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Neuroleptic-Induced Changes in the Anxiolytic and Myorelaxant Properties of Diazepam in the Rat

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TAUKULIS, H. K., M. T. FILLMORE AND J. L. RUGGLES. *Neuroleptic-induced changes in the anxiolytic and myorelaxant properties of diazepam in the rat.* PHARMACOL BIOCHEM BEHAV **41**(1) 13–21, 1992.—Diazepam (2.0 mg/kg) was injected (IP) into rats 30 min before chlorpromazine (2.5, 5.0, or 10.0 mg/kg) on ten occasions. All doses of chlorpromazine enhanced the capacity of diazepam to increase rats' exploration of the exposed arms of an elevated plus-maze, an animal screening test for anxiolytic and anxiogenic substances. When maze testing occurred during each of the ten diazepam→chlorpromazine trials (after diazepam but before chlorpromazine), this enhancement effect appeared on Trial 6 and persisted thereafter. Haloperidol (3.0 mg/kg, IP) changed diazepam-elicited plus-maze activity in the same manner as chlorpromazine; however, thioridazine (10.0 mg/kg) and pimozide (2.0 mg/kg) were ineffective. Additionally, haloperidol, like chlorpromazine, was found to reduce diazepam's muscle relaxation effect (inclined plane test) as a consequence of diazepam→haloperidol pairings; once again, thioridazine and pimozide proved ineffective. These results suggested that not all neuroleptics will alter diazepam activity, and also that dopamine blockade per se is not sufficient to induce such changes. While the reasons for the enhanced plus-maze effects of diazepam induced by haloperidol and chlorpromazine remain elusive, the diminished myorelaxant effect may be linked to a neuroleptic's capacity to induce muscular side effects: thioridazine and pimozide are far less likely to yield such effects than are chlorpromazine and haloperidol. Haloperidol administered chronically by itself was found to have an effect on diazepam-induced myorelaxation. Administration of this butyrophenone either orally (2.0 mg/kg daily for 22 days) or in depot form (haloperidol decanoate, 60.0 mg/kg IM once a month for four months) caused a diminished effect of diazepam in rats subjected to the inclined plane test. Research into this phenomenon may yield insights into the nature of the diminution of diazepam myorelaxation that results from diazepam→haloperidol pairings.

Diazepam Chlorpromazine Haloperidol Thioridazine Pimozide Haloperidol decanoate Anxiolytic
Myorelaxant Plus-maze Inclined plane Conditioning Interactions

TAUKULIS and Brake (32,33) discovered that the effect of diazepam (DZ) in an animal model of anxiety can be potentiated when animals are given repeated exposures to a combination of diazepam and the phenothiazine chlorpromazine (CPZ). In a 22-day treatment phase, rats were injected with the two drugs, 30 min apart, on ten occasions. During a test trial, the animals were given DZ alone 30 min prior to placement in an elevated plus-maze. This maze is comprised of two "open" (exposed) arms and two "enclosed" arms (surrounded by high walls). DZ will usually increase the amount of time the animals spend in the open arms and also the number of entries into these areas. Previous DZ→CPZ pairings substantially increased this effect. However, rats that had experienced the drugs in reverse order (CPZ→DZ) exhibited no such response; their plus-maze activity was comparable to that of control animals that had received only DZ during ten treatment sessions. It was suggested (33) that this difference between the forward and backward drug-paired groups indicated the occurrence of a learning process in which the diazepam came to elicit a conditional response because it had signalled the imminent effects of chlorpromazine.

In addition to altered activity in the plus-maze, Taukulis and Brake (33) found that animals with a DZ→CPZ history exhibited a diminished muscle relaxation response to DZ, as measured by their ability to maintain a fixed position on an inclined plane. Thus a single conditioning procedure was shown to cause a simultaneous enhancement and diminution of DZ's efficacy, depending upon the specific effect being measured.

Chlorpromazine, like most neuroleptics, is a complex substance that, either directly or indirectly, affects a variety of neurotransmitter systems (7,11). Its ability to alter the muscle relaxation effect of DZ may stem from its effect upon dopaminergic pathways in the corpus striatum and substantia nigra. Chronic administration of this agent causes an upregulation of striatal dopamine receptors (6) and, as an apparent consequence,

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a reduction of activity in GABAergic axon terminals within the nigrostriatal system. Inhibition of nigrostriatal GABA activity, whether induced by the destruction of pathways in this region (15,39) or by the administration of certain dopamine-blocking agents (1,14), is linked to concomitant increases in GABA binding sites within the substantia nigra.

Benzodiazepines (BZDs) like diazepam bind to acceptor sites on a macromolecular complex that includes a GABA receptor (9, 17, 21, 27). Certain neuroleptics like chlorpromazine may in some way alter the benzodiazepine-GABA interaction. In fact, they may directly enhance (or interfere with) the binding of diazepam to the BZD receptor (38).

Given that the neuroleptics affect neuronal systems involved in muscle control, and given that the muscle relaxation produced by BZDs may also stem from their activity here, it may be that the diminution in DZ-induced muscle tone observed by Taukulis and Brake (33) is attributable to a conditional response mediated by these systems. With each DZ→CPZ pairing, diazepam's effects signal the onset of chlorpromazine's effects. With repeated pairings, the organism develops a conditional response to DZ, a response that reflects the organism's anticipation of CPZ. This conditional response manifests itself as an attenuation of the muscle relaxation normally elicited by DZ.

It is far more difficult to speculate about the neural substrates of the putative conditional response that is presumed to account for the effect of DZ→CPZ pairings on plus-maze activity. The difficulty arises, in part, because the neurotransmitter systems underlying the "anxiolytic" activity of BZDs have not yet been definitively identified. Although GABAergic systems have been implicated, other systems may also play a role [e.g., (8,36)]. It has been suggested, for example, that dopamine-sensitive pathways in mesolimbic and mesocortical regions of the brain may be involved. BZDs reduce dopamine turnover here, though this effect may be an indirect function of their action at GABAergic sites (36). CPZ, like other neuroleptics, is a dopamine antagonist. At low doses, neuroleptics can reduce anxiety in some types of patients (3); and a recent study of the effects of selected neuroleptics on rats in an open-field test of emotional reactivity has supported the hypothesis of DA involvement in anxiolysis (4).

If the critical event signalled by DZ in DZ→CPZ pairings is dopamine antagonism, then substituting other neuroleptics for CPZ should also enhance DZ's effectiveness in the plus-maze test. Thus far, no neuroleptic other than CPZ has been tested in this "conditional interaction" paradigm. The present experiments were designed to serve several purposes. The first was to replicate the DZ→CPZ interaction with several parametric variations. The second was to explore the generality of the phenomenon and shed some light on its determinants by substituting each of the following neuroleptics for CPZ: haloperidol (HAL), thioridazine (THI), and pimozide (PIM). Haloperidol was selected because of its known interaction with the GABA-benzodiazepine-chloride ionophore complex, its similarity to CPZ as indicated by a variety of behavioral measures, and its potency as a dopamine blocker (23). Pimozide, a neuroleptic of the diphenylbutylpiperidine class, is a relatively selective, highly potent antagonist at dopamine receptors. Thioridazine, a piperidinalykyl derivative, is often referred to as "atypical" because it produces few extrapyramidal effects due to its potent anticholinergic properties. Atypical neuroleptics like thioridazine appear to have selectively greater effects on mesolimbic as opposed to nigrostriatal dopamine systems (2,20).

GENERAL METHOD

Subjects

Long-Evans male rats (Charles River Canada, St. Constant, Quebec) were used as subjects. Unless otherwise specified, they ranged in weight from 325 to 450 g at the beginning of the experiments. All were housed in suspended steel and wire mesh cages in a room maintained at 22–24°C with a 10/14-h light/dark cycle. Purina Lab Chow and tap water were available ad lib (except as specified below). Within each experiment, groups of animals receiving different treatments were equated as closely as possible on the basis of their body weights.

Apparatus

Plus-maze. This maze was constructed of black-painted, urethane-coated wood. Of its four arms (50 × 10 cm), arranged in the form of a cross, two were bordered with 40 cm high walls ("enclosed" arms) and two were left exposed ("open" arms). The entire maze was elevated to a height of 82.0 cm. Activity in the maze was videotaped while an observer sat in an adjoining room from which a rat could be watched while it traversed the open arms. Shortly before placement in this maze, all animals were transferred from their home cages to an open field. This consisted of a glass and steel enclosure whose floor measured 61.5 × 72.0 cm.

Inclined plane. Muscle tone was assessed in the manner described by Taukulis and Brake (33). The test apparatus for this consisted of a rigid corkboard (45.5 × 60.5 cm) and a large protractor. The board was raised manually with its shorter edge resting against a brace affixed to the surface of a table. Its longer edge moved along the surface of the protractor against which the angle of incline was determined.

Drugs. Diazepam (Valium, Roche) and haloperidol (Haldol, McNeil Pharmaceutical) were obtained in injectable form (ampules containing 5.0 mg/ml drug in a liquid vehicle). Chlorpromazine, thioridazine, and pimozide (all obtained from Sigma Chemical Company) were dissolved in physiological saline (CPZ and THI) or 0.6% tartaric acid (PIM) to concentrations that permitted all injections to be a uniform 2.0 ml/kg. For chronic administration studies, oral haloperidol solution (Haldol, 2.0 mg/ml, McNeil Pharmaceutical) and haloperidol decanoate (Haldol LA, 100 mg/ml, McNeil Pharmaceutical) were employed. All injections were IP.

EXPERIMENTS 1 AND 2

The first experiment was designed to replicate the enhancement of DZ-stimulated plus-maze activity by DZ→CPZ pretreatment as described by Taukulis and Brake (33). While these investigators used a 10.0 mg/kg dose of CPZ, doses in Experiment 1 ranged from 2.5 to 10.0 mg/kg. Experiment 2 was an attempt to determine how many DZ→CPZ pairings are required before a conditional response to DZ can be detected.

Procedure

Experiment 1. Fifty rats were divided into groups of ten. Over a twenty-three-day period, the rats received 10 drug treatment sessions spaced either 48 or 72 h apart. On 10 other occasions (24 h after each drug treatment session), saline injections were administered to minimize any association between the injection procedure itself and the drug effects. On each drug treatment day, three of the groups received a DZ injection (2.0 mg/kg) followed 30 min later by an injection of CPZ at a dose of either 2.5, 5.0, or 10.0 mg/kg (DZ→CPZ groups). Of the remaining two groups, one received a backward pairing of CPZ (10.0 mg/kg) and DZ (Group CPZ→DZ), and one received DZ followed by an injection of saline (Group DZ→SAL). In the latter group, the saline injections were equivalent in volume to the

CPZ injections received by the other groups.

Three to four days after the tenth such treatment, each rat was tested in an identical fashion in the plus-maze. A single injection of DZ (2.0 mg/kg) was administered and the animal was returned to its home cage. Twenty-five minutes later, the animal was moved to the open field where it remained for 5 min. Immediately thereafter, it was placed into the center "start" area of the plus-maze and its activity was recorded for 5 min. This test was repeated 72 h later, except that saline was substituted for the DZ injection. After a further 72 h, the test was performed a third time, but once again with DZ (2.0 mg/kg).

Experiment 2. Twenty rats were assigned to two groups (n = 10). Group DZ→CPZ received repeated pairings of DZ (2.0 mg/kg) with CPZ (10.0 mg/kg), while Group DZ→SAL received a saline injection after DZ. For each group, each drug treatment day was also a test day. That is, 25 min after the DZ injection, each animal was placed into the open field for 5 min and then tested in the plus-maze as described above. Upon removal from the maze, the animal was immediately injected with CPZ or SAL as determined by its group assignment. This procedure was repeated on 10 occasions spaced 96 h apart.

Two test sessions followed this treatment procedure. In the first, performed at 96 h after the tenth treatment day, rats in both groups were injected with saline 30 min before placement into the open field (5 min) and subsequently the plus-maze (5 min). No injections were administered following removal from the maze; the animals were simply returned to their home cages. Ninety-six hours thereafter, this procedure was repeated, except that all rats received DZ (2.0 mg/kg) rather than saline.

RESULTS AND DISCUSSION

An alpha level of 0.05 was adopted for all statistical analyses.

Experiment 1

Statistical analyses (Dunnett tests in which each group was compared with the DZ→SAL control) were performed on the following measures: mean percent of time spent in the open arms of the plus-maze relative to total arm exploration time, mean percent of entries into open arms, and total number of entries into both arms. The last measure is an index of hyperactivity or sedation. The results are displayed in Table 1. In the first plus-maze test (with DZ), analyses of variance yielded $F(4,45) = 3.55$, $p<0.02$ for the percent time spent in the open arms and $F(4,45) = 3.40$, $p<0.02$ for the percent entries into the open arms. Subsequent comparisons revealed that the DZ→CPZ groups that had received 5.0 and 10 mg/kg of CPZ (but not 2.5 mg/kg) each differed from Group DZ→SAL in terms of the percent time and percent entries measures. The behavior of the backward control group, Group CPZ→DZ, was equivalent to that of the DZ→SAL group; these two groups did not differ on any variable. Overall, no group differences were found in terms of the total number of entries into both types of arms, $F(4,45) = 0.99$, $p>0.05$. The pattern for the second plus-maze test with DZ was the same except that the DZ→CPZ group that had received 2.5 mg/kg of CPZ now exhibited behavior similar to that of the other two DZ→CPZ groups.

In the plus-maze test that intervened between the two DZ tests, only saline was administered. On this occasion, although all groups exhibited much reduced percentages of time in and entries into the open arms, no group differences appeared.

The results of Experiment 1 showed once again that DZ→CPZ pairings can enhance DZ's effect on open-arm exploration in the plus-maze, a replication of the phenomenon reported by Taukulis and Brake (32,33). The experiment also demonstrated that this enhancement can be achieved with doses of CPZ as low as 2.5 mg/kg. It is not clear why, with the 2.5 mg/kg dose, the effect did not appear until the second test. Correlations between the measures taken in the plus-maze on two different test days have been high in this laboratory, although effects have been known to appear during the second exposure to the maze [e.g., (34)] for reasons that are not yet understood.

TABLE 1

EFFECTS OF DIAZEPAM OR SALINE ON ACTIVITY IN AN ELEVATED PLUS-MAZE AFTER REPEATED PRETREATMENT WITH DIAZEPAM AND CHLORPROMAZINE

Pretreatment	% Time on Open Arms	% of Open Arm Entries	Total Arm Entries
Diazepam Test 1			
DZ→SAL	26.8 (4.2)	30.4 (3.1)	18.6 (1.3)
DZ→CPZ (2.5)	33.2 (4.6)	36.7 (2.9)	16.0 (1.9)
DZ→CPZ (5.0)	47.1 (6.5)*	48.1 (5.5)*	19.1 (1.3)
DZ→CPZ (10.0)	50.8 (6.8)*	45.2 (5.2)*	16.0 (2.2)
CPZ→DZ	29.8 (6.0)	30.8 (4.6)	16.3 (1.2)
Saline Test			
DZ→SAL	14.6 (3.7)	21.5 (5.2)	8.7 (1.0)
DZ→CPZ (2.5)	20.8 (4.1)	28.5 (4.2)	10.3 (0.7)
DZ→CPZ (5.0)	16.8 (5.2)	24.9 (5.0)	10.0 (1.2)
DZ→CPZ (10.0)	19.4 (6.1)	27.2 (4.7)	9.2 (1.2)
CPZ→DZ	14.5 (3.3)	21.8 (5.1)	8.1 (0.8)
Diazepam Test 2			
DZ→SAL	21.4 (4.0)	23.3 (4.8)	8.6 (0.8)
DZ→CPZ (2.5)	56.5 (6.1)*	45.8 (4.2)*	11.3 (1.8)
DZ→CPZ (5.0)	52.1 (9.1)*	47.3 (9.7)*	8.1 (1.4)
DZ→CPZ (10.0)	52.0 (10.3)*	54.0 (4.6)*	9.4 (1.4)
CPZ→DZ	28.5 (6.2)	27.1 (4.7)	8.4 (0.7)

All rats were tested in the plus-maze 30 min after a 2.0 mg/kg injection of diazepam. Each animal had previously experienced ten treatment sessions with the drug combination indicated by the abbreviations in the group designations: SAL (saline); DZ (diazepam, 2.0 mg/kg); CPZ (chlorpromazine, doses as indicated in mg/kg for DZ→CPZ groups; 10.0 mg/kg for Group CPZ→DZ). Values represent means ± SEM (in parentheses), n = 10 rats per group.

*$p<0.05$, comparison with DZ→SAL control group, Dunnett test.

Experiment 2

The results of the repeated plus-maze trials of this experiment are shown in Figs. 1 to 3. Statistical analyses of group differences in activity patterns on each of the treatment/test days were performed using the Mann-Whitney U-test. Groups DZ→CPZ and DZ→SAL exhibited similar patterns of plus-maze activity in terms of percent time in and entries into the open arms for the first five trials, but diverged on Trial 6, a difference that persisted to Trial 10. On Trial 11 (saline test) the groups continued to differ, as they did on Trial 12 (DZ test). The groups also tended to differ in terms of the total entries into all arms. This difference disappeared during the saline test but reappeared in the DZ test.

Ten drug→drug pairings have been the norm for many experiments of this type, but this number was arbitrarily chosen. In fact, what little data on the subject exists has suggested that fewer trials may suffice. Revusky (26), in an experiment in which a conditioned taste aversion was the measure of a drug→drug (pentobarbital→lithium chloride) association, found that two pair-

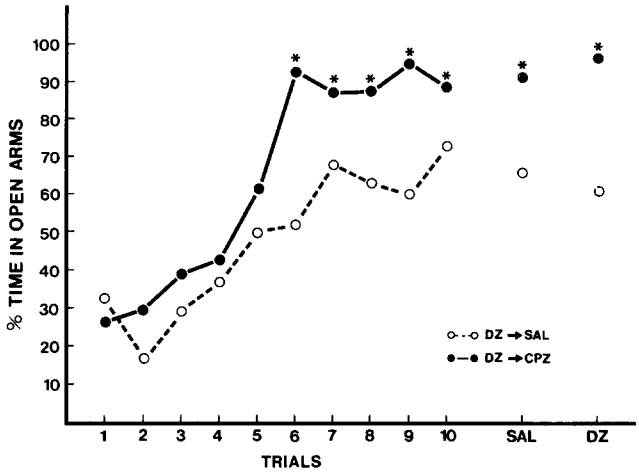

FIG. 1. Results of Experiment 2. All animals were injected with DZ (2.0 mg/kg) 30 min prior to placement in the plus-maze. Each point represents the mean percent of time spent by each group in the open arms of the maze relative to the total time spent in both arms. Asterisks indicate that the groups differed significantly at $p<0.05$ or less, one-tailed, Mann-Whitney U-test.

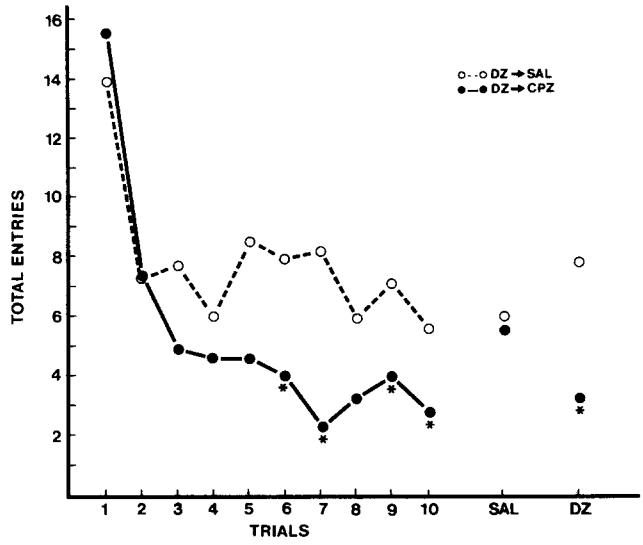

FIG. 3. Results of Experiment 2. Each point represents the mean total entries into both the open and enclosed arms of the plus-maze made by each group 30 min after a DZ injection (2.0 mg/kg). *$p<0.05$, Mann-Whitney U-test.

ings were sufficient for a detectable effect to appear. In Experiment 2 of the present series, the animals were repeatedly tested in the plus-maze 30 min after an injection of DZ. A reliable difference between the DZ→CPZ and DZ→SAL groups did not emerge until Trial 6, suggesting that at least five DZ→CPZ trials may be necessary for an enhancement of open-arm exploration to develop. We cannot be entirely certain of this because the experimental design ensured that, for Group DZ→CPZ, the plus-maze itself served as a cue for CPZ, since the test trial always preceded a CPZ injection. It could be argued, therefore, that the change in behavior was the product of a place-drug association. This interpretation is supported by the fact that, on Trial 11, when saline rather than DZ preceded the plus-maze test, the difference between groups persisted (see Figs. 1 and 2), suggesting that the animals were not responding differently to the DZ but rather to the maze cues. However, a comparison of the total entries into both types of arms made by the two groups (see Fig. 3) suggests that the groups were, in fact, reacting in different ways to the DZ. This measure, which served as an index of general activity, showed that, across Trials 3–10, Group DZ→CPZ made fewer entries into the arms of the maze, a difference that disappeared when they were tested with saline (Trial 11) and reappeared when DZ once again preceded placement into the plus-maze (Trial 12). This suggests that DZ may have been serving a cue function which was separate from that served by the maze.

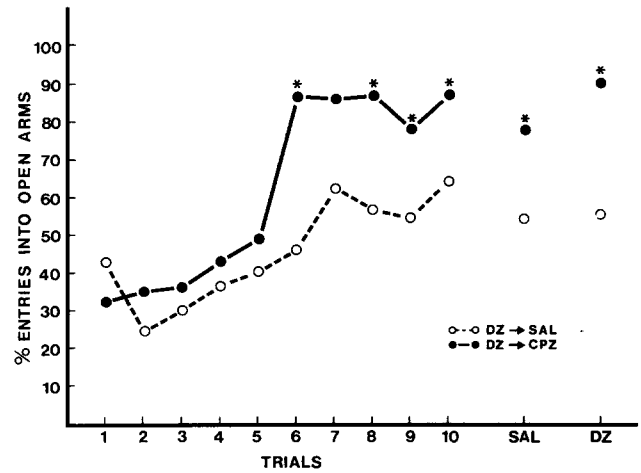

FIG. 2. Results of Experiment 2. Each point represents the mean percent of entries into the open arms of the plus-maze relative to the total entries into both arms 30 min after an injection of DZ (2.0 mg/kg). *$p<0.05$, one-tailed, Mann-Whitney U-test.

Although we were aware that the plus-maze cues were reliable predictors of CPZ and were therefore a confounding variable (25), we assumed that conditioning to these cues would be weak at best, so that the two groups would not differ on Trial 11 when the DZ cue was not present. Our assumption may have been incorrect, although other explanations for the saline test results are also tenable. For example, both groups showed a gradually increasing inclination to explore the open arms, an effect that became greater for Group DZ→CPZ than for Group DZ→SAL. Responses to an environment that develop while an animal is under the influence of DZ can persist once the drug is no longer administered (35). This fact alone could account for the Trial 11 difference without the further complexity of a maze→CPZ association. Nevertheless, a more precise analysis of the relationship between number of pairings and strength of conditioning awaits a more complex parametric experiment in which different groups are given varying numbers of DZ→CPZ pairings and are tested in the plus-maze thereafter.

Experiment 3

In this study, haloperidol, thioridazine, and pimozide were substituted for CPZ in an experimental design resembling that of Experiment 1. A major difference here was that a test of the animals' muscle relaxation response to DZ was performed after all plus-maze tests were completed.

Procedure

Eighty rats were assigned to eight groups (n = 10). Except that different neuroleptics were administered, the treatment and test procedures were exactly like those used in the first experiment described in the previous section. All animals received ten drug treatment sessions and were then tested on three occasions in the plus-maze, twice with diazepam and once with saline. During the 10-session treatment period, three of the eight groups received DZ (2.0 mg/kg) followed 30 min later by an IP injection of haloperidol (3.0 mg/kg), thioridazine (10.0 mg/kg) or pimozide (2.0 mg/kg). For another three groups, these drugs preceded the DZ injection. And finally, one group received a saline injection followed by haloperidol (3.0 mg/kg), and one group received a DZ injection followed by saline.

Beginning 72 h after the second DZ test in the plus-maze, two more drug treatment sessions and saline→saline injection sessions were carried out. The drug treatment sessions were spaced 48 h apart. Approximately 48 h after the second of these sessions, all animals were subjected to an inclined plane test to determine the degree to which DZ would elicit muscle relaxation. Thirty minutes after an IP injection of DZ (2.5 mg/kg), they were placed on the inclined plane with their bodies oriented towards the top edge of the plane. The angle of incline was increased at a rate of approximately 2 degrees per second until the animal began to slide off the board. Four such trials were performed in each session, and a mean was calculated from these. If an animal's body orientation changed during a trial, it was repeated until four successful trials were completed.

RESULTS AND DISCUSSION

Plus-Maze Tests

The percent of time on/entries into open arms for each of the eight groups on the first DZ test day of Experiment 3 is shown in Figs. 4 and 5. Each group was compared with Group DZ→SAL (Dunnett test). Only Group DZ→HAL exhibited significantly more open-arm activity; Groups HAL→DZ, SAL→HAL, DZ→THI, THI→DZ, DZ→PIM, PIM→DZ, and SAL→SAL yielded patterns equivalent to Group DZ→SAL. Similar analyses of total arm entries revealed no significant differences among the various groups. Entries into both types of arms for each group were as follows, with standard errors in parentheses: DZ→SAL, 15.1 (1.3); DZ→HAL, 17.3 (1.1); HAL→DZ 15.7 (1.1); SAL→HAL, 16.2 (1.4); DZ→THI, 12.7 (0.9); THI→DZ, 13.6 (1.7); DZ→PIM, 15.0 (0.9); PIM→DZ, 14.4 (2.2). This pattern of results was the same on the second DZ test day (not shown). And finally, analyses of plus-maze activity on the intervening saline test day revealed no differences among groups on any of the three measures of activity.

At the doses of HAL, PIM, and THI selected for use in this experiment, only HAL was effective in yielding a conditional interaction. These three neuroleptics, while different in several ways, all share the characteristic that they are dopamine antagonists. In terms of their ability to displace ^{3}H-spiroperidol at dopamine receptors in the caudate nucleus (23) and the corpus striatum (5), PIM is the most potent and HAL only a little less so. THI is considerably weaker and is roughly comparable to CPZ. The doses of the drugs that we chose to use reflected these relationships. It would seem from these results that dopamine blockade by itself is insufficient to ensure a shift in DZ response, although differential interaction with different dopamine receptor subtypes (D1 and D2) may be a critical factor.

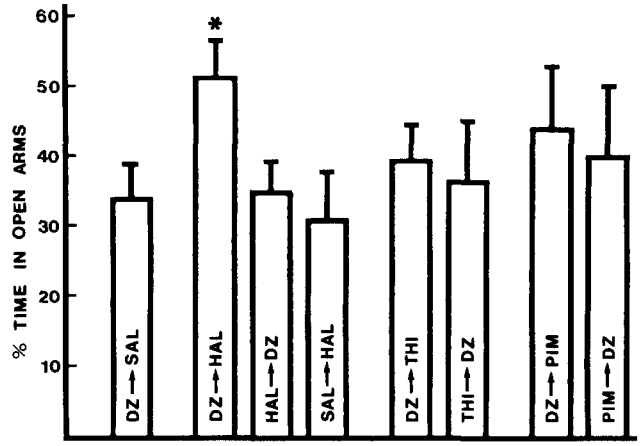

FIG. 4. Results of Experiment 3. All animals were tested in the plus-maze 30 min after an injection of DZ (2.0 mg/kg). The mean percent of time spent in the open arms of the maze relative to the total time spent in both arms is shown for each of the eight groups. *Statistically significant when compared with the DZ→SAL control using the Dunnett test, with alpha set at $p<0.05$.

Inclined Plane Test

The mean angle of inclined plane tolerated by each group (Fig. 6) was compared with the angle obtained for Group DZ→SAL (Dunnett test). This revealed that Group DZ→HAL clearly differed from DZ→SAL, and the means obtained for Groups HAL→DZ and SAL→HAL approached significance. No other differences were detected.

Taukulis and Brake (33) found that, while DZ→CPZ pairings enhanced the anxiolytic effect of DZ, its myorelaxant prop-

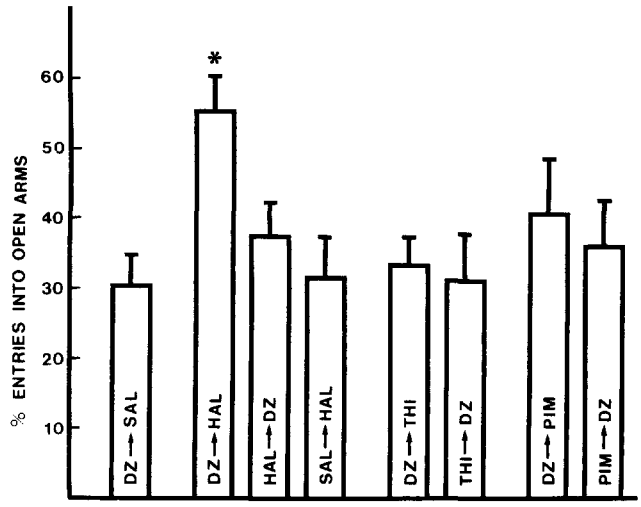

FIG. 5. Results of Experiment 3. The mean number of entries into the open arms of the plus-maze relative to the total number of arm entries 30 min after a DZ injection (2.0 mg/kg) is shown for each group. *$p<0.05$, Dunnett test.

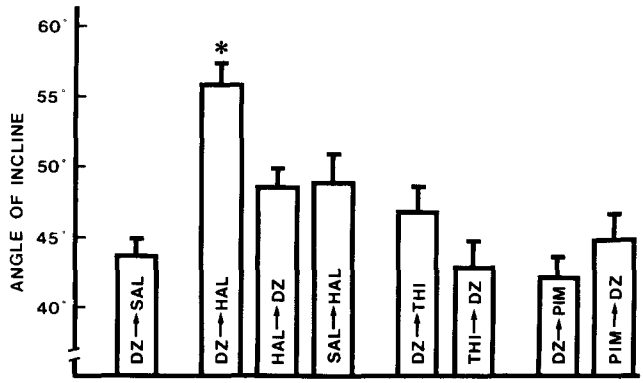

FIG. 6. Results of Experiment 3: Inclined plane test. The mean angle of incline tolerated by each group 30 min after an injection of DZ (2.5 mg/kg). *$p<0.05$, Dunnett test.

erty was diminished. In the inclined plane test performed in that study, their CPZ→DZ control group yielded a mean tolerated angle of incline (50.0 degrees) that was intermediate between that of their DZ→CPZ group (55.0 degrees) and their DZ alone group (44.6 degrees). In the present study, a similar pattern of results emerged. The fact that Groups SAL→HAL and HAL→DZ exhibited means that were intermediate relative to those of Group DZ→HAL and DZ→SAL suggested to us that perhaps mere exposure to HAL alone might affect the animal's myorelaxation response to DZ. The conditional response to DZ resulting from DZ→CPZ pairings may be superimposed upon the effect of HAL exposure, resulting in an even greater diminution of DZ-induced myorelaxation.

EXPERIMENTS 4 AND 5

Experiments 4 and 5 were prompted by the inclined plane results of Experiment 3. It is known that chronic and subchronic administration of haloperidol will induce a supersensitivity of GABAeric mechanisms in certain areas of the brain involved in motor control. Affected GABAergic pathways have been found in the nigrostriatal system (10, 13, 18), the basal ganglia (28) and in striatopallidal efferents (12). Because benzodiazepine activity is intimately linked to GABAergic function, changes to GABA systems may have consequences for the myorelaxation effect of a benzodiazepine like DZ.

Oral haloperidol and haloperidol decanoate (a depot version of the drug that is injected IM) were used here in order that the temporal pattern of drug absorption, distribution, and excretion might more closely resemble a typical pattern of clinical use of this drug.

Procedure

Experiment 4: Oral haloperidol. Twenty rats (240–255 g) were familiarized with a feeding regimen in which they were deprived of free access to food but were weighed and fed on a daily basis. Each day, they were given approximately 9.0 g of wet mash (powdered Purina rat chow mixed with an equal part of water) in a glass Stender dish. Immediately after they had finished eating this, they were provided with approximately 10.0 g of dry chow in pellet form. This was gradually increased to about 20.0 g daily over a 5-day period. This routine ensured that they would gain weight at a steady rate but would eat the wet mash readily when it was offered to them. At no time did a rat not finish the mash within minutes after it was placed in its cage.

On Day 6 after this feeding regimen was begun, the animals were assigned to two groups (n = 10). For one of the groups, 2.0 mg/kg of haloperidol solution for oral administration was mixed with the wet mash. For the other group, an equivalent volume (1.0 ml/kg) of distilled water was added to the food. This procedure was carried out once daily on each of Days 6–26. On Day 27, both groups were given wet mash, but HAL was withheld from the drug treatment group. On Day 28, all rats were tested in the plus-maze following an injection of DZ at 2.0 mg/kg. The test procedure was the same as that described in Experiment 1. Later that same day, wet mash mixed with HAL or saline was provided to the two groups as before. Days 29–31 saw a continuation of the procedure of Days 6–26. On Day 32, the drug was again withheld from the drug treatment group, and on Day 33 all animals were tested on the inclined plane 30 min after an injection of DZ (2.0 mg/kg). The same procedure was employed as that described in Experiment 3.

In order to be certain that the two groups did not differ in terms of their mean tolerated angle purely as a function of their exposure or nonexposure to HAL, the procedure of Days 29–33 was repeated on days 34–38, with the change that an injection of physiological saline (2.0 ml/kg) was administered 30 min prior to the inclined plane test. In contrast with the previous DZ test, the rats were far more active and therefore less likely to remain still on the test plane. More trials were therefore required in order to obtain four "good" trials (i.e., trials during which a rat did not turn sideways or grip the top of the plane) from which a mean could be derived for each animal. A record was kept of the number of "false" trials per subject.

Experiment 5: Haloperidol decanoate. Twenty-four animals (163–293 g) were assigned to two groups (n = 12). Rats in one group were given a 60.0 mg/kg intramuscular injection of haloperidol decanoate on four occasions spaced thirty days apart. The rats in the other group were injected with an equivalent volume of propylene glycol. On the thirtieth and thirty-second days after the last injection, the rats were tested for muscle tone using the inclined plane procedure. Six animals from each group received a saline injection prior to the first test and a DZ injection (2.5 mg/kg) prior to the second; and for the remaining subjects the order of injections was reversed. Fewer problems of the type noted in Experiment 4 were encountered in this experiment when the animals had received saline. In comparison with those of Experiment 4, the animals in the present experiment were far less active and tended to remain in position on the plane more readily. The reason is probably that they were much larger by the time they were tested (mean weight = 522 g, s.d. = 63).

For the next 28 days, no further injections were administered. On the twenty-eighth day after the second inclined plane test, the procedure was repeated; but this time the subjects were tested only once, with 2.5 mg/kg of DZ.

RESULTS AND DISCUSSION

Experiment 4

As the left portion of Fig. 7 shows, the rats that had received haloperidol in daily oral doses exhibited significantly less muscle relaxation in response to DZ relative to the control group that had not been given the neuroleptic. That is, the average angle of incline that the experimental rats were able to tolerate before sliding off the inclined plane was greater than that of the control group, $t(18) = 2.20$. The two groups did not differ on the

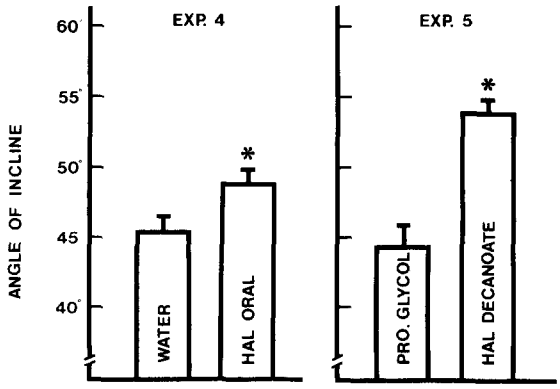

FIG. 7. Results of Experiments 4 and 5: Inclined plane test. Left: At 30 min after a DZ injection (2.0 mg/kg), mean angle of incline tolerated by the two groups fed wet mash mixed with either water alone or oral haloperidol solution. $*p<0.05$, t-test. Right: At 30 min after a DZ injection (2.5 mg/kg), mean angle of incline tolerated by rats injected with either propylene glycol or haloperidol decanoate. $*p<0.001$, t-test.

saline test: the obtained means were 54.6(±1.9) degrees for the water group and 53.7(±1.1) degrees for the HAL group, $t(18)<1$.

An analysis of plus-maze activity (t-tests) revealed that the groups did not differ on any of the three measures taken (see Table 2).

Experiment 5

The rats that had been given haloperidol decanoate responded to DZ with a weaker muscle relaxation response relative to controls that had not ingested the neuroleptic, $t(22)=5.83$. This difference is illustrated in the right portion of Fig. 7, which shows the mean angle of incline tolerated by each group. No significant difference was obtained when the test injection was saline: the means were 58.5(±2.0) degrees for the propylene glycol control group and 60.3(±1.3) degrees for the HAL group, $t(22)<1$. In the second DZ test, performed 28 days after the animals' second experience with the inclined plane, the HAL group no longer exhibited a diminished muscle response, $t(22)<1$.

As anticipated, these experiments showed that chronic exposure to HAL will decrease the myorelaxation effect of DZ. This phenomenon was stronger in Experiment 5, perhaps because the duration of exposure was longer or perhaps because the depot version of HAL, which releases the drug at a continuous, steady rate, is more effective than the oral form. Future studies will examine parameters like the relationship between duration of exposure and the intensity of the effect, as well as the length of time that the effect can be expected to persist. Some progress towards this end was made in Experiment 5, where it was shown that a difference between the drug exposure and control groups was no longer detectable at 60 days after the last HAL injection.

TABLE 2

EFFECTS OF DIAZEPAM ON ACTIVITY IN AN ELEVATED PLUS-MAZE AFTER CHRONIC PRETREATMENT WITH ORAL HALOPERIDOL OR WATER

Pretreatment	% Time on Open Arms	% of Open Arm Entries	Total Arm Entries
HAL	33.8 (6.9)	35.5 (5.1)	14.4 (1.8)
Water	35.2 (6.7)	41.1 (6.6)	17.6 (1.1)

GENERAL DISCUSSION

Experiments 1 and 2 replicated one of the findings of Taukulis and Brake (33): that DZ→CPZ pairings will conditionally enhance open-arm activity elicited by DZ in a plus-maze animal model of anxiety. In Experiment 3 it was found that HAL, but not THI or PIM, can effect such a conditional change in the animals' response to DZ. Admittedly, any conclusions drawn from the failure to detect an effect with the latter two drugs must be considered tentative due to the fact that only one dose of each was employed. Additionally, the doses of HAL (3.0 mg/kg) and PIM (2.0 mg/kg) were quite high, intentionally selected so as to maximize the probability of an effect. In rats, behaviourally significant actions can often be obtained with doses substantially less than 1.0 mg/kg of either of these neuroleptics, and such lower doses should be tested in the present design.

Assuming for the moment that the failure to obtain changes in DZ efficacy when this drug was paired with either THI or PIM is indicative of a general ineffectiveness of these two neuroleptics regardless of dose, one possible explanation for the differences among the neuroleptics tested may be found in new evidence that, while all of these substances are dopamine antagonists, they vary widely in the more subtle details of their activity on dopaminergic systems. For example, Tecott, Kwong, Uhr and Peroutka (37) found that chronic haloperidol significantly increases dopamine D2 receptor binding of ^{3}H-spiperone in rat striatum, while pimozide decreases it. Dopamine and GABA systems interact in intricate ways (19), and differences in neuroleptic activity at dopamine receptor sites may translate into differences in how GABA systems (and ultimately, perhaps, benzodiazepines) act. Neuroleptic-induced changes in GABAergic activity within the striatonigral, mesocortical, and mesolimbic areas are believed to be secondary to neuroleptic modification of dopamine function within these areas (10, 29, 30). Therefore, selectivity of action within these areas by different neuroleptics may account for differences in their influence on the various properties of benzodiazepines.

Lane and Blaha (20) have shown that THI increases the firing rate of DA neurons in the ventral tegmental (A10) area of the mesolimbic CNS but not within the substantia nigra (A9). HAL, in contrast, increases activity of DA neurons in both regions. On the basis of this finding, it might have been predicted that THI would not alter DZ's effect on the inclined plane test, assuming that the nigrostriatal system is implicated in DZ's muscular effect. The drug's failure to affect DZ's impact on plus-maze activity suggests (among other things) that 1) the dopamine system within the mesolimbic cortex is not directly involved in this effect, or 2) the effect is primarily mediated by other neurotransmitter systems (noradrenergic or serotonergic, perhaps) which are differentially affected by HAL and THI. Evidence in support of both noradrenergic and serotonergic substrates for anxiety and its alleviation can be found [e.g., (8, 16, 24, 31, 40)] and are worthy of consideration in any analysis of enhanced DZ anxiolysis in animal models like the plus-maze.

With regard to the conditional diminution of DZ-induced muscle relaxation found in Group DZ→HAL of Experiment 3 and also reported by Taukulis and Brake (33), it is possible that this effect does not reflect a direct change in DZ's activity at its own receptor site, nor its modulating effect on GABAergic or dopaminergic transmission. Rather, the drug may simply serve a cue function, eliciting a conditional response that is competitive with its own effect. That is, it may elicit a conditional response in the nigrostriatal system (or another, as yet unspecified, re-

gion) which manifests itself as increased muscle tension. This effect may compete with the DZ-induced decrease, and the observed outcome may reflect an algebraic summation of these two competing effects. This analysis implies that any cue drug that predicts HAL or CPZ activity in a central muscle control system may elicit enhanced muscle tension. If this drug has no muscular effects of its own, then rats primed with this cue before inclined plane testing may actually tolerate a greater than normal angle of incline. We are currently testing this possibility.

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