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Effects of drug discrimination history on anti-punishment properties of chlordiazepoxide in rats

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Abstract

Several studies have indicated that acquiring discriminative stimulus control for a certain anxiolytic drug influences its subsequent anti-conflict properties. To further elaborate on the question whether drug discrimination procedures affect behaviour in a conflict paradigm, a classical two-lever drug discrimination procedure was combined with an operant conflict procedure within the same animals. To this extent, rats were trained to discriminate the anxiolytic chlordiazepoxide (CDP, 30 mg/kg, po) from saline (SAL), and subsequently punished responding periods were introduced within the same session. In addition to the rats that were trained to discriminate CDP from vehicle, a group of rats was trained on a random relationship between CDP and the rewarded lever. CDP and alprazolam completely substituted for CDP, whereas mianserin did not. Responding during punished components in a session was increased by CDP and alprazolam, but not by mianserin in rats that were trained to discriminate CDP from vehicle and in randomly trained rats. The data indicate that rats can be reliably trained and tested in drug discrimination and conflict procedures within a single session and that CDP's discriminative stimulus does not alter its anti-conflict effects. © 2000 Elsevier Science Inc. All rights reserved.

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Drug discrimination (Järbe, 1989) and the Geller–Seifter conflict procedure (Geller and Seifter, 1960) are two procedures widely used to characterize the discriminative stimulus properties and putative anxiolytic effects of drugs, respectively. Recently, a combination of these two behavioural paradigms, within a single session, has been described in both pigeons (Li and McMillan, 1998; McMillan et al., 1997) and rats (Wiley and Balster, 1999). It was concluded that the discriminative stimulus and anti-conflict properties of drugs can be studied reliably in the same animal in a single session. Furthermore, in the studies by Li and McMillan (1998) and McMillan et al. (1997), using pigeons trained to discriminate pentobarbital from vehicle, there was a close correspondence between these two effects for pentobarbital and benzodiazepines, but less for other types of drugs (e.g. buspirone, phencyclidine). However, Wiley and Balster (1999), using rats trained to discriminate diazepam from vehicle, found a dissociation between the discriminative stimulus and anti-punishment effects of diazepam. Diazepam substituted completely for itself but had in only some of the rats anti-punishment effects.

The present experiments were designed to further investigate the relationship between drug discrimination and conflict responding using the anxiolytic benzodiazepine chlordiazepoxide (CDP) as training drug. However, a number of adaptations were made. In the study by McMillan et al. (1997), drug discrimination and conflict procedures were presented together only during test and not during training sessions. For the present experiment, animals were trained first in a drug discrimination procedure and after acquiring the discrimination, were further trained with both procedures in the same session, in line with the Wiley and Balster (1999) study. In the latter, however, drug discrimination and conflict procedures were represented in different levers, two levers for drug discrimination and one separate lever for the conflict procedure, thereby possibly dissociating both pro-

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cedures. For the present study, the same levers were used for both drug discrimination and conflict training, and the drug discrimination and conflict procedures were therefore not dissociated environmentally.

Moreover, a group of animals was included that was trained with a random relationship between drug cue and rewarded lever. By comparing a trained and a randomly trained group, the effect of a drug acquiring discriminative stimulus control on its subsequent anti-conflict effects can be investigated. This is an important question concerning the validity of the combination of these procedures, if it is going to be used for investigating new putative anxiolytic drugs.

Dose–response curves were determined for CDP and another benzodiazepine receptor agonist, alprazolam. In addition, a dose–response curve was determined for the nonselective 5-HT_2 receptor antagonist mianserin, that has been shown to possess anti-conflict activity in the Geller–Seifter conflict procedure (Kennett et al., 1994). This drug was included to investigate the nature of the CDP discriminative stimulus itself. To date, only for pentylenetrazole there is evidence that its discriminative stimulus is mediated by a specific psychological effect, i.e. a state of fear (see for instance, Gauvin et al., 1996; Gauvin and Holloway, 1991b). It has been suggested that the CDP discriminative stimulus might be mediated by relaxation (or anxiolysis (Gauvin et al., 1989; Gauvin and Holloway, 1991a)). It was expected that if the discriminative stimulus of CDP was related to its anti-punishment properties, a dose-dependent substitution of mianserin for CDP might occur. However, if the two effects are dissociable, we would expect mianserin to have anti-conflict activity but not to substitute for the CDP discriminative stimulus.

1. Method

The work reported here was conducted with the approval of the Ethics committee of the Faculties of Pharmacy, Biology and Chemistry of the Utrecht University.

1.1. Subjects

Initially, 24 male Wistar rats, weighing approximately 300 g at the start of the experiment were obtained from GDL (Utrecht, The Netherlands). Rats were housed individually under a nonreversed 12 h light–dark cycle (lights on at 7:00 a.m.), room temperature varying between 21°C and 23°C and humidity between 50% and 60%. Subjects were maintained at approximately 85% of their expected free-feeding weights by restricting food intake to 15 g per day (Hope Farms, Woerden, The Netherlands). Water was available ad libitum.

All experiments were carried out with the approval of the Ethics committee of the Faculties of Pharmacy, Biology and Chemistry of the Utrecht University.

1.2. Apparatus

Experiments were conducted in eight ventilated operant chambers (MED Associates, East Fairfield, USA) housed in sound-insulated boxes. The chambers were equipped with two retractable levers, a pellet dispenser which delivered 45 mg food pellets (Noyes, NH, USA) in a tray placed between the levers, a house light and three stimulus lights; lights were located above each lever and above the food tray. Each chamber had a grid floor, through which scrambled electric shocks could be delivered by a shock generator (MED Associates). Schedule contingencies and data collection were programmed using a microcomputer (IBM 386) through a MED interface (MED Associates).

1.3. Procedure

Prior to the training procedure, rats were randomly assigned to either a discrimination group ($n=12$) or a random group ($n=12$).

1.3.1. Training procedure

Initially, rats in both groups were trained to lever-press for food according to a fixed ratio 1 (FR1) schedule of reinforcement. Once acquired, the FR1 schedule was gradually replaced by a variable-interval 30-s (VI30″) schedule of reinforcement. The discrimination group was trained to discriminate between CDP (30 mg/kg, po) and saline (SAL), according to a 2-weekly alternating schedule (CDP–SAL–CDP–CDP–SAL, SAL–CDP–SAL–SAL–CDP). Depending on the injection condition, reinforcement could be obtained by pressing the CDP-appropriate lever or the SAL-appropriate lever; responding on the inappropriate lever never delivered food. The position of CDP and SAL levers was counterbalanced across rats. This training sequence was repeated until the subjects reached a stable criterion such that the percentage of responding on the correct lever in the discrimination group was equal to or greater than 80% during six consecutive sessions. Rats in the random group were also injected with CDP and SAL, but there was no correspondence between the injection condition and the correct lever. After both drug and SAL administration sometimes left lever presses were rewarded and sometimes right lever presses were rewarded, always one lever during a specific session. After stimulus control for the discrimination group was established, the Geller–Seifter conflict procedure, adopted from Schreiber and De Vry (1993), was introduced for both groups. In the conflict procedure, rats were trained to lever-press under a multiple schedule: periods with a VI30″ schedule for food (unpunished period) were alternated with periods with a fixed-ratio 10 (FR10) schedule for food + shock (punished period). A training session (duration 30 min) always started with a 2-min VI30″ period and there were four additional VI30″ periods (duration 5 min each), alternating with four FR10 periods (2 min each). Lights above both levers were

turned on to signal the FR10 periods. During the FR10 periods, responses produced food reward paired to a 0.5 s scrambled foot-shock. The intensity of the electrical shock used to suppress responding during the FR10 was adjusted for each rat separately. All rats began punishment training with shock intensity at 0.10 mA. Subsequently, shock intensities were titrated individually by 0.10 mA steps (mean = 0.67 mA; range 0.30–0.90 mA). Training continued until unpunished responding was at least 0.3 responses/s, and punished responding was less than 0.1 responses/s. During training, during the first interval of the first VI30″ period, before the first food reward, lever responses were recorded to serve as an indication of the accuracy of discrimination.

1.3.2. Testing procedure

Test sessions (duration 30 min) were carried out on Wednesdays and Fridays. On the remaining days, the training procedure was continued. Test sessions were similar to training sessions but they always started with 60 s of extinction during which lever presses were not reinforced but were recorded to serve as a measure for drug discrimination. Hereafter, a 1-min period of VI30″ responding was followed by punished and unpunished periods alternated in which responses on both levers (not just one as during training) were rewarded, with either food for unpunished periods or food + shock for punished periods.

1.4. Statistics

Discrimination results are expressed as the mean number of drug-lever responses as a percentage of total number of lever responses, recorded during the first 60 s during testing. Complete stimulus generalization was said to occur when 80% or more of the responses were made on the drug-appropriate lever. For the random group, the mean percentage of left-lever responses was calculated. Generalization and substitution data were only included when the response rates for a subject during the first 5 min of a test session was at least 0.10 responses/s.

For the Geller–Seifter conflict procedure, the number of punished and unpunished responses were recorded, and the results are expressed as mean number of responses/s. The data obtained in the punished periods of responding were tested separately from data obtained in the unpunished periods. Since a dose-dependent effect of CDP, alprazolam and mianserin on punished responding was expected, only for punished responding was the significance of the linear component of this effect by means of linear regression analysis tested. To further test for group differences, data were analysed with an analysis of variance for repeated measures with Dose as a within-subject variable and Group as a between-subject variable. The data obtained in the unpunished periods were analysed by means of an analysis of variance for repeated measures with Dose as a within-subject variable and Group as a between-subject variable. In all analyses, the criterion for statistical significance was set at $P < .05$.

In addition, consistent with the Wiley and Balster (1999) study, data were further divided in responders and nonresponders. Responders are rats that showed an increase in punished responding to one or more doses of the test drug, and nonresponders are rats in which a test drug did not induce anti-conflict effects in any dose. Because of the small numbers of animals in either the nonresponder and/or responder group, statistical analyses were not performed on these data.

1.5. Drugs

Chlordiazepoxide-hydrochloride (Pharbita, Zaandam, The Netherlands) and mianserin-hydrochloride (RBI, Natick, USA) were dissolved in 0.9% physiological saline. Alprazolam (Upjohn, Kalamazoo, USA) was suspended in a 0.5% gelatin/5% mannitol solution. CDP and mianserin were administered orally (po) 30 min prior to training and testing; alprazolam was injected subcutaneously (sc) 30 min prior to testing. All doses of drugs are given as salts and drug solutions were freshly prepared daily and injected in a volume of 2 ml/kg.

2. Results

All animals in the discrimination group (10 out of 12 rats) learned to discriminate CDP from SAL after a training period of a total of 42 sessions for all animals in this group. Additional training for acquisition of conflict responding lasted in a total of 59 sessions for all animals in both groups to reach criterion.

Fig. 1 shows generalization and substitution test data for both groups. In the discrimination group, a complete, dose-dependent, generalization with CDP and alprazolam was obtained, 96.8% and 93.0% at the highest doses, respectively. Mianserin did not substitute for CDP to any extent. For rats in the random group, approximately 50% of all responses were made on either the right and left lever for all drugs tested, except for the intermediate dose of CDP (10 mg/kg), which elicited 72.5% left-lever responding. More importantly, there is no difference between SAL and the training dose of CDP, or between any of the doses of alprazolam or mianserin and SAL.

Fig. 2 shows the effects of drugs on punished and unpunished responding. CDP elicited a dose-dependent linear increase in punished responding during the conflict periods [$F(1,70) = 29.91$, $P < .001$]. Furthermore, groups differed in punished responding, however, there was no Dose × Group interaction effect [$F(1,16) = 6.14$; $P = .025$ and $F(3,48) = 1.87$, $P = .15$, NS, respectively]. CDP did affect responding during unpunished periods [$F(3,48) = 7.04$, $P = .001$], but there was no difference between groups nor a Dose × Group interaction effect

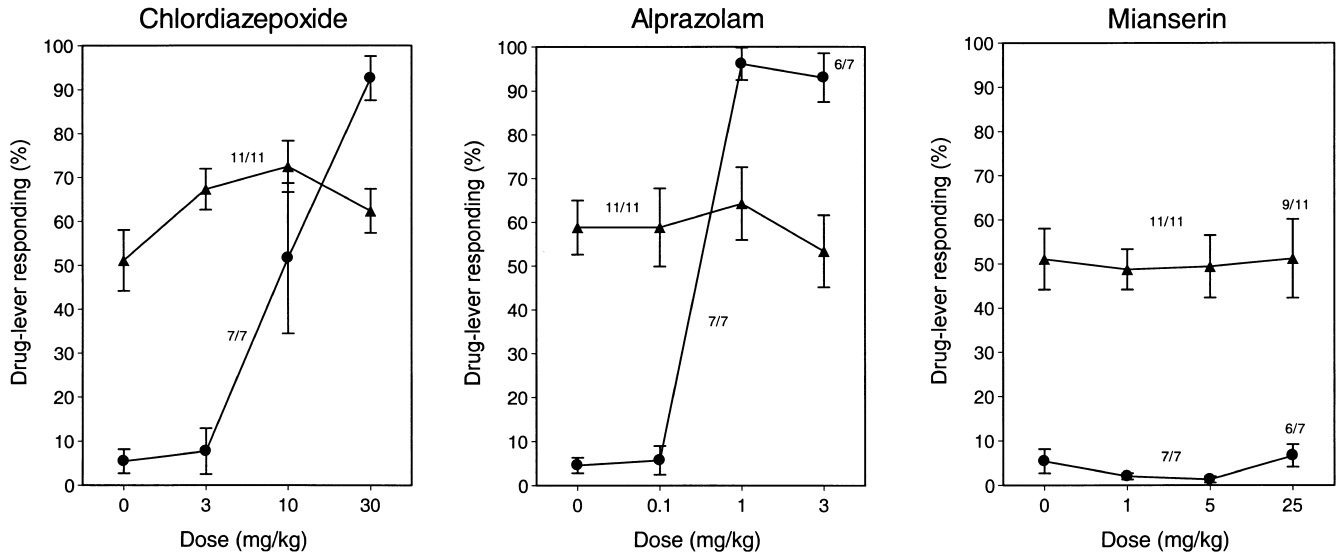

Fig. 1. Results of generalization and substitution tests (±S.E.M.) with CDP, alprazolam and mianserin rats trained (circles) and rats randomly trained (triangles) to discriminate between CDP (30 mg/kg) and SAL. Ordinate: Mean percentage of responses on the drug (for trained rats) and left lever (for randomly trained rats). Abscissa: Dose in milligrams per kilogram. The number of subjects responding and the number of subjects tested is expressed as the ratio adjacent to the graph or each of the points.

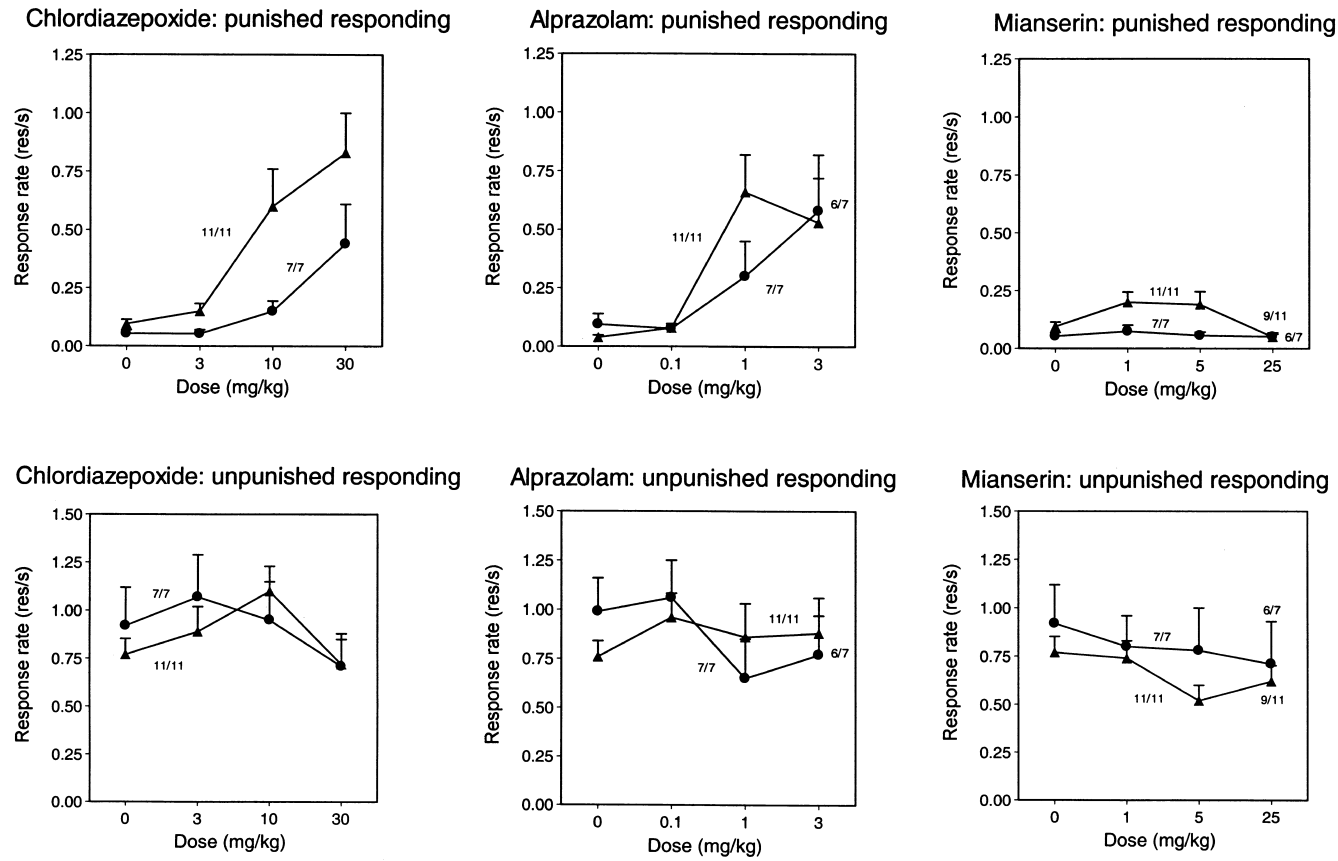

Fig. 2. Upper panel: Effects of CDP, alprazolam and mianserin on punished responding during conflict periods (±S.E.M.) in rats trained (circles) and rats randomly trained (triangles) to discriminate between CDP (30 mg/kg) and SAL. Lower panel: Effects of CDP, alprazolam and mianserin on unpunished responding during conflict periods (±S.E.M.) in rats trained (circles) and rats randomly trained (triangles) to discriminate between CDP (30 mg/kg) and SAL. Ordinate: Response rate in number of responses made per second. Abscissa: Dose in milligrams per kilogram. The number of subjects responding and the number of subjects tested is expressed as the ratio adjacent to the graph or each of the points.

Table 1
Drug discrimination and conflict responding data in responders and nonresponders: discrimination group

Drug	Dose (mg/kg)	N[a]	% CDP lever (S.E.M.)[b]	FR10 (S.E.M.)[c]	VI30″ (S.E.M.)[d]
Responders					
Chlordiazepoxide	0	5/7	6.6 (3.7)	0.05 (0.02)	0.8 (0.2)
	3.0	5/7	10.2 (7.1)	0.05 (0.02)	0.9 (0.2)
	10.0	5/7	53.2 (21.7)	0.2 (0.05)	1.0 (0.2)
	30.0	5/7	89.6 (6.7)	0.6 (0.2)	0.7 (0.2)
Alprazolam	0	4/7	4.0 (3.1)	0.1 (0.08)	1.0 (0.3)
	0.1	4/7	7.2 (5.6)	0.1 (0.03)	1.0 (0.3)
	1.0	4/7	93.5 (6.5)	0.5 (0.2)	0.7 (0.2)
	3.0	4/7	91.5 (8.5)	0.9 (0.3)	0.9 (0.2)
Mianserin	0	2/7	12.0 (8.0)	0.05 (0.04)	0.9 (0.3)
	1.0	2/7	3.5 (0.5)	0.2 (0.04)	0.9 (0.4)
	5.0	2/7	1.0 (1.0)	0.1 (0.01)	1.0 (0.5)
	25.0	2/7	7.5 (4.5)	0.07 (0.05)	0.8 (0.5)
Nonresponders					
Chlordiazepoxide	0	2/7	2.5 (1.5)	0.07 (0.03)	1.3 (0.7)
	3.0	2/7	1.5 (0.5)	0.07 (0)	1.5 (0.7)
	10.0	2/7	47.5 (37.5)	0.04 (0.02)	0.8 (0.7)
	30.0	2/7	100 (0)	0.02 (0.01)	0.7 (0.6)
Alprazolam	0	3/7	5.3 (1.5)	0.04 (0.01)	1.0 (0.3)
	0.1	3/7	3.7 (2.7)	0.05 (0.02)	1.2 (0.3)
	1.0	3/7	99.7 (0.3)	0.02 (0.01)	0.6 (0.4)
	3.0	2/7	96.0 (4.0)	0.03 (0.01)	0.5 (0.4)
Mianserin	0	5/7	2.8 (1.7)	0.05 (0.02)	0.9 (0.3)
	1.0	5/7	1.4 (0.9)	0.04 (0.02)	0.8 (0.2)
	5.0	5/7	1.4 (1.0)	0.04 (0.01)	0.7 (0.3)
	25.0	4/7	6.3 (3.5)	0.04 (0.01)	0.7 (0.3)

[a] Number of subjects responding/number of subjects tested.
[b] Percentage of responses made on the CDP lever.
[c] Response rate (responses/s) during punished responding (FR10) periods.
[d] Response rate (responses/s) during unpunished responding (VI30″) periods.

[$F(1,16)=0.053$, $P=.82$, NS and $F(3,48)=2.06$, $P=.12$, NS, respectively].

Alprazolam produced a dose-dependent linear increase in punished responding during the conflict periods [$F(1,69)=15.60$, $P<.001$], however, there were neither group differences nor Dose × Group interaction effects [$F(1,15)=0.139$; $P=.71$, NS and $F(3,45)=1.18$, $P=.33$, NS, respectively]. Alprazolam also affected unpunished responding during conflict periods [$F(3,45)=3.09$, $P<.05$], with neither group differences nor a Dose × Group interaction effect [$F(1,15)=0.002$, $P=.96$, NS, and $F(3,45)=2.50$, $P=.072$, NS, respectively].

Punished responding during the conflict periods was not significantly increased by mianserin [$F(1,69)=3.45$, $P=.068$, NS]. In addition, there were no differences between groups in punished responding, nor Dose × Group Group interaction effects [$F(1,13)=2.66$, $P=.13$, NS and $F(3,39)=1.19$, $P=.33$, NS, respectively]. Mianserin affected unpunished responding [$F(3,39)=4.16$, $P=.012$], and inspection of the data indicates that this is a reduction in unpunished responding. There were no differences between groups, nor significant Dose × Group interaction effects [$F(1,13)=0.491$, $P=.50$, NS and $F(3,39)=2.18$, $P=.11$, NS, respectively].

Tables 1 and 2 show data in the discrimination group (Table 1) and random group (Table 2) when divided in responders and nonresponders. As can be seen from Table 1, the doses of CDP and alprazolam that produced anti-conflict effects in responders (i.e. response rate during FR10 >0.10 responses/s) in the discrimination group also increased drug-lever responding. The single dose of mianserin (1.0 mg/kg) that only slightly increased punished responding did not produce drug lever responding. Table 2 shows data from the random group, and as can be seen, CDP had anti-conflict effects in all animals (all responders) in one or more doses. Alprazolam had anti-conflict effects in nearly all animals (10 out of 11 animals), whereas mianserin did increase punished responding, but only moderately compared to CDP and alprazolam, in some animals at the low and the intermediate dose (1.0 and 5.0 mg/kg, respectively).

3. Discussion

In the present study, a two-lever operant drug discrimination paradigm was combined with the Geller–Seifter conflict paradigm. A combination of these two operant paradigms within the same subjects has previously been

Table 2
Drug discrimination and conflict responding data in responders and nonresponders: random group

Drug	Dose (mg/kg)	N [a]	% left lever (S.E.M.)[b]	FR10 (S.E.M.)[c]	VI30″ (S.E.M.)[d]
Responders					
Chlordiazepoxide	0	11/11	51.1 (6.9)	0.1 (0.02)	0.8 (0.08)
	3.0	11/11	67.3 (4.7)	0.15 (0.03)	0.9 (0.1)
	10.0	11/11	72.5 (5.9)	0.6 (0.2)	1.1 (0.1)
	30.0	11/11	62.4 (6.7)	0.8 (0.2)	0.7 (0.1)
Alprazolam	0	10/11	56.2 (6.2)	0.04 (0.01)	0.8 (0.09)
	0.1	10/11	59.5 (9.9)	0.08 (0.01)	1.0 (0.1)
	1.0	10/11	62.6 (9.0)	0.7 (0.2)	0.9 (0.2)
	3.0	10/11	55.0 (8.9)	0.6 (0.2)	0.9 (0.2)
Mianserin	0	6/11	44.5 (10.4)	0.07 (0.01)	0.7 (0.1)
	1.0	7/11	48.6 (5.6)	0.3 (0.06)	0.7 (0.1)
	5.0	7/11	56.9 (7.8)	0.3 (0.08)	0.7 (0.09)
	25.0	4/11	56.8 (11.5)	0.04 (0.01)	0.6 (0.1)
Nonresponders					
Chlordiazepoxide	all animals are responders				
Alprazolam	0	1/11	85	0.01	0.6
	0.1	1/11	52	0.04	0.6
	1.0	1/11	81	0.05	0.4
	3.0	1/11	37	0.01	0.3
Mianserin	0	5/11	59.0 (8.5)	0.1 (0.04)	0.9 (0.1)
	1.0	5/11	49.0 (8.5)	0.09 (0.02)	0.7 (0.1)
	5.0	5/11	39.0 (12.5)	0.06 (0.02)	0.3 (0.07)
	25.0	5/11	46.8 (14.0)	0.05 (0.02)	0.7 (0.1)

[a] Number of subjects responding/ number of subjects tested.
[b] Percentage of responses made on the left lever.
[c] Response rate (responses/s) during punished responding (FR10) periods.
[d] Response rate (responses/s) during unpunished responding (VI30″) periods.

described in pigeons (Li and McMillan, 1998; McMillan et al., 1997) and in rats (Wiley and Balster, 1999), but these studies did not include a group of randomly trained animals.

The results demonstrate that CDP and alprazolam, but not mianserin, substitute completely for CDP in rats trained to discriminate between CDP and vehicle compared to randomly trained rats. In the latter group rats all drugs tested elicited approximately 50% responding on either lever and no dissociation between SAL and the training dose of CDP or any of the doses of the other drugs was found, clearly showing that CDP did not acquire stimulus control.

CDP and alprazolam did increase punished responding, consistent with previous data in rats (Fontana et al., 1999; Hascoet and Bourin, 1997; King et al., 1997) and in pigeons (Kleven and Koek, 1999). There were, however, no significant interaction effects between drug-dose and group, and therefore, the different discrimination training conditions did not affect the anti-conflict activity of both benzodiazepines. There was a group difference with CDP, indicating that responding in the randomly trained group was overall somewhat higher than in the discrimination group. This was not confirmed with alprazolam, however, and this might have been a chance finding since there was considerable variation among the individual animals. Inspection of the individual data indicated that in some animals, CDP and alprazolam did not produce anti-conflict effects as reported earlier (Wiley and Balster, 1999). Interestingly, in the discrimination group, these were the same animals, whereas in the random group, only alprazolam did not produce anti-conflict effects in only one animal. Whether this difference between discrimination and random group in the number of nonresponders is caused by drug discrimination history cannot be answered by these limited data.

In the present study, the same levers were used for both drug discrimination and conflict training, thereby making a dissociation between the procedures based on spatial (and possibly olfactory) cues impossible. This strongly supports the notion that the anti-conflict effects can be studied reliably without interference from drug discrimination. A related question is whether the discriminative stimulus properties might be altered as a result of conflict training. The present experiments cannot answer this question since no comparisons were made between generalization curves before and after conflict training, but it might be worthwhile to study this in future experiments.

All three drugs affected unpunished responding. However, thorough inspection showed that CDP and alprazolam did not have a clear dose-dependent effect (either increase or reduction), whereas only mianserin showed a, although small, dose-related reduction in response rates.

The present study did not indicate whether the discriminative stimulus properties of CDP are related to its anti-conflict effects. From the outset, it was expected that mianserin would show an anti-conflict effect, as reported

previously (Cervo and Samanin, 1995; Griebel et al., 1997; Kennett et al., 1994), and depending on the level of substitution for CDP, one might argue whether the anti-conflict effects are related to the discriminative stimulus effects. However, in this experiment, mianserin did not substitute for CDP in the discrimination-trained rats. This precludes any conclusion about the behavioural or psychological nature of the CDP discriminative stimulus. One reason for the lack of anti-punishment effect might be related to the training with CDP. In humans, for instance, it has been noted that buspirone is less effective in people treated with benzodiazepines prior to buspirone (for a review Deakin, 1993). Whether something similar is happening with mianserin in the present study has to be investigated in future experiments with a control group that has no prior history of CDP training. Other explanations for the lack of anti-conflict effects of mianserin, although speculative, might be that the injection–test interval for the route of administration in our study was too short (po, −30 min) compared to the other studies [(Kennett et al., 1994): po, −60 min; (Cervo and Samanin, 1995): sc, −30 min; and (Griebel et al., 1997): ip, −30 min].

To summarize, the present results suggest that the same rats can be reliably trained and tested in a drug discrimination and Geller–Seifter conflict procedure within a single session and that CDP as a discriminative stimulus does not alter its anti-conflict effects.

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