Pharmacology Biochemistry & Behavior, Vol. 21, pp. 409–415, 1984. © Ankho International Inc. Printed in the U.S.A. 0091-3057/84 $3.00 + .00

Tolerance to the Depressant Effects of Diazepam in the Drug Discrimination Paradigm[1]

TROND HAUG

University of Trondheim, Department of Psychiatry
Östmarka Hospital, P.O. Box 3008, N-7001, Trondheim, Norway

Received 10 November 1982

HAUG, T. *Tolerance to the depressant effects of diazepam in the drug discrimination paradigm.* PHARMACOL BIOCHEM BEHAV 21(3)409–415, 1984.—Seven groups of rats (n=35) were run in operant drug experiments. All groups were trained on a Fixed Ratio 10 schedule to discriminate diazepam from saline. Two groups (n=7, n=6), after extensive drug discrimination training (doses of 2.0 and 3.0 mg/kg diazepam), were submitted to generalization experiments with various doses of the training drug. Two additional groups, (n=6, n=8) in the initial phase of drug discrimination, were trained on intermediate and high doses of diazepam (i.e., 5.0 and 10.0 mg/kg). The development of tolerance to the depressant effects of diazepam for these two groups was compared to the low dose sophisticated rats. Of the above-mentioned groups, two groups were given tests after a waiting period in drug discrimination training. In this test the two groups were compared to an additional group (n=8) in its initial phases of drug discrimination training. The results show that a large number of low doses (i.e., doses below 3.0 mg/kg) is not able to induce any tolerance to the depressant effects of diazepam in this particular paradigm. Intermediate doses of diazepam (i.e., 3.0 mg/kg), administered in a large number, induced some tolerance to the depressant effects, while another intermediate dose (5.0 mg/kg) and a high dose (10.0 mg/kg) rapidly induced a significant tolerance. Once developed, the tolerance persisted for 51 days.

Diazepam Drug discrimination Depressant effects Tolerance Rats

IN the drug discrimination paradigm, animals are typically exposed to a certain drug (the training drug), most often as repeated administrations of a fixed dose of the training drug. Usually, animals are trained for different but equivalent responses such as a choice of opposite directions in which to run or different levers to press [1].

Most of the data from this paradigm are derived from tests with novel drug doses, time intervals, and especially, different drugs in animals trained to discriminate a particular drug from vehicle. These tests determine to what degree the discrimination generalizes to other doses (i.e., of the particular training drug) as well as to doses of other drugs in relation to time intervals [1].

Sedatives have been extensively investigated in relation to their stimulus properties in the operant procedure [17]. Among this class of pharmacologically active drugs the benzodiazepines have also been submitted to test [1, 2, 5, 7, 9, 10, 11, 12].

The benzodiazepines (e.g., diazepam) have also been tested for agonist-antagonist activity in the same paradigm where other classes of drugs have been used as discriminative stimuli [3,18]. In these tests, it is determined whether or not the benzodiazepines are able to either abolish a discriminative stimulus complex produced by another drug (antagonist) or mimic this stimulus complex (agonist).

The fact that anti-anxiety agents depress operant responding was originally described by Margules and Stein [15]. In their study it was observed that the initial predominance of sedative effects could be demonstrated only when the animals were drug naive. In contrast, when the animals received drug treatments for several consecutive days, they became "drug experienced," the animals were tolerant to the sedative effects and maximally sensitive to the anti-conflict effect. These effects have been observed in other conflict situations [20,21], as well as in studies with human subjects [22].

The above-mentioned observations may suggest that the dose-response effects reported in the drug discrimination paradigm are a function of a number of factors related to drug sophistication, animal strain, weight, operant level and route of administration as well as time after administration and vehicle bearing the active drug.

The drug discrimination paradigm may well be suitable for the investigation of a drug's unconditional effects (i.e., depressant effects) as well as conditional effects (i.e., discriminative stimulus properties). This is because one is mostly dealing with well trained animals with a high level of drug sophistication. Often the unconditional effects of the drugs are reported together with the discriminative properties for the dose-range tested.

However, the various studies use considerably different dose ranges of the different benzodiazepines submitted to test. With regards to diazepam, a representative benzodiazepine drug, it has been shown that a dose of 5.0 mg/kg (IP) depresses operant responding to almost zero [9,10]. In another study [3], however, using basically the same procedure (described by Colpaert *et al.* [6,7]) a dose of 17.8 mg/kg diazepam as a supposed antagonist to the stimulus did not

[1]This research was supported by the Norwegian Research Council for Science and the Humanities (project no. C.35.65-2).

TABLE 1
DESCRIPTION OF THE DIFFERENT GROUPS WITH REGARDS TO TRAINING AND TESTING CONDITIONS

		Training		Test	
Exp.	Group	Dose (mg/kg)	No. expos.	Dose (mg/kg)	Sessions
1	1 (n=7)	2.0	80	0.1–6.0	167–189
	2 (n=6)	3.0	65	0.75–20.0	136–162
2	3 (n=6)	5.0	0	5.0	11–19
	4 (n=8)	10.0	0	10.0	13–21
3	5 (n=3)	3.0	117	0.078–20.0	321–355
	6 (n=4)	5.0	75	0.078–20.0	175–206
	7 (n=8)	—	0	0.078–20.0	20–49

Number of expositions refer to the number of doses of diazepam the rat received in training before it was submitted to the test doses. The range of the test doses is also indicated.

In addition, Group 5 had received the test doses reported for Group 2, plus test doses of ethanol and pentobarbital [12].

affect response rate significantly. This most extreme difference thus far reported within the same paradigm, may be related to differences in route of administration or rat strain. Browne [3] administered the drugs SC to Sprague-Dawley rats and used benzodiazepine as a supposed antagonist, while Haug and Götestam [9,10] administered the drug IP to Wistar rats and used the drug as a training drug.

The present study was designed to elucidate the effects of administration of a high number of small doses compared to the effects of administration of a low number of intermediate and high doses. One strain of rats and one training and testing procedure were used. In addition, the importance of different vehicles and animal body weight, and operant level were also studied. Finally, the established tolerance was tested in relation to a pause between drug exposures and number and size of doses earlier exposed to.

METHOD

Animals

Male Wistar rats (Möllegaard-Hansen avlslaboratorium, Skensved, Denmark) weighing between 200 and 510 g at the start of the experiments were housed in cages in a windowless room maintained at 22±1.5°C. The room was illuminated artificially from 7 p.m. to 7 a.m. The training and testing sessions started at 10 a.m.

Experimental Cages

The rats were placed in individual experimental cages, 30×30×40 cm, during the entire experiment. Two levers and a food pellet dispenser were positioned on one wall of the cage. Pellets (standard pellets, 45 mg) were delivered as a consequence of lever pressing according to the current reinforcement schedule. Lever impulses and pellet deliveries were registered on electronic counters (Lehigh Valley Electronics, Fogelsville, PA). The experimental cages, with dispensers, were in a sound attenuated case with constant ventilation, white noise, and constant lighting throughout the experimental sessions.

Drugs

Diazepam suspended in dimethylacetamide and cremophor EL was used. Physiological saline was used for control conditions. Dimethylacetamide and cremophore EL have been separately tested for the possibility of cueing properties in Experiment 1 and 2 [10,12].

In addition, diazepam prepared as for clinical use (i.e., Stesolid, Dumex Ltd, Copenhagen, Denmark) was tested for both cueing and response depressant effects and compared to the diazepam suspended in dimethylacetamide and cremophor EL. This was of interest since Group 3 and Group 4 were exclusively trained on Stesolid as the active drug.

General Procedure

All rats were shaped to bar press for food reinforcement and successively shifted to a fixed ratio schedule requiring ten lever presses for each reinforcement (FR 10). The animals were further trained according to the two lever fixed ratio 10 (FR 10) drug discrimination protocol described by Colpaert *et al.* [6] and Haug and Götestam [9, 10, 11, 12]. Thirty minutes prior to the 14-minutes session, diazepam or an isovolumetric dose of saline (1 ml/kg, except Group 4 which received 2 ml/kg) was administered IP.

The relation between training dose, number of expositions and test dose range, as well as session numbers for training and test, are shown in Table 1.

Experiment 1

In this experiment drug sophisticated rats trained on 2.0 or 3.0 mg/kg diazepam were tested. The animals were not exposed to any other benzodiazepine.

Group 1 (n=7), training dose 2.0 mg/kg, was submitted to test in a period where the animals were tested with different drugs with supposed agonist or antagonist activity [10].

Group 2 (n=6), training dose 3.0 mg/kg, consisted of rats from a group submitted to test in a period where the animals were tested with different drugs with supposed agonist or antagonist activity [12].

TABLE 2

THE SEQUENCE OF DIFFERENT DOSES OF DIAZEPAM AND THE RESPONSES CALCULATED AS PERCENT OF THE MOST PROXIMAL SALINE SESSION

Group 5			Group 6			Group 7		
Sequence	Dose	Response	Sequence	Dose	Response	Sequence	Dose	Response
1	1.25	95.8	1	1.25	140.7	1	1.25	107.1
2	0.31	97.7	2	0.31	125.4	2	0.31	105.8
3	10.0	44.6	3	10.0	70.9	3	10.0	16.7
4	0.63	102.15	4	0.63	95.9	4	0.63	105.2
5	5.0	34.7	5	5.0	126.0	5	5.0	14.7
6	0.078	86.1	6	0.078	110.9	6	0.078	96.9
7	0.156	100.3	7	0.156	110.6	7	0.156	104.5
8	5.0	70.7	8	5.0	149.6	8	5.0	56.7
9	15.0	33.8	9	15.0	105.3	9	15.0	27.4
10	20.0	2.9	10	20.0	37.4	10	20.0	26.0
11	5.0	95.0	11	5.0	103.9	11	5.0	57.7
12	5.0	92.5	12	5.0	183.3	12	5.0	61.7

The sequence of drug administrations was interspaced with saline sessions (not indicated in the table)

Experiment 2

In this experiment drug naive rats were tested on 5.0 and 10.0 mg/kg.

Group 3 (n=6) was in the initial phase of drug discrimination training when they received a fixed test dose of diazepam (5.0 mg/kg) alternating with saline.

Group 4 (n=8) was trained identical to Group 3 except for the dose concentration which was 10.0 mg/kg.

Experiment 3

In this experiment both drug sophisticated (Group 5 and 6) and drug naive (Group 7) animals were tested with a broad spectre of doses (0.078–20 mg/kg).

Group 5 (n=3) consisted of three rats from Group 2 and was submitted to test after receiving approximately a total of 117 doses of diazepam (3.0 mg/kg) in addition to the dose range test sessions carried out as Group 2 (Table 1). In Group 2 they also had received doses of barbiturates and alcohol [12]. A pause of 51 days in drug discrimination training with no discrimination retraining or drug exposure preceded this new test. The rats in this experiment were then exposed to the test doses in a sequence described in Table 2.

The group was given 12 sessions of operant training exclusively on the saline lever the last 14 days preceding the drug test. This was done to reestablish a relatively steady level of responding.

Group 6 (n=4) which was identical to four rats from Group 3, was again submitted to test after receiving approximately a total of 75 additional doses of diazepam (5.0 mg/kg).

An interval of 86 days in drug discrimination training with no discrimination retraining or drug exposure preceded this new test. The rats were then exposed to the test doses in a sequence described in Table 2.

The group was given 12 sessions of operant training exclusively on the saline lever the last 14 days preceding the drug test. This was done to reestablish a relatively stable level of responding.

Group 7 (n=8) was in the initial stage of drug discrimination training when they received variable test doses of the drug diazepam alternating with saline in a sequence described in Table 2. The range of variable doses was identical for Group 5, 6, and 7.

Statistics

Analyses of variance were performed for each experiment. In Experiment 3 a split-plot two-way (group × dose) analysis of variance was performed. In Experiment 1 and 2, since different doses were employed in the different groups, separate one-way analyses of variance were performed for each group [14]. Differences between means were subsequently tested by *t*-tests [14]. Missing data were estimated according to a least square solution [14].

In Experiment 3, product-moment correlations were computed between depressant effects on the one hand and body weight and operant level on the other.

Group means after drug administrations are expressed as percent of the mean of the most proximal saline session.

RESULTS

Experiment 1

Group 1 showed a median in responding (based on medians from six saline sessions, ranging from1289 to 1680) of 1421 lever presses during the test. The grand mean (based on the same sessions) was 1432.

The one-way analysis of variance was significant, $F(6,42)=17.51$, $p<0.001$, and *t* tests revealed differences between both 5.0 and 6.0 mg/kg diazepam when compared to saline (for 5.0 mg/kg, $t=10.99$, $p<0.001$; 6.0 mg/kg, $t=4.55$, $p<0.01$).

Group 2 showed a median in responding (based on medians from seven sessions, ranging from 1161 to 1483) of 1477 lever presses during the test. The grand mean (based on the same sessions) was 1266.

The one-way analysis of variance was significant (two missing data points were estimated, and the *df* was reduced from 8 to 6), $F(6,45)=10.56$, $p<0.001$. Responding reached a

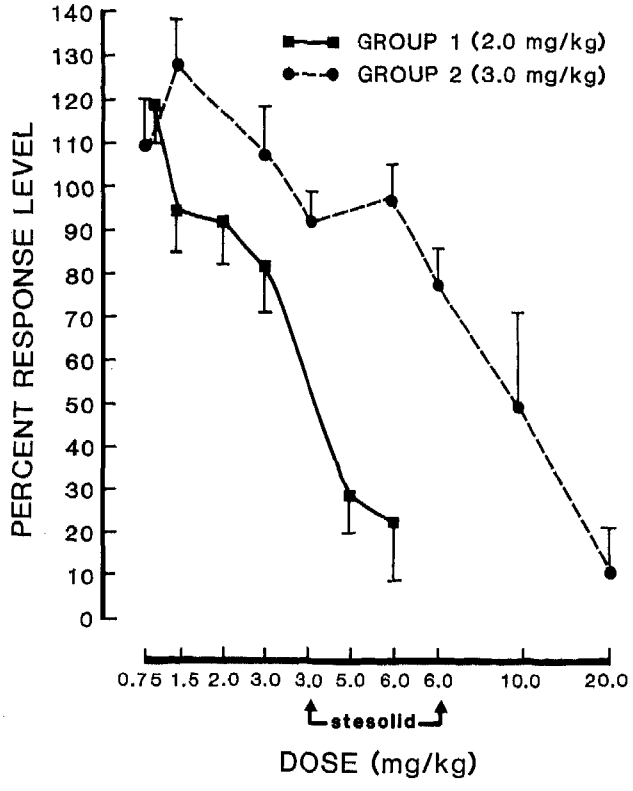

FIG. 1. Group 1 (n=7) and Group 2 (n=6); Depressant effects of different doses of diazepam. Group 1 was trained to discriminate 2.0 mg/kg diazepam from saline and the test was carried out during sessions 167–189. Group 2 was trained to discriminate 3.0 mg/kg diazepam and the test was carried out during sessions 136–162. Group 1 had received approximately 80 doses of diazepam (2.0 mg/kg) and Group 2 approximately 65 doses of diazepam (3.0 mg/kg). Percent response level is the total number of responses emitted during a specified drug session computed as percent of the most proximal saline session for the group. For Group 1 the differences in depression between saline and the two doses 5.0 and 6.0 mg/kg diazepam were significant. Group 2 reached significance first at a dose as high as 20 mg/kg. Brackets indicate SEM.

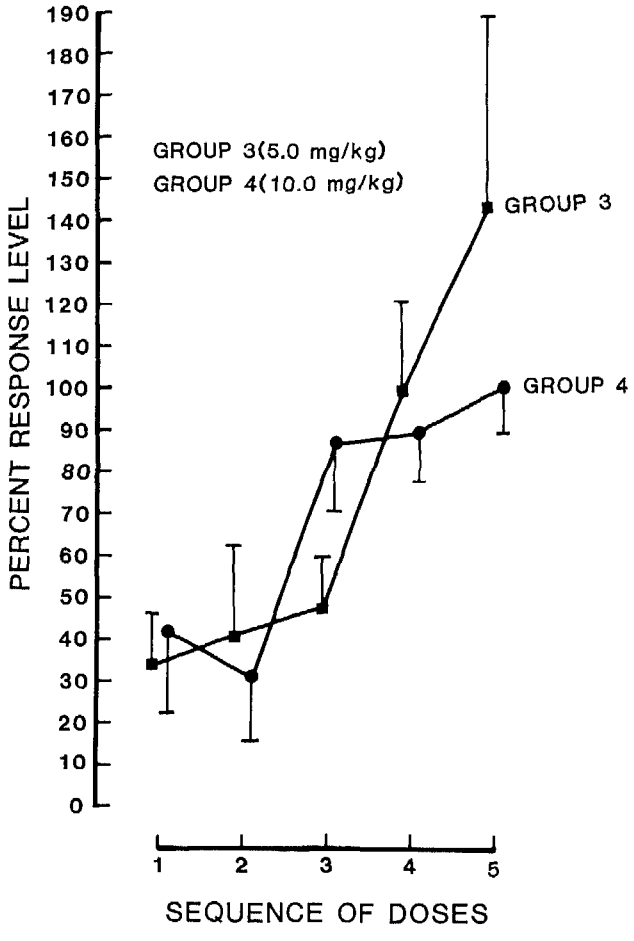

FIG. 2. Group 3 (n=6) and Group 4 (n=8): Depressant effects of 5.0 and 10.0 mg/kg diazepam during the first 5 administrations of the drug. The training drug was diazepam prepared as for clinical use (i.e., Stesolid, Dumex Ltd, Copenhagen, Denmark). Group 3 started the drug discrimination training in session 11. Group 4 started the drug discrimination training in session 13. For Group 3 there was a significant reduction of depressant effect after the fourth administration, while for Group 4 a significant decrease appeared already after the third administration.

low level (10.1%) compared to saline after administration of 20.0 mg/kg diazepam. This difference was highly significant ($t=8.40, p<0.001$).

The depression of operant responding after a dose of 10.0 mg/kg diazepam was not significantly different than after saline ($t=2.13, p>0.05$).

The depressant effects of 5.0 and 6.0 mg/kg in Group 1 was consistently more pronounced than both doses of 6.0 mg/kg in Group 2 (5.0 and 6.0 mg/kg in Group 1 compared to 6.0 mg/kg diazepam in dimethylacetamide and cremophor EL in Group 2: $t=6.87, p<0.001$, $t=3.79, p<0.01$) (5.0 and 6.0 mg/kg in Group 1 compared to 6.0 mg/kg diazepam as Stesolid in Group 2; $t=4.51, p<0.01$, $t=2.59, p<0.05$).

Experiment 2

Group 3 showed a median in responding (based on medians from seven saline sessions ranging from 397.5 to 970.0) of 704.5 lever presses during the test. The grand mean (based on the same sessions) was 712.4.

On the fourth administration of 5.0 mg/kg diazepam there was a significant increase in tolerance to the depressant effects. The one-way analysis of variance was significant, $F(4,25)=6.11, p<0.01$. The differences between the administrations Nos. 1 and 4, and between Nos. 1 and 5 were significant ($t=3.96, p<0.05$; $t=3.11, p<0.05$), while the differences between administrations Nos. 1 and 2 and between Nos. 1 and 3 were not ($t=0.99, p>0.05$; $t=0.23, p>0.05$).

Significant differences were also found between each of the first three administrations (Nos. 1, 2 and 3) when individually compared to the most proximal saline sessions ($t=4.77$, $p<0.01$; $t=2.58$, $p<0.05$; $t=4.97$, $p<0.01$). The differences between administration Nos. 4 and 5 were not significantly different from the most proximal saline session ($t=0.33, p>0.05$; $t=1.53, p>0.05$).

Group 4 showed a median in responding (based on me-

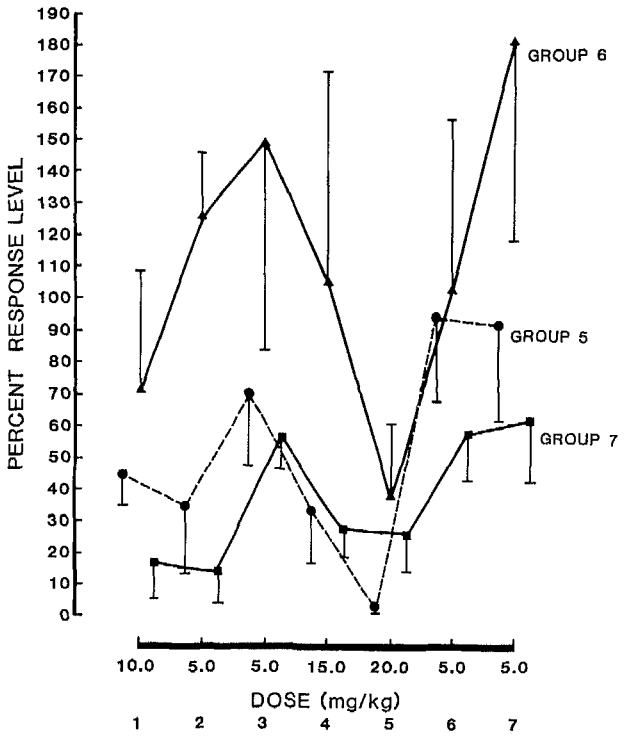

FIG. 3. The depressant effects of seven intermediate and high doses of diazepam in Group 5, 6 and 7, administered in a sequence shown in Table 2. Between Group 6 and 7 the differences in depressant effects were significant for all doses except administrations No. 5 (20.0 mg/kg) and 6 (5.0 mg/kg). Between Group 5 and 6 no differences were found, as was also true for the differences between Group 5 and 7.

dians from five saline sessions preceding the test doses, ranging from 744.5 to 988.0) of 837.0 lever presses. The grand mean (based on the same sessions) was 885.8.

The one-way analysis of variance was significant (one missing data point was estimated, and the *df* were reduced from 4 to 3) ($F(3,35)=5.26$, $p<0.01$).

On the third administration of 10.0 mg/kg of diazepam there was a significant increase in tolerance to the depressant effects. The differences between administrations Nos. 1 and 3, between 1 and 4, and between 1 and 5 were significant ($t=3.45$, $p<0.05$; $t=2.84$, $p<0.05$; $t=3.33$, $p<0.05$). The differences between the first two administrations and the most proximal saline session were also significant ($t=3.23$, $p<0.05$; $t=4.85$, $p<0.05$). The differences between administrations Nos. 3, 4 and 5 compared to the most proximal saline session were not significant ($t=0.84$, $p>0.05$; $t=0.73$, $p>0.05$; $t=0.02$, $p>0.05$).

Experiment 3

The split plot analysis of variance showed a significant group variable, $F(2,18)=25.54$, $p<0.001$, a significant dose variable, $F(7,126)=31.47$, $p<0.001$, and a significant interaction, $F(14,126)=6.76$, $p<0.01$.

Group 5 showed a median in responding (based on medians from seven saline sessions preceding the test doses ranging from 863.0 to 1376) of 1127 lever presses. The grand mean (based on the same sessions) was 1163. In this group the only dose exerting significant depressant effect compared to saline was 20.0 mg/kg diazepam ($t=17.08$, $p<0.01$).

Group 6 showed a median in responding (based on medians from seven saline sessions ranging from 515.5 to 927.0) of 591.5 lever presses. The grand mean (based on the same sessions) was 721.3. In Group 6 none of the doses tested depressed operant responding to a significantly different degree when compared with saline.

Group 7 showed a median in responding (based on medians from seven saline sessions ranging from 946.5 to 1275) of 1062 lever presses. The grand mean (based on the same sessions) was 1175. In Group 7 six of the seven doses tested depressed operant responding significantly (10.0 mg/kg: $t=8.36$, $p<0.001$; 5.0 mg/kg: $t=9.26$, $p<0.001$; 5.0 mg/kg: $t=4.79$, $p<0.01$; 15 mg/kg: $t=9.32$, $p<0.001$; 20 mg/kg: $t=6.58$, $p<0.001$; 5.0 mg/kg: $t=3.39$, $p<0.05$). One of the administrations (5.0 mg/kg) was not significantly different from saline ($t=1.52$, $p>0.05$).

Concerning differences among the three groups tested, several significant differences were found between Group 6 and 7 (10.0 mg/kg: $t=2.52$, $p<0.05$; 5.0 mg/kg: $t=6.20$, $p<0.001$; 5.0 mg/kg: $t=2.95$, $p<0.05$; 15.0 mg/kg: $t=2.97$, $p<0.05$; 20.0 mg/kg: $t=0.65$, $p>0.05$; 5.0 mg/kg: $t=2.2$, $p>0.05$ and 5.0 mg/kg: $t=3.56$, $p<0.01$). The only doses not significantly different were doses No. 5 (20.0 mg/kg) and 6 (5.0 mg/kg). Between Group 5 and 6 no differences were found between any doses tested. This was also true for the differences between Group 5 and 7.

The depressant effects of the highest doses (10.0, 15.0, and 20.0 mg/kg diazepam) were neither systematically related to body weight ($r_{xy}=0.44$, -0.39, 0.02, $p>0.05$), nor to operant level ($r_{xy}=0.39$, 0.28, -0.27, $p>0.05$) for individual rats in Group 5, 6, and 7.

DISCUSSION

In the present study it is shown that intermediate and high doses of diazepam (from 5.0 mg/kg and higher) very strongly depress operant responding in naive and low dose exposed Wistar rats (Figs. 1 and 2). More specifically, rats trained with a high number of low doses (Group 1, training dose 2.0 mg/kg) showed a depressant effect when submitted to doses of 5.0 and 6.0 mg/kg, comparable to naive rats. That is, the depressant effect of the first two doses of 10.0 mg/kg in Group 4 were not significantly different from the depressant effect of 5.0, $t(13)=0.61$, 0.14, $p>0.05$, and 6.0 mg/kg, $t(13)=0.57$, 0.21, $p>0.05$, in Group 1. These results are consistent with earlier observations in rats trained on a low dose (1.5 mg/kg) on a VI schedule [9].

The different profile in depressant effect which is seen in Group 2 is most certainly a result of repeated exposures to an intermediate dose of diazepam (3.0 mg/kg). This means that administrations of doses below 3.0 mg/kg may lack the ability to induce tolerance to the depressant effects at all. As seen in Table 2 the drug naive Group 7 shows the strongest depressant effects to the intermediate and high doses during the first eight administrations. This group shows a certain tolerance developed first after administration No. 8 which is the third administration of an intermediate dose (5.0 mg/kg). The result of the last administration of an intermediate dose (5.0 mg/kg, Fig. 3) was not significantly different from saline. The intermediate and high doses tested in Group 1 and Group 2 could be of no significance for the data in Fig. 1 since these doses were administered at the end of the test.

On the other hand, intermediate and high doses rapidly

induce tolerance to their depressant effect (Fig. 2). In Group 3 (exposed to 5.0 mg/kg diazepam) the depressant effect reaches zero level after the fourth administration while in Group 4 (trained on 10.0 mg/kg diazepam) the depressant effect reaches zero level after the third administration. A similar decrease in depressant effects of intermediate and high doses is also seen in Group 7 (Table 2).

When a certain tolerance to the depressant effect is established it is traceable after a long time with no drug exposures. This residual tolerance may be dose dependent since the responding in Group 6 was significantly less depressed for five of the seven doses tested (10.0 mg/kg: $t(10)=2.52$, $p<0.05$; 5.0 mg/kg: $t=6.20$, $p<0.001$; 5.0 mg/kg: $t=2.95$, $p<0.05$; 15.0 mg/kg: $t=2.97$, $p<0.05$; 20.0 mg/kg: $t=0.65$, $p>0.05$; 5.0 mg/kg: $t=2.20$, $p>0.05$; 5.0 mg/kg: $t=3.56$, $p<0.01$) compared to the control group (Group 7). This difference was not detectable in the comparison between Groups 5 and 7. Apart from the length of the pause in drug exposures, the difference between Groups 5 and 6 was mainly that the first group received a large number of intermediate doses of diazepam (3.0 mg/kg) together with a few high doses (up to 20.0 mg/kg) as in Group 2. In addition the group was exposed to a few doses of ethanol and pentobarbital which may contribute to the development of tolerance [12]. In spite of this, a low number of intermediate doses (5.0 mg/kg diazepam) for Group 6 seemed to be more effective in the establishment and maintenance of tolerance.

The general depressant effects seen in these groups of rats are considerably higher than the effects reported elsewhere [13]. This may be related to different factors, such as animal strain used, but may also be related to drug vehicle. Originally we tested Group 2 with two identical doses (3.0 and 6.0 mg/kg diazepam) with two different vehicles (Fig. 1). Although diazepam for clinical use (i.e., Stesolid) exerts a slightly more pronounced depressant effect [12], this was not significantly different from diazepam in dimethylacetamide and cremophor EL (3.0 mg/kg: dependent $t(5)=1.61$, $p>0.05$; 6.0 mg/kg: $t=1.67, p>0.05$). Further, Group 3 and 4 were trained on diazepam as Stesolid and the depressant effects of the first administrations were not as pronounced as the depressant effects of the high doses in Group 7 (Fig. 2, Table 2). Thus it was impossible with the present data to assess if either of the two vehicles are exerting more depressant effects on operant responding in this paradigm.

The effect of deprivation level on fixed ratio performance has been investigated in earlier experiments [8,19]. Its principal effect is on the post reinforcement pause, and the local rates of responding showed very little sensitivity to even wide ranges in deprivation [8]. The average duration of the post reinforcement pauses decreases with decreasing FR size [4] and as this pause is very short in FR 10 schedules, the effects of deprivation level may not be observable. This concurs with the findings in Group 2 where the variation in weight was 14 percent with very slight variation in rate in baseline performance. The effects of a dose of diazepam (6.0 mg/kg) did not show systematic variation related to weight and response rate. It should be noted that this dose level did not depress operant responding significantly as the group showed tolerance to intermediate doses of diazepam after prolonged training. A further investigation on groups that showed a more pronounced variation in baseline responding (Group 5, 6, and 7) confirmed this finding also for doses that depressed operant responding significantly.

The correlations between body weight and depressant effects were not significant ($r_{xy}=0.44$, -0.39, 0.02; $p>0.05$).

The medians based on a high number of sessions showed a considerable variation for the different groups (591.5 for Group 6 and 1477 for Group 2) a finding also true for the means (712.4 for Group 3 and 1432 for Group 1). The benzodiazepines may exert schedule actions on operant responding, but as the important variables that lead to these schedule dependent actions have not yet been assessed clearly, these actions are very difficult to consider [16]. There may be reason to believe that the benzodiazepines (i.e., chlordiazepoxide) depress operant responding of different control rates after administration of high doses [16] and also stimulates responding of different control rates after administration of low doses [5,16]. As the response rate may not be the major determinant of the depressant effect, it is very difficult to argue that Group 3 (with the fastest tolerance development) and Group 6 (least affected by the doses administered) are demonstrating their tolerance as a function of lowest baseline response rate.

In relation to Group 1 and 2 it is further very difficult to use the baseline responding as explanation for the differences in depressant effects. The medians were almost identical (Group 1: 1421, Group 2: 1477) and the means slightly different (Group 1: 1432, Group 2: 1266).

When research laboratories report dose response effects for certain drugs, this is most certainly an effect not solely produced by the active compound itself. While the vehicle, rat strain, route of administration and time and schedule dependent factors still need extensive investigation, the sequence of doses tested may be of utmost importance.

Specifically, when a large number of randomized doses are tested, the exposures to a few intermediate and high doses probably have an impact on the depressant effects of the subsequent doses tested. Thus, the responding that is measured may be a mixture of naive sensitivity and newly acquired tolerance. In other words, the responding to the last doses tested are based on a certain tolerance level and is thus not easily comparable with the responding to the initial doses without tolerance.

All of the mentioned effects should be taken into consideration when dose response curves are reported in the drug discrimination procedure. In addition to this, when related compounds are submitted to test, a cross tolerance effect may be one more variable to take into consideration.

ACKNOWLEDGEMENTS

K. Gunnar Götestam is gratefully acknowledged for supervision throughout the study.

REFERENCES

1. Barry, H., III and E. C. Krimmer. Pharmacology of discriminative drug stimuli. In: *Drug Discrimination and State Dependent Learning*, edited by B. T. Ho, D. W. Richards, III and D. L. Chute. New York: Academic Press, Inc., 1978, pp. 3–29.

2. Barry, H., III and E. C. Krimmer. Similarities and differences in discriminative stimulus effects of chlordiazepoxide, pentobarbital, ethanol, and other sedatives. In: *Stimulus Properties of Drugs: Ten Years of Progress*, edited by F. C. Colpaert and J. A. Rosecrans. Amsterdam: Elsevier/North Holland Biomedical Press, 1978, pp. 31–51.

3. Browne, R. G. Anxiolytics antagonize yohimbine's discriminative stimulus properties. *Psychopharmacology (Berlin)* **74:** 245–249, 1981.
4. Catania, A. C. Reinforcement schedules. In: *Learning,* edited by A. C. Catania. Englewood Cliffs, NJ: Prentice-Hall, Inc, 1979, p. 182.
5. Colpaert, F. C., L. K. C. Desmedt and P. A. J. Janssen. Discriminative stimulus properties of benzodiazepines, barbiturates and pharmacologically related drugs: Relation to some intrinsic and anticonvulsant effects. *Eur J Pharmacol* **37:** 112–123, 1976.
6. Colpaert, F. C., C. J. E. Niemeegers and P. A. J. Janssen. Theoretical and methodological considerations on drug discrimination learning. *Psychopharmacologia* **46:** 169–177, 1976.
7. Colpaert, F. C. Discriminative stimulus properties of benzodiazepines and barbiturates. In: *Discriminative Stimulus Properties of Drugs, Advances in Behavioral Biology,* vol 22, edited by H. Lal. New York: Plenum, 1977, pp. 93–106.
8. Ferster, C. B. and B. F. Skinner. Fixed ratio. In: *Schedules of Reinforcement,* edited by C. B. Ferster and B. F. Skinner. New York: Appleton-Century-Crofts, Inc, 1957, p. 71.
9. Haug, T. and K. G. Götestam. Two opposite effects of diazepam on fear by differential training in the CER-paradigm. *Psychopharmacology (Berlin)* **75:** 110–113, 1981.
10. Haug, T. and K. G. Götestam. The diazepam stimulus complex. Specificity in a rat model. *Eur J Pharmacol* **80:** 225–230, 1982.
11. Haug, T. and K. G. Götestam. Onset and offset of the diazepam stimulus complex. *Pharmacol Biochem Behav* **17:** 1171–1174, 1982.
12. Haug, T. Neuropharmacological specificity of the diazepam stimulus complex based on selected agonists and antagonists. *Eur J Pharmacol* **93:** 221–227, 1983.
13. Kilts, C. D., R. L. Commisaris and R. H. Rech. Comparison of anti-conflict drug effects in three experimental animal models of anxiety. *Psychopharmacology (Berlin)* **74:** 290–296, 1981.
14. Kirk, R. E. *Experimental Design: Procedures for the Behavioral Sciences.* Belmont, CA: Brooks and Cole, 1968.
15. Margules, D. L. and L. Stein. Increase of antianxiety activity and tolerance of behavioral depression during chronic administration of oxazepam. *Psychopharmacologia* **13:** 74–80, 1968.
16. Sanger, D. J. and D. E. Blackman. Rate-dependence and the effects of benzodiazepines. In: *Advances in Behavioral Pharmacology,* vol 3, edited by T. Thompson, P. B. Dews and W. A. Mc Kim. New York: Academic Press, 1981, pp. 1–20.
17. Schechter, M. D. Stimulus properties of ethanol and depressant drugs. In: *Drug Discrimination and State Dependent Learning,* edited by B. T. Ho, D. W. Richards, III and D. L. Chute. New York: Academic Press, Inc., 1978, pp. 103–117.
18. Shearman, G. and H. Lal. Discriminative stimulus properties of pentylenetetrazol in the rat. In: *Stimulus Properties of Drugs: Ten Years of Progress,* edited by F. C. Colpaert and J. A. Rosecrans. Amsterdam: Elsevier/North Holland Biomedical Press, 1978, pp. 181–188.
19. Sidman, H. and W. C. Stebbins. Satiation effects under fixed-ratio schedules of reinforcement. *J Comp Physiol Psychol* **47:** 114–116, 1954.
20. Soubrié, P., L. Schoonhoed, P. Simon and J. R. Boissier. Conflict behavior in a heated-floor maze: Effects of oxazepam. *Psychopharmacologia* **26:** 317–320, 1972.
21. Soubrié, P., P. Simon and J. R. Boissier. Newer or more operational models and/or methods for studying anti-anxiety drugs in animals. In: *Neuro-Psychopharmacology,* edited by J. R. Boissier, H. Hippus and P. Pichot. Amsterdam: Excerpta Medica, 1975, pp. 770–778.
22. Warner, R. S. Management of the office patient with anxiety and depression. *Psychosomatics* **6:** 347–351, 1965.