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High-Dose Discrimination Training with Midazolam: Context Determines Generalization Profile

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ATOR, N. A. *High-dose discrimination training with midazolam: Context determines generalization profile.* PHARMACOL BIOCHEM BEHAV **64**(2) 237–243, 1999.—In previous work, greater differentiation among ligands for the benzodiazepine site was found in rats trained to discriminate among vehicle, 0.32, and 3.2 mg/kg midazolam than in animals trained to discriminate a single midazolam dose from vehicle (i.e., virtually all test drugs occasioned low-dose midazolam-appropriate responding, but most did not occasion high-dose midazolam-appropriate responding even at high test doses). A possibility was that merely training with 3.2 mg/kg–midazolam (not previously studied) would result in greater selectivity than training with lower midazolam doses. In the present study, rats were trained to discriminate 3.2 mg/kg IP midazolam from no drug under a two-lever, food-maintained, procedure; and drugs from the previous three-lever studies were tested. Triazolam, bretazenil, clonazepam, lorazepam, midazolam, zolpidem, chlordiazepoxide, pentobarbital, and flurazepam all dose-dependently occasioned >80% responding on the midazolam-appropriate lever in roughly that order of potency. Only triazolam had occasioned midazolam 3.2 mg/kg–appropriate responding in the previous work. The greater differentiation among these drugs in the dose-vs.-dose procedure likely was due to a training dose context rather than to the high training dose per se. © 1999 Elsevier Science Inc.

Benzodiazepines Bretazenil Chlordiazepoxide Clonazepam Drug discrimination Flurazepam Lorazepam Midazolam Pentobarbital Rats Time course of discriminative effects Training dose in drug discrimination Triazolam Zolpidem

RATS, pigeons, and monkeys trained to discriminate a midazolam dose from vehicle typically have generalized to other benzodiazepines (Bzs), barbiturates, and other sedatives, and to novel agonist and partial agonist ligands for the Bz site [review in (14,15)]. In a three-lever procedure, however, Sannerud and Ator (15) reported greater differentiation among the discriminative effects of ligands for the Bz site in rats trained to discriminate among vehicle and two doses of midazolam (0.32 and 3.2 mg/kg). In that study, only midazolam itself, diazepam, and triazolam dose dependently occasioned >80% of total test session responding on the 0.32 mg/kg-appropriate lever (at low and intermediate doses) and on the 3.2 mg/kg-appropriate lever (at intermediate to high doses). In tests with bretazenil, chlordiazepoxide, flurazepam, lorazepam, and zolpidem, the rats responded on the midazolam 0.32 mg/kg-appropriate lever but, even at the highest doses, generally did not respond on the midazolam 3.2 mg/kg-appropriate lever. These results are summarized in Table 1. Interestingly, clonazepam did not occasion full midazolam-appropriate responding, even on the 0.32 mg/kg-paired lever, in almost half the rats, which differed markedly from results of other studies in which rats had been trained to discriminate midazolam (11,20).

In another study, Sannerud and Ator (14) found that pentobarbital produced partial generalization on both the midazolam 0.32 and 3.2 mg/kg-appropriate levers; but, similar to the tests with Bzs described above, there was more responding on the lower than the higher dose lever (Table 1). This was particularly interesting because rats trained to discriminate midazolam from the no-drug condition had not shown qualitative differences between the discriminative effects of midazolam and barbiturates across a couple of training doses; yet studies in pigeons and nonhuman primates had shown such a difference [review in (14)]. The results of the three-lever dose-vs.-dose discrimination study thus suggested that the apparent species difference in the generalization profile for midazolam-trained animals was likely instead to be a function of differences in effective training dose (i.e., that training doses in pigeons and monkeys had been effectively higher than those in rats).

It was tempting to attribute the unique generalization profile obtained by Sannerud and Ator (14,15) to a greater sensi-

TABLE 1

PEAK PERCENTAGES OF RESPONDING ON THE MIDAZOLAM 0.32 AND 3.2 mg/kg-APPROPRIATE LEVERS IN RATS TRAINED UNDER A THREE-LEVER PROCEDURE

	Dose Range	0.32-mg/kg Lever		3.2-mg/kg Lever	
Test Drug	mg/kg, IP	%	mg/kg	%	mg/kg
Benzodiazepine full agonist					
Chlordiazepoxide	0.1–18	80	18.0	0*	—
Clonazepam	0.1–10	60	3.2	5	10.0
Diazepam	0.032–18	99	1.0	96	18.0
Flurazepam	0.01–10	77	10.0	20	10.0
Lorazepam	0.032–1.0	76	1.0	19	1.0
Midazolam	0.1–10	85	0.32	98	5.6
Triazolam	0.0032–3.2	69†	0.1	100	3.2
Benzodiazepine partial agonist					
Bretazenil	0.01–32	99	1.0	5	0.32
Imidazopyridazine, $GABA_A$-a_1-subtype selective					
Zolpidem	0.032–3.2	97	3.2	13	1.0
Barbiturate					
Pentobarbital	0.32–18.0	71	10.0	33	18.0

Data are group means from Sannerud and Ator (14,15). If a peak percentage occurred at more than one dose, the lower dose is listed.

*One of the 11 rats made 99% 3.2-mg/kg–appropriate responses at 32 mg/kg; no other rat responded.

†All rats responded 80% or more on the 0.32 lever, but peaked at different doses.

tivity of the dose-vs.-dose training context itself, but a strong possibility was that merely being trained to discriminate 3.2 mg/kg midazolam would result in greater selectivity. Manipulation of the Bz training dose, including midazolam, typically has not resulted in qualitative differences in generalization profiles except for the probability of generalization to the Bz antagonist flumazenil (5,8,9,17). Tang and Franklin (19), however, showed differential selectivity for Bz-site partial agonists in rats trained to discriminate 1.0 mg/kg diazepam compared to rats trained to discriminate 10 mg/kg: less diazepam-appropriate responding occurred in the higher training dose condition. Those results suggested that it was important to determine the generalization profile for the high midazolam dose by itself before concluding that the dose-vs.-dose context had been critical to the results obtained by Sannerud and Ator.

In the present study, rats learned to discriminate 3.2 mg/kg midazolam from vehicle. They were tested with triazolam, and all drugs that occasioned midazolam 0.32 mg/kg-appropriate but not midazolam 3.2 mg/kg-appropriate responding in the studies by Sannerud and Ator (14,15). In addition, a time course manipulation was used to examine duration of effect of high test drug doses to aid interpretation of the role a cumulative dosing procedure might have played in the pattern of results obtained by Sannerud and Ator.

METHOD

Subjects

Six male Long–Evans hooded rats (Harlan–Sprague–Dawley, Indianapolis, IN) were obtained at 6 weeks of age and individually housed in a vivarium (average temperature = 70°F; average relative humidity = 40%). They were permitted free access to Purina laboratory rodent diet until initial training began a month after arrival. At the beginning of training, weights ranged from 300 to 344 g across rats. Weights were not reduced to any percentage of this free-feeding weight. Rather, the rats were fed a measured amount of food once per day, and weights were stabilized at 330 g (±10 g) for the dose-effect determinations. Supplemental feeding was 30–45 min after the experimental session, and feeding occurred about the same time of day on days when there were no sessions. Water was continuously available in the individual home cages. A nonreversed 12-h light/dark cycle was maintained; experimental sessions were conducted during the light phase.

Apparatus

Experimental sessions were conducted in six custom-made operant chambers (27.7 × 30.3 × 53.2 cm high), which were enclosed in sound-attenuating cubicles. On one wall of each chamber, two stainless steel levers (Gerbrands Corp., Arlington, MA) were mounted 13 cm apart and 5 cm above the floor. A cue light with a translucent, colored cap was centered 6 cm above each lever (cap colors differed across chambers, but were the same within chambers). An electromechanical pellet dispenser delivered 45-mg food pellets ("precision" pellets, P. J. Noyes Co., Lancaster, NH) into a food cup on the wall opposite the levers. The outer enclosure was equipped with a ventilation fan and a speaker, through which white noise (55–60 dB as measured in each chamber) for further masking extraneous sounds was delivered. Experimental con-

trol and data collection were accomplished with computers programmed in a MED-PC state notation (MED Associates, St. Albans, VT). An event recorder plotted lever operations (excluding those in time outs) and pellet delivery.

Procedure

Each rat's experimental sessions always were conducted in the same chamber; sessions occurred simultaneously 5 or 6 mornings/week.

Discrimination training. The rats (designated 44-1, 2, 3, 4, 5, 6) were habituated to the chambers, trained to approach the food cup at the sound of the feeder, and lever pressing was shaped first on the lever designated as the no-drug lever. For the even-numbered rats, this was the left lever, and for the odd-numbered it was the right-hand lever. Once shaping was completed, experimental sessions lasted 20 min. The response requirement was raised across sessions to four consecutive lever presses per pellet. A response on the alternate lever caused the response requirement on the appropriate lever to reset. The lights above both levers were illuminated whenever the reinforcement contingencies were in effect. Coincident with delivery of the food pellet, a time out began, during which the chambers were dark and lever presses had no programmed consequences. The duration of the time out gradually increased from 1 to 10 s. Sessions were preceded by a time out, which was lengthened from 1 to 15 min. The rats were given saline injections before one or two of these training sessions to habituate them to the injection procedure. Next, injections with 3.2 mg/kg midazolam were given immediately before the 15-min presession time out and pressing the opposite lever was shaped. The response requirement and postpellet time-out duration were raised over sessions to the previous values. Double alternation of no-drug and midazolam training sessions began, across which the response requirement was raised to 10 consecutive responses per pellet. Single alternation of no-drug and midazolam training sessions began. The following criteria had to be met in each of four consecutive training sessions before the first test: <10 consecutive responses on the inappropriate lever before the first pellet delivery and 95–100% of the total responses on the appropriate lever.

Generalization testing. Test sessions were also 20 min, and were identical to training sessions except that 10 consecutive responses on either lever produced food. The first two test sessions were those with midazolam 3.2 mg/kg and with its vehicle (i.e., tests for stimulus control). After stimulus control was shown in the midazolam 3.2 mg/kg and vehicle tests, tests with novel doses of midazolam were conducted. Training sessions alternated between test sessions, except that the type of training session after a test was the same as the one before it to ensure that test sessions were preceded equally often by midazolam and no-drug training sessions. If criterion level performance occurred in two consecutive training sessions after a test, then a test was conducted in the next session. If criterion level performance did not occur in any one training session, then such performance was required in four consecutive training sessions before the next test.

Dose–effect determinations were made for the novel drug conditions in the following order: pentobarbital, flurazepam, zolpidem, clonazepam, bretazenil, chlordiazepoxide, lorazepam, triazolam. Before each set of dose–effect determinations, stimulus control by midazolam 3.2 mg/kg and its vehicle were again demonstrated under test conditions for each rat. Rats 44-4 and 44-3 became ill with chronic respiratory disease after the study with midazolam and pentobarbital, respectively; they were removed from the experiment for treatment with antibiotic upon the advice of the veterinarian. Rat 44-3 recovered and rejoined the experiment; rat 44-4 did not. With all test drugs, injections occurred immediately before the 15-min presession time out, except that with lorazepam, the injection was 45 min before the 15-min presession time out. All dose–effect curves included each drug's own vehicle. Test doses were studied in a generally ascending and somewhat mixed order across rats. Doses generally were given once to each rat (i.e., they were repeated in cases of procedural problems), and except for 5.6 mg/kg chlordiazepoxide (which was not tested in rat 44-3), each rat was tested with all doses.

Seventy-five-minute pretreatment condition. Sannerud and Ator (14,15) had used a cumulative dosing procedure. Practical considerations in the laboratory weighed against using cumulative dosing in the present study, and there was no evidence that qualitative differences in generalization profiles derived from cumulative dosing procedures. However, the possible contribution of metabolism of cumulating drug to the low probability of responding on the 3.2-mg/kg midazolam-appropriate lever in those studies that used cumulative dosing seemed important to consider. When all the dose–effect curves were completed, the rats were tested 75 min after dosing with saline, midazolam 3.2 mg/kg, and high test drug doses to see whether there would be a failure to respond on the midazolam-appropriate lever. The 75-min time interval was selected to coincide with the time at which the final dose increment would have been given under Sannerud and Ator's cumulative procedure.

Drugs. Midazolam maleate (Hoffmann–LaRoche, Basel, Switzerland), flurazepam hydrochloride (Hoffmann–LaRoche, Nutley, NJ), chlordiazepoxide hydrochloride, and pentobarbital sodium (both from Sigma Chemical Company, St. Louis, MO), and zolpidem tartrate (Research Biochemicals Inc., Natick, MA) were dissolved in 0.9% saline. All were prepared within an hour of injection, except midazolam and zolpidem were maintained in stock solutions. Bretazenil (Hoffmann–LaRoche, Inc., Basel, Switzerland) was suspended in a 96% saline/4% Tween 80 vehicle within an hour of injection. Clonazepam (Hoffmann–LaRoche, Nutley, NJ) was dissolved in a stock solution of 20:80 ethanol (95%w/v):propylene glycol; lorazepam (Wyeth–Ayerst Research, Princeton, NJ) was dissolved in a stock solution of 20:80 propylene glycol:polyethylene glycol; triazolam (Upjohn, Kalamazoo, MI) was dissolved in a stock solution of propylene glycol. Those stocks were maintained for up to 30 days. They were diluted 50% with 0.9% saline for injection; diluted stocks were maintained up to 7 days. All doses were calculated in terms of the form of the drug given above. Injections were intraperitoneal in a volume of 1 ml/kg, except that 3.2 and 10 mg/kg clonazepam were in 2 and 3 ml/kg, respectively.

Data analysis. The dependent measures to be reported are percentage of total responses on the midazolam 3.2 mg/kg-appropriate lever and the response rate on both levers combined. Responses and time elapsed during time outs were excluded from both measures. A percentage was included in the group mean only if the rat completed the response requirement and obtained at least one pellet; however, the response-rate data were not excluded. Consistent with Sannerud and Ator (14,15) and previous studies, a drug was considered to share discriminative stimulus effects with midazolam 3.2 mg/kg if the peak percentage of midazolam-appropriate responding was at least 80%; and absence of midazolam discriminative stimulus effects was concluded if midazolam-appropriate responding was 20% or less. The doses at which the mean generalization gradient crossed 50 and 80% midazolam-appropriate responding (ED_{50} and ED_{80}) were determined by

dropping a line from the gradient to the $\log_{10}$-scale × axis (7). A dose was judged to have had a significant effect on response rate if the group mean response rate fell outside the confidence interval formed by 2 SE of the drug vehicle mean.

RESULTS

Stimulus Control Tests

Once criterion-level performance had occurred in four consecutive training sessions, all six rats showed criterion level performance in their first test sessions with the midazolam vehicle, despite not having regularly received injections of saline prior to no-drug training sessions. This result is consistent with previous experience in our laboratory in training animals to discriminate sedative/anxiolytic drugs using the drug versus no drug procedure (3,5,14–16). Good stimulus control was shown in the vehicle and 3.2-mg/kg midazolam test sessions that preceded study of each test drug: across all those tests, the grand mean midazolam-appropriate responding after the training dose and its vehicle were 99.6% (range 98–100%) and 0.2% (range 0–0.4%), respectively.

Generalization Gradients

Figure 1 shows that all novel test drugs fully shared discriminative stimulus effects with 3.2 mg/kg midazolam. The group mean peak percentage of midazolam-appropriate responding was 95% or above for every drug. The relative potency of the drugs for occasioning some midazolam-appropriate responding was in the order shown in the key to Fig. 1. The ED_{50}s (mg/kg) were: bretazenil [0.02], triazolam [0.04], clonazepam [<0.1], lorazepam [0.18], midazolam [0.8], zolpidem [1.1], chlordiazepoxide [2.6], pentobarbital [6.2], flurazepam [10]. The relative potency for full generalization (and the ED_{80}, in mg/kg, obtained by interpolation from the mean gradient) was: triazolam [0.07] > bretazenil [0.1] > lorazepam [0.3] > clonazepam [1.0] > midazolam [1.4] > zolpidem [1.6] > chlordiazepoxide [4.5] > pentobarbital [8.5] > flurazepam [20].

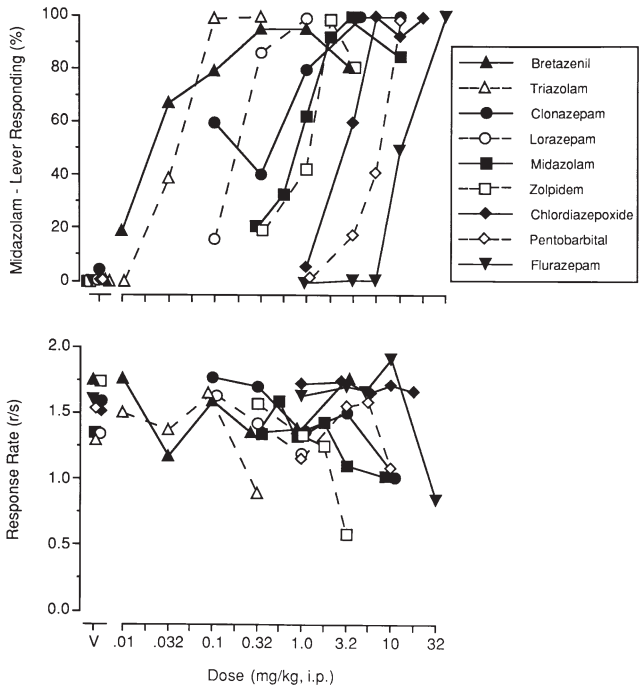

FIG. 1. Group mean generalization gradients (upper panel) and mean response rates (lower panel) in a group of rats trained to discriminate midazolam 3.2 mg/kg IP from the no-drug condition. The data are means for single-test sessions for each rat. The same five rats were tested with every drug, except six that were tested with midazolam, and only four were tested with flurazepam. Three rats were tested with 13.3 mg/kg pentobarbital (data not shown: % = 93; rate = 1.3). See text for information on rate effects for all drugs relative to a 95% confidence interval.

Each individual rat's peak percentage of midazolam-appropriate responding exceeded 95% for every drug with two exceptions. Rat 44-5' s peak percentage for bretazenil was 76% (at 0.32 and 1.0 mg/kg). Rat 44-2's peak percentage for pentobarbital was 2% (at 5.6 mg/kg); this rat failed to respond sufficiently to obtain even one pellet after 10 mg/kg and also after 7.8 mg/kg (which was tested to try to obtain evidence for generalization). The individual generalization gradients, based on single determinations of each dose, showed predominantly quantal responding (i.e., <20% > 80% midazolam-appropriate responding). Intermediate percentages of responding at intermediate doses occurred for one or two rats in tests with bretazenil, chlordiazepoxide, lorazepam, and midazolam.

Response Rates

Although the training dose of midazolam initially suppressed lever pressing and produced ataxia, this effect no longer occurred after the first few injections during the training phase. Figure 1 shows that response rates generally were unaffected at the lower test doses. The SE of the mean response rate was 0.3 for the vehicle tests for bretazenil, chlordiazepoxide, clonazepam, flurazepam, lorazepam, and zolpidem; and it was 0.2 for vehicle tests for midazolam, pentobarbital, and triazolam. Relative to a 95% confidence interval formed by 2 SE on either side of each vehicle control rate (not shown), no response rates were significantly increased by drug. Rates tended to be decreased at the highest dose of each drug; but only rates after the highest doses of clonazepam (10 mg/kg), flurazepam (32 mg/kg), pentobarbital (10 mg/kg), triazolam (0.32 mg/kg), and zolpidem (3.2 mg/kg) were decreased significantly. Even when overall response rates were decreased significantly with respect to vehicle, they still generally remained above 0.5 response/s. No rat failed to obtain pellets in test sessions after any test dose except as described above for rat 44-2 with pentobarbital and also for rat 44-5 at both 10 mg/kg clonazepam and 3.2 mg/kg zolpidem.

Time Course

Figure 2 presents the percentage of midazolam-appropriate responding for individual rats in the tests conducted 75 min after drug. After saline, there was <5% midazolam-appropriate responding. After the drug injections, the rats made ≥80% of their responses on the midazolam-appropriate lever with a couple of exceptions. Rat 44-5 made 13, 2, and 0.2% midazolam-appropriate responses after 3.2 mg/kg midazolam and 10 mg/kg flurazepam, respectively. Rats 44-1 and 44-5 made <1% midazolam-appropriate responses after 3.2 mg/kg zolpidem. Rats 44-2, 44-5, and 44-6 all failed to respond sufficiently to obtain a single pellet in the test session 75 min after 18 mg/kg pentobarbital. This pentobarbital dose had not been tested at the 15-min pretreatment because full generalization occurred at 10 and 13.3 mg/kg, and in our pre-

vious experience 18 mg/kg likely would have suppressed responding completely. [This had been the highest dose tested by Sannerud and Ator (14), however.] Although no lever choice could be evaluated in the present study due to rate suppression, the very fact of the rate suppression indicated that pentobarbital's effect was present after 75 min.

Response rates for most rats in the tests 75 min after dosing were >1 response/s. Aside from the extremely low rates after pentobarbital, only rat 44-6 responded at an extremely low rate in any test session (after 10 mg/kg clonazepam). After saline, the mean response rate was 1.1 response/s, and the 95% confidence interval ranged from 0.8 to 1.5 response/s. The mean rate for pentobarbital coincided with this lower limit, but no other mean rate was as low. In fact, the mean rates for chlordiazepoxide (1.5) and bretazenil (1.7 responses/s) fell at or slightly above the confidence interval.

DISCUSSION

The purpose of the present study was to determine whether the generalization profile for rats trained to discriminate the high 3.2 mg/kg dose of midazolam under a conventional two-lever procedure would be the same as the generalization profile obtained with respect to the midazolam 3.2 mg/kg lever under the three-lever procedure of Sannerud and Ator (14,15). The result of the present study was a generalization profile that was very different from the one obtained in the studies with the three-lever procedure. Only the results with triazolam were the same across studies (i.e., triazolam produced full midazolam 3.2 mg/kg-appropriate responding in both studies); plausible reasons for this difference are not clear at present. Unlike the three-lever studies, chlordiazepoxide, clonazepam, flurazepam, lorazepam, bretazenil, zolpidem, and pentobarbital also fully shared discriminative effects with 3.2-mg/kg midazolam. Therefore, the greater differentiation found by Sannerud and Ator (15) among compounds that enhance γ-aminobutyric acid (GABA) through $GABA_A$ modulatory sites likely was a function of midazolam dose-vs.-dose training rather than of greater selectivity conferred by training with a higher midazolam dose per se.

There were a number of differences between the studies that used the three-lever procedure and the present two-lever study, however, other than whether or not rats were trained to discriminate a low from a high dose of midazolam. Among the other salient differences are the strain of rat (Sprague–Dawley vs. Long–Evans hooded in the present study), the route of midazolam training dose (SC vs. IP in the present study), and the use of the cumulative dosing procedure by Sannerud and Ator. The first two differences seem unlikely to have produced such dramatic differences in generalization. Woudenberg and Slangen (20) used midazolam-trained Wistar rats and obtained qualitatively similar results for pentobarbital and Bzs to those of the present study and to Garcha et al. (11), who trained Lister hooded rats. Also, Woudenberg and Slangen (20) directly compared SC and IP routes of administration with respect to generalization to chlordiazepoxide and diazepam, and did not find qualitative differences; nor did Sannerud and her colleagues with respect to midazolam itself (15,16).

It is more difficult to rule out the cumulative dosing procedure as a factor in the failure of even the highest doses of most of the test drugs to occasion midazolam 3.2 mg/kg-appropriate responding in the Sannerud and Ator (14,15) studies. The present study did investigate one aspect of the cumulative dosing procedure: the lengthy interval across which drug dose was incremented in the studies by Sannerud

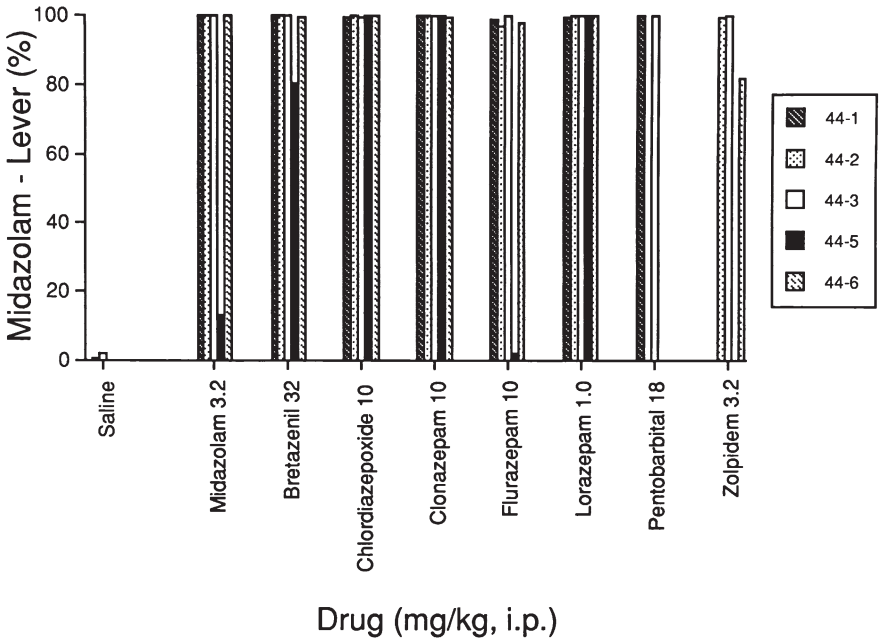

FIG. 2. Percentage of total responses on the midazolam–3.2-mg/kg–appropriate lever in single test sessions that were conducted 75 min after injection. Each column represents the data for one of five rats. All rats were tested in all conditions except for saline for rat 44-1; no data appear for some rats because percentages were zero (saline, zolpidem) or the rat did not respond sufficiently (pentobarbital). See text for information on response rates.

and Ator. Although only the highest dose of the test drugs was studied, the discriminative effects did indeed last for 75 min, which was the full length of time it would have taken for the cumulative doses in the Sannerud and Ator studies to increment to that level. To the extent that pharmacokinetics may be dose dependent over this time span in rats, the 75-min time-course study must be interpreted cautiously. However, rats in the present study responded predominantly on the midazolam-appropriate lever 75 min after injection, which seems to be a reasonable test of the possible duration of discriminative effects of these compounds. That drugs, including quickly eliminated compounds, can occasion switching to the 3.2 mg/kg-appropriate lever under the cumulative dosing procedure was indeed demonstrated with triazolam, midazolam, and diazepam in that study (15).

The present results with pentobarbital are of particular interest. Across studies, rats have generalized from midazolam to pentobarbital (11,20), even when the role of midazolam training dose was investigated directly (3); but pigeons and monkeys have not (4,10,18). Furthermore, drug discrimination studies that have investigated whether manipulation of training dose for other Bzs would decrease (8,17) or increase (5) the probability of generalization to pentobarbital have not shown such an effect. The failure of pentobarbital to occasion responding on the midazolam 3.2 mg/kg-appropriate lever under the three-lever procedure had been interpreted by Sannerud and Ator (14) to indicate that effective training dose may have been critical in the lower probability of generalization to barbiturates in midazolam-trained pigeons and monkeys compared to rats. They speculated that training rats with a higher dose of midazolam than previously had been used in two-lever procedures might decrease generalization to pentobarbital. The present results show that this was not the case (at least for 3.2-mg/kg midazolam), and reestablish the general conclusion that there appears to be a species difference in the probability of generalization from midazolam to barbiturates. Once again, dose-vs.-dose training under the three-lever procedure is what appears to have determined the lower probability of generalization to pentobarbital (14).

The present study extends the range of Bzs and related compounds that have occasioned midazolam-appropriate responding under two-lever procedures. Zolpidem, an imidazopyridazine that is selective for a subtype (Bz_1/ω_1) of the Bz binding site had not been reported previously in other two-lever procedures in midazolam-trained rats. Generalization to this selective ligand by midazolam-trained animals is consistent with the results obtained with zolpidem in midazolam-trained baboons (4). [Apparently, midazolam has not been tested in zolpidem-trained animals; cf., (13)]. With respect to Bzs tested in midazolam-trained rats, the present study extends these to include flurazepam, lorazepam, and triazolam. Results of previous studies that tested chlordiazepoxide, clonazepam, diazepam, and flunitrazepam (11,20) were comparable to the present findings.

Results with Bzs in rats are consistent with results in midazolam-trained pigeons and monkeys (4,10,18). The present results showing full generalization to the partial agonist bretazenil, however, are not consistent with results in pigeons. That is, Acri et al. (2) found only partial generalization to bretazenil and other Bz partial agonists in midazolam-trained pigeons. Furthermore, when pigeons and rats were trained to discriminate bretazenil and tested with midazolam, there was full generalization to midazolam in the rats but virtually none in the pigeons (1). Generalization to bretazenil in rats in that study and in the present study replicates the findings of Rijnders et al. (12) for rats trained to discriminate 1.0-mg/kg midazolam. However, the present results do not extend to midazolam the differentiation of partial from full Bz agonists that was conferred by using a very high (10 mg/kg) diazepam training dose (19) or by training rats to discriminate lorazepam (6).

Being trained to discriminate one dose of a drug from another appears to be a critical determinant of greater differentiation among compounds having a common mechanism of action. The pattern of results under the dose-vs.-dose discrimination under the three-lever procedure seem to be a function of that juxtaposition rather than being a composite of results that would be obtained under each training dose condition separately. This lends further credence to speculation that dose-vs.-dose training promotes greater sensitivity to differences among drugs that may correspond to differences in intrinsic efficacy or to other differences in molecular mechanisms of action [review in (15)]. It remains to be seen, however, whether dose vs. dose training with all Bz ligands produces greater differentiation among test drugs than two-lever procedures, or whether enhanced sensitivity under three-lever procedures is indeed related to differences in identifiable molecular mechanisms of action.

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