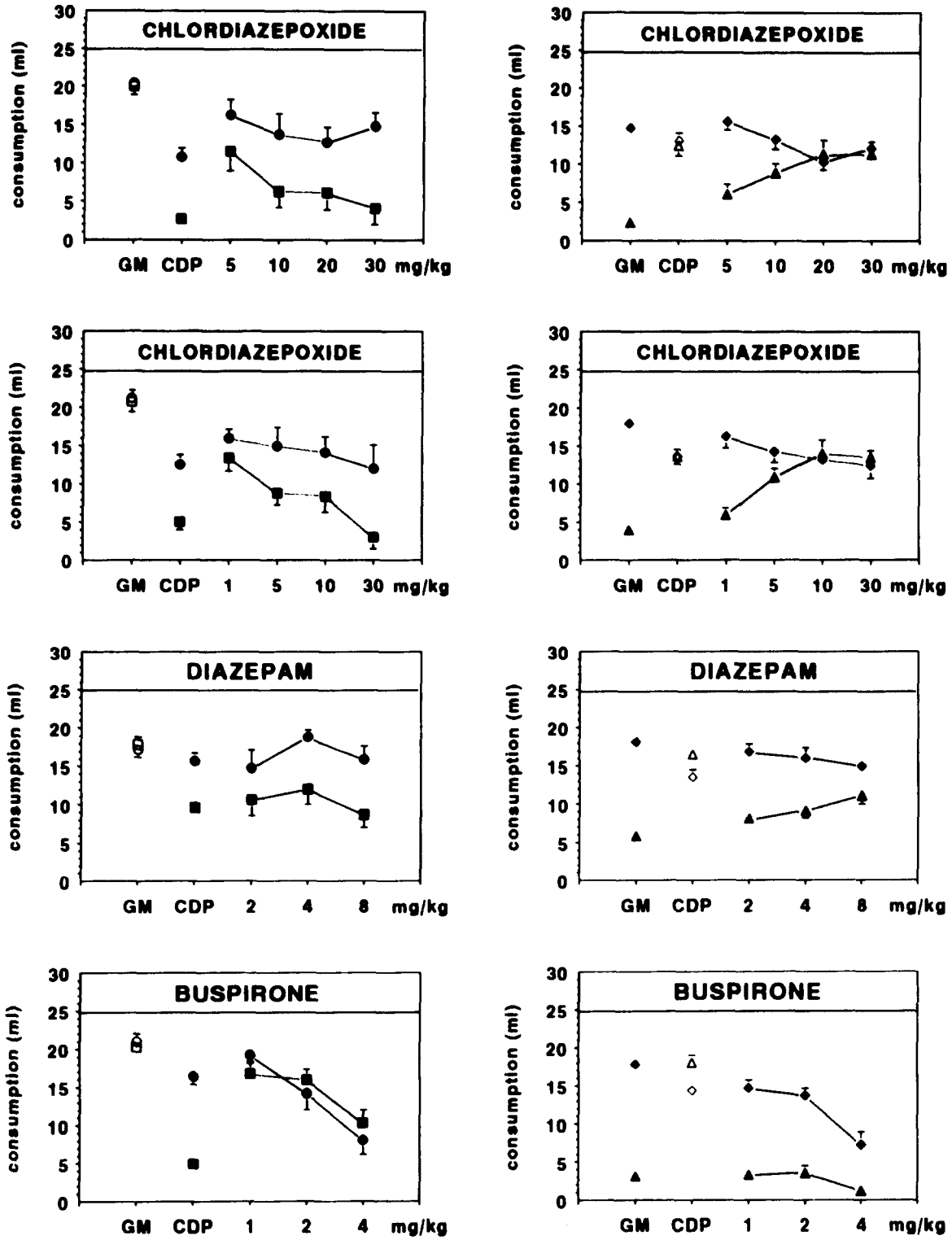

FIG. 2. Dose-response determinations for subjects trained to discriminate CDP from vehicle (GM). The left panels show data for subjects exposed to CDP-LiCl pairings during training (■, □); the right panels show data for subjects exposed to GM-LiCl pairings (▲, △); their appropriate controls are also represented (●, ○, ♦, ◇, respectively).

intake following CDP treatment was significantly more suppressed in experimental subjects than in controls, $F(1, 14) = 29.39$.

The results for subjects that were trained to refrain from drinking following vehicle injections (groups E_v and C_v) mirrored the results of groups E_d and C_d, but differences between groups were also observed during NaCl sessions, $F(1, 14) = 13.87$. The experimental subjects consumed less water following CDP treatment than their matched controls, $F(1, 14) = 107.78$. However, between-group comparisons of the differences between consumption in LiCl and NaCl sessions revealed that experimental subjects consumed less than their matched controls, suggesting that the vehicle came to serve as a discriminative stimulus cue for subjects in group E_v, $F(1, 14) = 13.87$.

Comparisons between groups showed that subjects drank less, $F(1, 28) = 12.08$, and acquired the discrimination more rapidly, $F(4, 112) = 3.99$, when CDP was paired with LiCl than when it was paired with NaCl. As can be inferred from Fig. 1, subjects in group E_d consumed ± 15 ml during both LiCl and NaCl sessions and then gradually learned to refrain from drinking in the presence of CDP. Subjects in group E_v, on the other hand, showed a generalized suppression of saccharin consumption immediately after the first LiCl treatment. Saccharin consumption during safe trials then slowly recovered in the presence of CDP and diminished in the presence of NaCl.

Substitution

Following acquisition, a dose-response curve was determined for CDP. These data are depicted in the upper left and upper right panels of Fig. 2. Subjects were injected with different doses of CDP (5.0, 10.0, and 30.0 mg/kg). All subjects received all doses once in a random order. Treatment with increasing doses CDP produced a neat generalization curve. Subjects in group E_d dose dependently refrained from saccharin drinking and subjects in group E_v showed increased saccharin consumption after they were given increasing doses of CDP. Saccharin consumption in control groups decreased after the higher doses of CDP, but less than the decrease that was observed for subjects in group E_d, and may have been due to nonspecific effects of CDP on fluid intake.

Following CDP dose-response testing, all subjects were reexposed to the acquisition procedure for two complete cycles (Fig. 1, session blocks 6–7). Reacquisition was necessary because subjects in group E_d consumed an increasing amount (more than 5 ml) of saccharin water after they were treated with CDP. Another CDP dose-response curve (Fig. 2, left and right panels in the second row from above) was then established to investigate whether the curve obtained during the first dose-substitution phase could be replicated. Comparison between the first and second rows of panels in Fig. 2 reveals an overall decrease in saccharin consumption for all groups when the dose-response curve was redetermined. Qualitative differences between the first and second dose-response curves were not observed.

Finally, subjects were injected with increasing doses of diazepam (2.0, 4.0, and 8.0 mg/kg) and buspirone (1.0, 2.0, and 4.0 mg/kg) 15 min prior to saccharin exposure. These data are depicted in the lower panels of Fig. 2. Saccharin consumption following buspirone treatment did not differ from consumption following vehicle. Diazepam dose dependently decreased saccharin intake in subjects trained to avoid saccharin water following CDP administration and increased consumption in subjects trained with CDP as a safe signal for drinking. Further deterioration of baseline behavior prevented generalization testing with other compounds.

EXPERIMENT 2

METHOD

Subjects

Thirty-two males were obtained from Harlan (Zeist, The Netherlands) when they were approximately 5 weeks old. Housing conditions were identical to those described for subjects participating in Experiment 1.

Drugs

All drugs were freshly prepared before use. 8-OH-DPAT (RBI, Natick, MA), ipsapirone, (Solvay Duphar, The Netherlands), and TFMPP (Solvay Duphar) were dissolved in physiological saline (0.9% NaCl in distilled water). All drugs were administered IP in a volume of 2 ml/kg. LiCl was dissolved in distilled water and injected in a volume of 8 ml/kg.

Adaptation

The adaptation procedure was identical to the adaptation phase employed in Experiment 1. Subjects drank an average of 18.8 (± 3.53) ml saccharin water on the last day of adaptation.

Acquisition

Subjects were 7 weeks old and weighed an average of 221.00 (± 17.55) g at the start of experimentation. The conditioning procedure was basically identical to the procedure used in Experiment 1. Subjects in group E_d were treated with 0.4 mg/kg 8-OH-DPAT prior to saccharin–LiCl pairings and with vehicle when saccharin exposure was followed by NaCl. Subjects in group E_v were exposed to a reversed procedure, that is, they were given NaCl–LiCl or 8-OH-DPAT–NaCl pairings. Subjects in control groups C_d and C_v were repeatedly injected with vehicle or 8-OH-DPAT prior to saccharin water exposure, but they were never treated with LiCl.

Substitution

Test sessions were interspersed between training sessions. Subjects were injected with test drug on days 1, 5, and 8 of the conditioning cycle (Table 2). Saccharin consumption on these days was never followed by LiCl treatment. Subjects received 8-OH-DPAT or vehicle prior to saccharin exposure on the other remaining days of the conditioning cycle.

RESULTS

Acquisition

Figure 3 shows the mean saccharin consumption for subjects exposed to 8-OH-DPAT–LiCl (group E_d, left panel) and vehicle–LiCl pairings (group E_v, right panel) and their appropriate controls in blocks of four sessions. Open symbols show mean saccharin consumption when saccharin exposure was followed by NaCl injections. Solid symbols depict consumption when subjects in experimental groups received LiCl injections following consumption, whereas control subjects re-

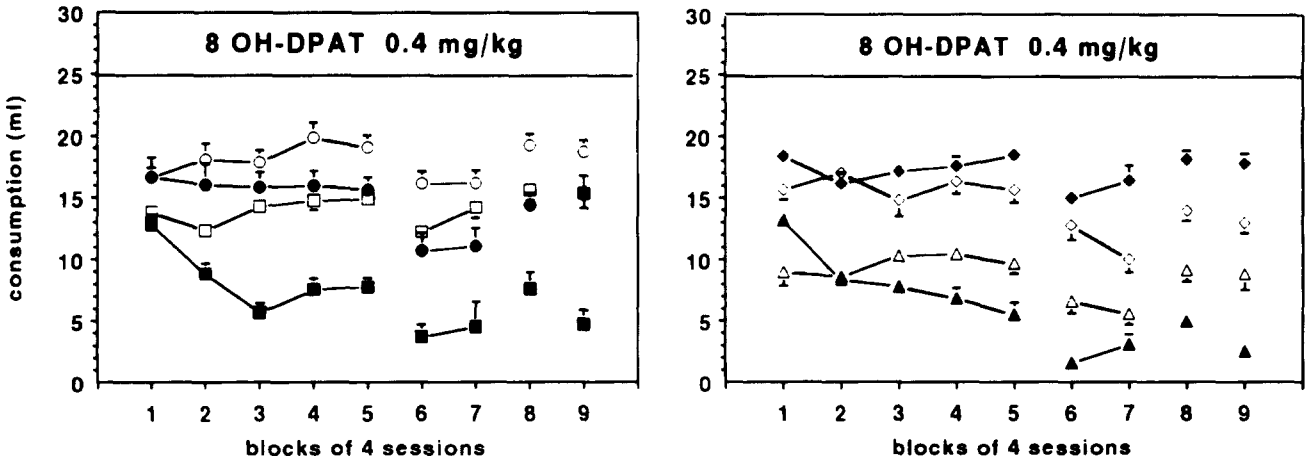

FIG. 3. Mean (±SEM) saccharin consumption for subjects trained to discriminate 8-OH-DPAT from vehicle. Left: Consumption on days when both the experimental (□) and control (○) groups received NaCl following saccharin exposure and on days when experimental groups (■) received LiCl while control groups (●) received NaCl following saccharin consumption. Right: Consumption on days when both the experimental (△) and control (◇) groups received NaCl following saccharin consumption and on days when experimental groups (▲) received LiCl while control groups (◆) received NaCl following saccharin consumption.

ceived NaCl injections. These later sessions are referred to as LiCl sessions. Data analysis was accomplished by means of ANOVA as described in Experiment 1.

Training was continued until all subjects had completed four cycles (sessions 1–40). Subjects in experimental groups consumed less than their controls when consumption was followed by LiCl treatment, $F(1, 28) = 96.69$. However, experimental subjects also showed a reduction in intake on NaCl sessions as compared to controls, albeit to a lesser extent than on LiCl sessions, $F(1, 28) = 22.08$. Control subjects consumed less saccharin following 8-OH-DPAT injections than during vehicle sessions, $F(1, 7) = 17.99$. Nonspecific drug effects on water intake accounted for a 2.1-ml reduction during drug sessions irrespective of whether subjects received LiCl or NaCl following saccharin intake. As such, saccharin intake on NaCl and LiCl sessions for subjects in group E_d was compared to the corresponding values for subjects in group C_d and subjected to ANOVA to differentiate between conditioning effects on the one hand and nonspecific drug effects on the other. This analysis revealed that saccharin intake following 8-OH-DPAT injections was significantly more suppressed in experimental subjects than in controls, $F(1, 14) = 7.94$. In addition, it was shown that the aversion was more rapidly established when drug, as opposed to vehicle, injections preceded LiCl toxicosis, $F(1, 28) = 39.31$.

Despite the fact that consumption preceding LiCl treatment was suppressed, subjects never completely acquired the discrimination during the first 40 sessions of the acquisition phase. All experimental subjects still consumed more than 5 ml saccharin water during LiCl sessions at the completion of the fourth cycle. Therefore, subjects were given free access to tapwater between 3:30–4:00 p.m. during sessions 41–56 (sessions blocks 6–7). This manipulation was instated to see whether the failure to obtain good discrimination performance was due to dehydration. Saccharin intake decreased by an overall average of 3.4 ml, but the suppression was observed to the same extent for all groups and treatment conditions, $F(1, 28) < 1.0$, NS. During sessions 57–64 (session block 8), the original conditioning procedure was then reinstated and subjects were no longer given access to water during the afternoon. Saccharin intake increased again to baseline levels. Finally, during sessions 65–72 (session block 9) the doses of LiCl following saccharin exposure was no longer dependent upon saccharin consumption but was fixed at 1.2 mEq (48.0 mg/kg). Subjects in both experimental groups showed good discrimination performance at the completion of this acquisition phase, drinking less than 5 ml saccharin water during saccharin exposure preceding LiCl treatment, $F(1, 2) = 40.04$.

ANOVA on body weights showed that subjects exposed to LiCl treatment gained considerably less weight in the course of training as compared to control subjects, $F(8, 240) = 14.46$.

Substitution

Next, a dose–response curve was obtained for 8-OH-DPAT (Fig. 4, upper row of panels). Subjects were injected with different doses (0.1, 0.2, and 0.8 mg/kg) of 8-OH-DPAT on days 1, 5, and 8 of the conditioning cycle. Subjects in group E_d dose dependently decreased their saccharin intake after increasing amounts of 8-OH-DPAT, but the decrease as observed after treatment with the highest (0.8 mg/kg) dose can be attributed to nonspecific drug effects on fluid intake as a similar decrease was also observed for subjects in groups C_d and C_v. Subjects in group E_v showed a dose-dependent increase in saccharin intake after treatment with increasing doses of 8-OH-DPAT.

Substitution tests with TFMPP (0.2, 0.4, and 0.8 mg/kg) and ipsapirone (2.0, 4.0, 8.0, and 16.0 mg/kg) are depicted in the lower part of Fig. 4. It was shown that the 8-OH-DPAT cue generalized to ipsapirone but not to TFMPP. Again, subjects from both experimental as well as control groups showed decreased saccharin intake at the higher doses of both drugs.

GENERAL DISCUSSION

The results of the present experiments confirmed the findings of others, showing that rats are able to discriminate between drug and saline when they are exposed to a CTA procedure. Subjects in the present experiment learned to refrain from saccharin drinking in the presence of CDP or 8-OH-DPAT when the drug condition was followed by LiCl-induced

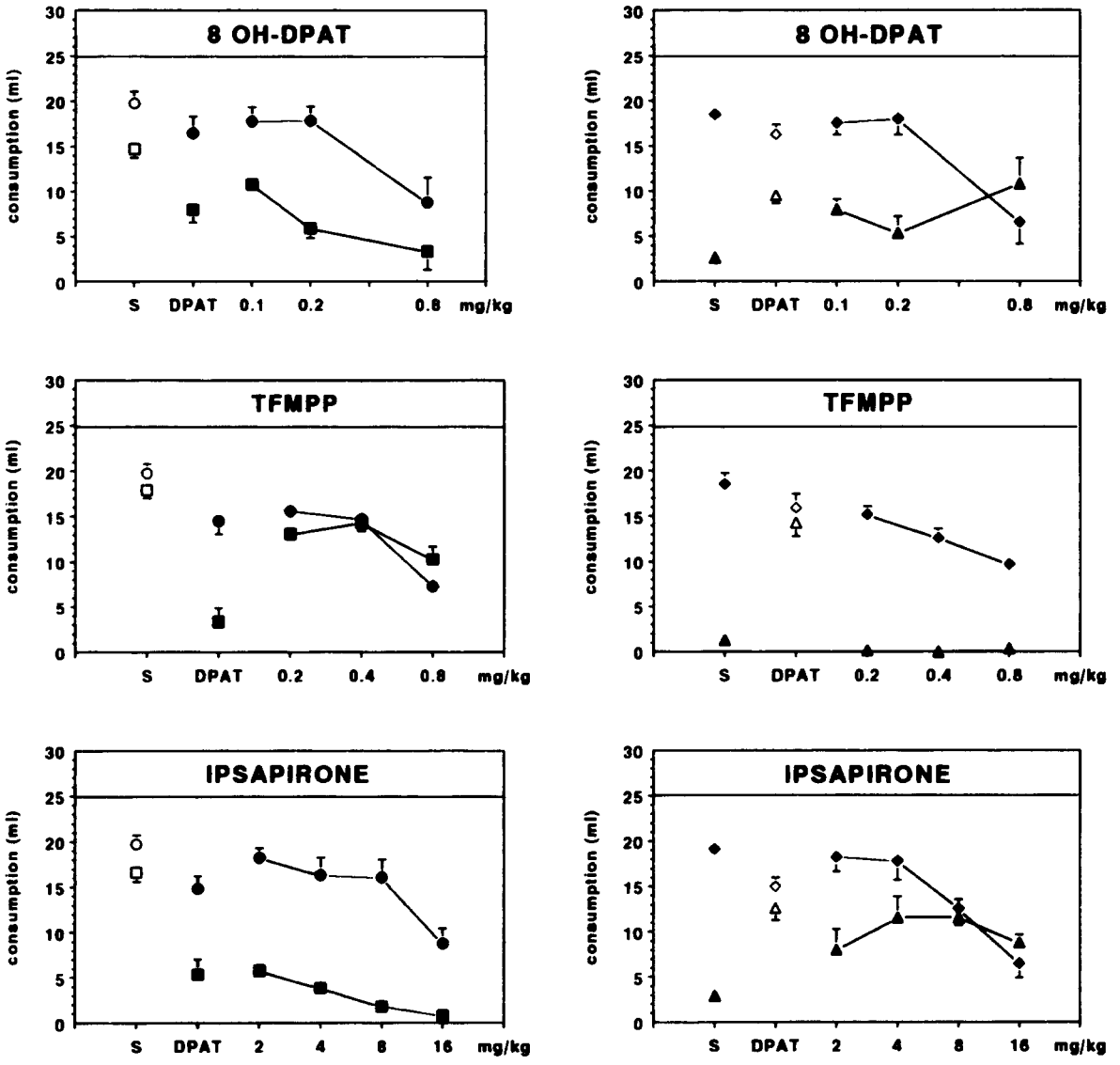

FIG. 4. Dose-response determinations for subjects trained to discriminate 8-OH-DPAT from vehicle (S). The left panels show data for subjects exposed to 8-OH-DPAT-LiCl pairings during training (■, □); the right panels show data for subjects exposed to NaCl-LiCl pairings (▲, △); their appropriate controls are also represented (●, ○, ♦, ◇, respectively).

sickness. In addition, it was shown that drug treatment may come to serve as a safe signal for saccharin intake, as separate groups of subjects showed saccharin aversion following vehicle treatment but not after they were treated with drug. Furthermore, we were able to obtain dose-response and generalization curves roughly similar to the curves obtained with two-lever operant procedures as are frequently found in the literature. Diazepam, a benzodiazepine anxiolytic drug, generalized, at least partly, to the CDP cue. Buspirone is a novel anxiolytic drug that does not exert its therapeutic effects via the benzodiazepine receptor but most likely via its actions on the 5-HT_{1A} receptor. As such, it was not surprising that the buspirone cue did not generalize to CDP. These results are in agreement with findings from the literature (11,17). Rats trained to avoid saccharin water after treatment with the selective 5-HT_{1A} ligand 8-OH-DPAT avoided the saccharin solution after treatment with the 5-HT_{1A} agonist ipsapirone, but not when they had received an injection with the 5-$HT_{1B/C}$ agonist TFMPP. Again, these results do not differ from the results reported previously by others (24,27).

Recently, Woudenberg and Hijzen (29) reported that rats were able to discriminate 20 mg/kg CDP from saline after seven CDP-LiCl pairings which were given over a 17-day training period. Our results are in complete agreement with this observation. The filled squares at session blocks 1 and 2 in the left panel of Fig. 1 depict a total of eight CDP-LiCl pairings for subjects trained to refrain from drinking in the presence of CDP. Comparisons with control subjects that received CDP without LiCl (filled circles) show almost complete suppression of drinking in experimental subjects after eight CDP-LiCl pairings. Training was discontinued at that point in the Woudenberg study, but not in the present study, because subjects that were exposed to the reversed condition (vehicle-LiCL and CDP-NaCl pairings) were still unable to

discriminate between the presence vs. absence of the drug. A general decrease in consumption after CDP treatment that was independent of conditioning contingencies was observed in both the Woudenberg as well as in the present study and has been attributed to the sedative effects of CDP (29).

As such, the results of the present experiment do not contribute anything new to the already existing firm body of literature on stimulus properties of benzodiazepine-like and serotonergic drugs. The results are, however, important from a methodological point of view. Training subjects to discriminate a drug from its vehicle employing two-lever operant procedures in the Skinnerbox is often very time consuming in the sense that a large number of experimental sessions are required before subjects attain the discrimination. Over the past few years, a number of investigators have sought alternative approaches to speed the training procedure [e.g., (2,4,10, 20,21,23,25)]. Most recently, the CTA procedure was brought up as an alternative, faster method to train subjects to discriminate between a drug and its vehicle. It was reported that conditioning of the discrimination occurred much more rapidly and required less frequent drug exposures than more traditional techniques. The differential effects on saccharin consumption could already be observed after only two to three pairings of the drug stimulus with LiCl injections (14). Second, CTA may not only provide a more rapid but also a more sensitive index than traditional procedures. Kautz and coworkers (13) reported that rats were able to discriminate low (0.3 mg/kg) doses of the opiate antagonist naloxone, a drug that fails to support discrimination training in two-lever operant procedures. In addition, Riley et al. (22) reported that the pentobarbital dose-response curve was shifted to the left, suggesting that subjects are able to discriminate pentobarbital at lower doses using CTA as compared to two-lever operant techniques. Another advantage is that the CTA procedure does not require expensive equipment.

The results of the present experiment do, however, show that there may also be disadvantages to the use of the CTA procedure. Stable baseline performance was obtained only after a large number of sessions (40 daily training sessions for CDP discrimination, 72 sessions for 8-OH-DPAT). The behavior of rats exposed to CDP discrimination deteriorated after the establishment of a dose-response curve, probably due to the omission of LiCl treatment during the dose-substitution sessions. Additional training (20 sessions) was necessary, but could not prevent a further deterioration of baseline behavior after substitution testing, thus preventing further generalization studies. The same problems were recently described by de Beun and coworkers (5). 8-OH-DPAT training succeeded only at the highest dose of LiCl, a finding that is in agreement with the work of Jaeger and Mucha (12). Large day-to-day fluctuations in individual subjects' drinking behavior, and the fact that we were unable to maintain the discrimination for a prolonged period of time, suggest that the CTA procedure may not yield results as robust as those obtained with the two-lever operant procedure. Furthermore, it was shown that the speed of discrimination acquisition was dependent upon whether the drug signalled safety or LiCl toxicosis. Such asymmetry strongly suggests that the results of discrimination learning using CTA not only depend upon the specificity of the stimulus cue but may be confounded by procedural variables.

Lucki and South (15) argued that the use of CTA procedures circumvents the problem of drug effects on rate of responding when rats are pressing a lever to obtain food. The use of CTA procedures, however, introduces the complication of nonspecific drug effects on fluid intake. In the present experiment, both CDP and 8-OH-DPAT unconditionally suppressed drinking, thereby attenuating discrimination performance. This problem was previously also discussed by Kautz et al. (13). As such, separate groups of rats are needed to control for nonspecific drug effects, thereby doubling the number of subjects in each experiment. Furthermore, the effects of other manipulations that affect fluid intake, for example, level of deprivation, saccharin concentration, and rebound effects, may thus be expected to interfere with discrimination performance, but have so far not been thoroughly studied.

A final point of criticism is that the test is laborious for the experimentator and may be very bothersome to the experimental animal. The daily testing routine (preparing drug and vehicle solutions, preparing the saccharin solution, filling, calibrating, and reading off the drinking tubes) takes much time. Since data registration is not automated, errors can easily occur. As for experimental subjects, they are injected twice daily (as opposed to only one injection with Skinnerbox procedures) and are regularly subjected to the sickening effects of LiCl. Our experimental subjects gained considerably less weight over the course of the training procedure than control subjects, an observation that may be indicative of possible hazardous effects of chronic LiCl treatment.

Taken together, on the basis of the results of the present experiments we feel that the CTA procedure does not have sufficient advantages over two-lever operant procedures to replace the latter in drug discrimination research.

ACKNOWLEDGEMENTS

Part of the results were previously presented during the Third Meeting of the European Behavioral Pharmacology Society, Noordwijkerhout, June 1990, and the Conference on Animal Models in Psychopharmacology, Amsterdam, July 7-8, 1990. The authors express their thanks to M. van Drimmelen and K. Pietersma for expert technical assistance.

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