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Effects of Four Antipsychotics on Punished Responding in Rats

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WILEY, J. L., A. D. COMPTON AND J. H. PORTER. *Effects of four antipsychotics on punished responding in rats.* PHARMACOL BIOCHEM BEHAV 45(2) 263–267, 1993.—In Experiment 1, the benzodiazepine chlordiazepoxide (CDP), two typical antipsychotics, haloperidol (HAL) and chlorpromazine (CPZ), and the atypical antipsychotic clozapine (CLZ) were evaluated for antipunishment effects in rats in a modified Geller–Seifter conflict procedure [MULT fixed interval (FI) 60-s, fixed ratio (FR) 1 (food + shock)]. In Experiment 2, CDP and thioridazine (THD) were similarly tested. CLZ (2.5 and 5.0 mg/kg), but not HAL, CPZ, or THD, selectively increased punished responding, although the magnitude of effect was smaller than that observed for CDP. Possible serotonergic mechanisms for CLZ's action in this model and the possible importance of serotonergic activity for the development of other atypical antipsychotic drugs are discussed.

Clozapine Haloperidol Chlorpromazine Thioridazine Chlordiazepoxide Neuroleptics Antipsychotics Antipunishment Serotonin

THE recent introduction of the antipsychotic clozapine for clinical use has furthered interest in the search for other atypical antipsychotics that do not share the extrapyramidal motor side effects seen with typical antipsychotic drugs [e.g., chlorpromazine; see (5,14)]. Antipsychotic drugs may be differentiated from each other based upon biochemical (e.g., chemical structure and binding profile) and/or behavioral properties. While typical antipsychotics may differ in binding profiles, they tend to have similar effects in traditional behavioral screening procedures for antipsychotic drugs (13), whereas atypical antipsychotics are distinguished by their lack of effect in these screening procedures (19,26,31). Because a distinction between typical and atypical antipsychotics can be shown with differences in behavioral effects (8,14,33), it is of interest to determine whether or not these drugs can be differentiated in other behavioral paradigms not specifically designed to detect neuroleptic action.

One aspect of a drug's behavioral profile is its effect on punished responding in conflict procedures (7,10). Almost 20 years ago, Cook and Davidson (10) suggested that anticonflict procedures may be " . . . useful in differentiating effects even among compounds classified as neuroleptics" (p. 343). Yet, antipsychotics have not been tested systematically in the context of a single study for anticonflict effects, and results from scattered reports of previous studies that used an antipsychotic as a control are inconsistent (16,18,23). The purpose of Experiment 1 in the present study was to investigate whether the effects of antipsychotics on punished behavior would be correlated with differences in their binding profiles, chemical structures, or other behavioral properties. Two typical antipsychotics, haloperidol (HAL) and chlorpromazine (CPZ), prototypes of the butyrophenone and phenothiazine classes, respectively, and an atypical antipsychotic, clozapine (CLZ), a dibenzazepine, were tested in a modified Geller–Seifter conflict procedure that is commonly used for screening drugs for anxiolytic properties (10). CLZ differs from typical antipsychotics such as HAL and CPZ in chemical structure as well as in its effects in many of the traditional behavioral screening procedures (1); however, its binding profile is similar to that of CPZ (13). HAL and CPZ produce similar behavioral effects but differ in both chemical structure and binding profile (13).

Based upon the results of Experiment 1, a second experiment was conducted with a second atypical antipsychotic, thioridazine (THD). THD is chemically related to the typical antipsychotic CPZ and shares its binding profile (13); however, in traditional screening procedures THD produces behavioral effects similar to those seen with CLZ (19,26,31). Clinically, THD also differs from most typical antipsychotics in that it does not cause motor side effects at doses that ameliorate psychosis (29). Thus, THD shares commonalities with both typical and atypical antipsychotics. For comparison, the benzodiazepine anxiolytic chlordiazepoxide (CDP) was also tested in each experiment. In this type of procedure, CDP

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usually produces increases in punished responding without increasing unpunished responding (7).

METHOD

Subjects

Adult, male Sprague-Dawley rats (260–320 g) obtained from Dominion Breeders (Dublin, VA) were housed individually in an animal colony room maintained at 22°C with a 6:00 a.m. light/6:00 p.m. dark cycle. Rats were maintained at 80% of their free-feeding body weights by adjusting their daily ration of food. Water was available ad lib in the home cages throughout the study. Animals in both experiments had prior training and testing with chlordiazepoxide and other drugs on the schedule described in the Procedure section. Animals in Experiment 1 were 16 months old at the beginning of the experiment; those in Experiment 2 were 11 months old.

Apparatus

Four standard operant chambers (Model SEC-002, BRS/LVE, Laurel, MD) were housed in sound-attenuating cubicles. A lever was mounted in these chambers on the left side of the

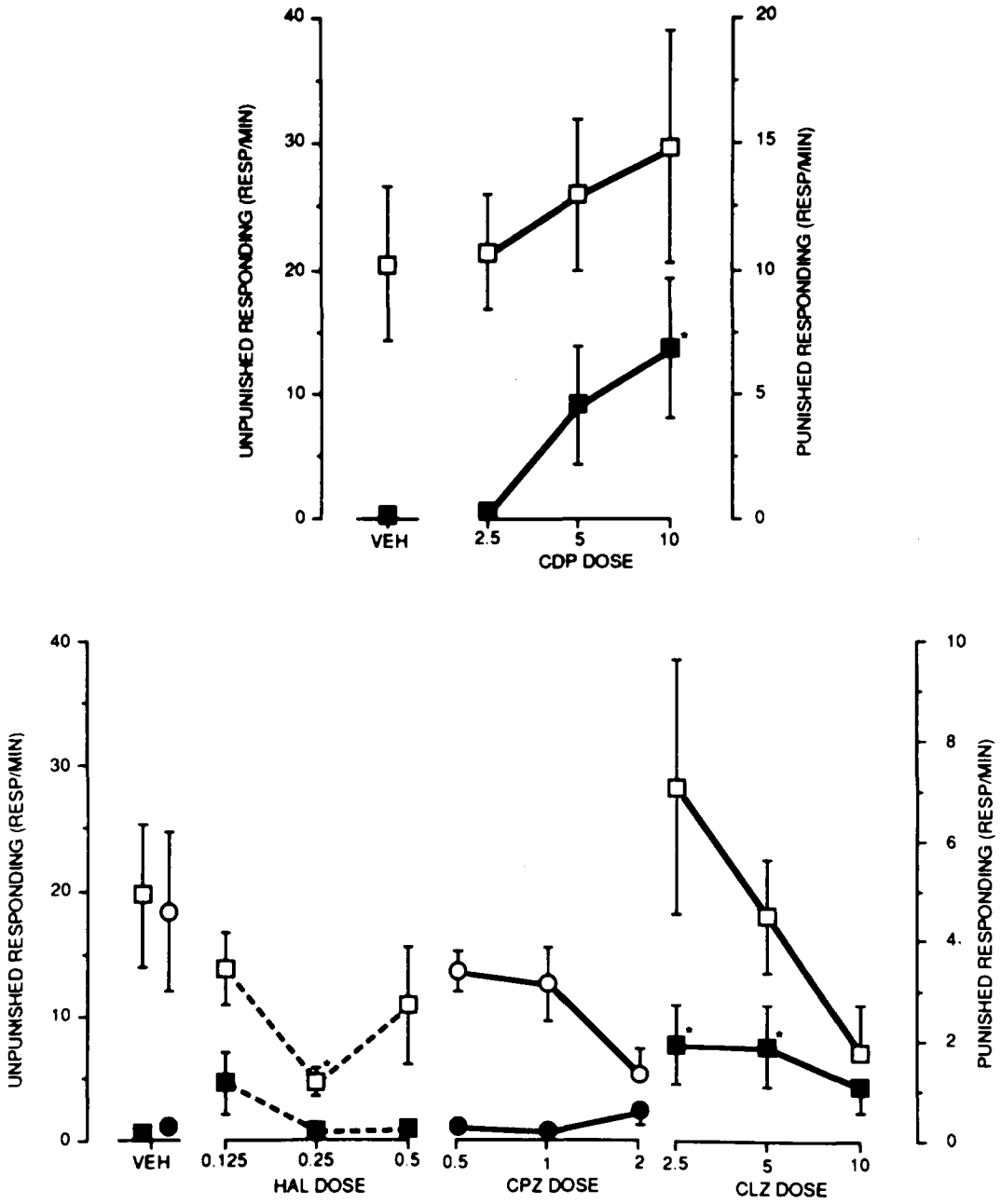

FIG. 1. Mean response rates during CDP (n = 8) tests (upper panel) and during CLZ (n = 8), HAL (n = 8), and CPZ (n = 7) tests (lower panel). Open symbols indicate unpunished responding. Filled symbols indicate punished responding. Asterisks indicate significant differences ($p < 0.05$) from the corresponding vehicle tests.

intelligence panel, 5 cm above the floor. A pellet dispenser (PDC/PPD series, BRS/LVE) delivered 45-mg BIO SERV (Frenchtown, NJ) food pellets to a food cup located in the center of the panel, 1.6 cm above the grid floor. Fan motors and white noise provided masking noise for each chamber. A 7-W houselight was located 24 cm above the floor in the center of the intelligence panel. Direct constant-current shock was provided by a shock source (Model 26170, Stoelting Co., Chicago, IL) and passed through a shock scrambler (Model 26171, Stoelting) before being sent to the grid floor of the operant chamber. Solid-state programming equipment that controlled the experimental contingencies and recorded lever presses was located in an adjacent room.

Drugs

CLZ and HAL were prepared in a vehicle solution of 85% lactic acid (5–10 drops) and distilled water. CDP HCl, CPZ HCl, and THD HCl were dissolved in distilled water. All injections were given IP at a volume of 1 ml/kg. Doses for each drug were administered in a counterbalanced order. CLZ, CPZ, and THD were injected 1 h presession; HAL, 45 min presession; and CDP, 30 min presession (injection times were based upon previous research in this laboratory). Doses of CDP, CPZ, and THD refer to the salts. Doses of CLZ and HAL refer to the free bases.

General Procedure

Training procedures similar to those previously described (24) were used in each experiment. Drug-experienced male Sprague-Dawley rats, maintained at 80% free-feeding body weights, were trained to lever press under a multiple fixed-interval (FI) 60-s (food reward only), fixed ratio (FR) 1 (food + shock) reinforcement schedule in standard operant chambers. Three 7-min FI components alternated with 3-min FR components for a total session length of 30 min. The shock level was titrated for each rat until lever pressing during the FR components was suppressed to between 1 and 10 responses for the entire session. Shock levels ranged from 0.3–1.4 mA; duration was 0.5 s. Drug testing was conducted twice weekly with a minimum 2-day nondrug period between tests. To be tested, a rat was required to earn more than 14 (of 21 possible) reinforcers during the FI components and between 1 and 10 reinforcers during the FR components on the preceding control day.

Experiment 1. CDP (2.5–10 mg/kg) and its vehicle were tested first in Experiment 1. CDP served as a standard anxiolytic compound to which the other drugs were compared. Only CDP-sensitive animals that showed an increase in punished responding without a concomitant increase in unpunished responding following administration of any dose of CDP were included in further tests. After testing with CDP was completed, drug tests were conducted with CLZ (2.5–10 mg/kg), HAL (0.125–0.5 mg/kg), and then CPZ (0.5–2 mg/kg). One rat died during the course of the experiment and was not tested with CPZ. The vehicle solution for CLZ and HAL was identical; thus, a single vehicle test was performed for the dose–response determinations for these drugs. A separate vehicle test was conducted for the CPZ curve.

Experiment 2. As in Experiment 1, chlordiazepoxide (1.25–10 mg/kg) was used as a standard and was tested first. Animals that showed a CDP-induced selective increase in punished responding were included in tests with THD (2.5–10 mg/kg). Separate vehicle tests were conducted for CDP and THD during each dose–effect determination.

Data Analysis

Data for Experiments 1 and 2 were collected and analyzed separately according to the following procedure. The number of responses was recorded separately for punished and unpunished components during each test session. For the purposes of data analysis, these data were converted to response rates (resp/min). Two repeated-measures analyses of variance (ANOVAs) were conducted for each drug, comparing mean response rates across doses for punished and unpunished components, respectively. Duncan posthoc tests ($\alpha = 0.05$) were used to specify differences revealed by significant ANOVAs (3).

RESULTS

Experiment 1

CDP and CLZ produced dose-dependent, selective increases in punished responding [CDP, $F(3, 21) = 4.06$, $p < 0.02$; CLZ, $F(3, 21) = 3.65$, $p < 0.03$]. The highest dose of CDP (10 mg/kg) (Fig. 1, upper panel) and the two lower doses of CLZ (2.5 and 5 mg/kg) (Fig. 1, lower panel) significantly increased punished responding ($p < 0.05$) without significantly affecting unpunished responding [CDP, $F(3, 21) = 0.74$, $p > 0.05$; CLZ, $F(3, 21) = 0.08$, $p > 0.05$]. Neither HAL nor CPZ had any significant effects on punished responding [HAL, $F(3, 21) = 2.42$, $p > 0.05$; CPZ, $F(3, 18) = 1.64$, $p > 0.05$]. While CPZ did not significantly affect unpunished responding, $F(3, 18) = 2.40$, $p > 0.05$, HAL produced a significant suppression of unpunished responding, $F(3, 21) = 4.28$, $p < 0.02$. Posthoc analyses revealed that the 0.25-mg/kg dose of HAL significantly decreased ($p < 0.05$) unpunished responding (Fig. 1, lower panel).

Experiment 2

CDP produced dose-dependent changes in both punished and unpunished responding, $F(4, 24) = 3.13$, $p < 0.04$; $F(4, 24) = 4.71$, $p < 0.007$, respectively. The 10-mg/kg dose of CDP produced a significant increase in punished responding ($p < 0.05$) and significantly ($p < 0.05$) decreased unpunished responding (Fig 2, upper panel). THD did not produce any significant changes in punished responding at any dose, $F(3, 18) = 0.17$, $p > 0.05$; however, the highest dose of THD (10 mg/kg) produced a significant decrease ($p < 0.05$) in unpunished responding, $F(3, 18) = 6.31$, $p < 0.005$ (Fig. 2, lower panel).

DISCUSSION

Clozapine produced a dose-dependent selective increase in punished responding. The magnitude of this antipunishment effect was considerably smaller for CLZ than for CDP. These results are consistent with previous reports that CLZ produces moderate increases in punished responding in conflict procedures with rats, pigeons, and squirrel monkeys (21,27,28). Interestingly, in the present study low doses of CLZ produced antipunishment effects whereas a higher dose was ineffective. Combined with early clinical reports that low doses of antipsychotics of the butyrophenone (9) and phenothiazine (25) classes produce anxiolytic effects in humans, these findings suggest that low doses of clozapine may produce similar anti-anxiety effects, although its potential for toxic effects on bone marrow would require periodic blood testing (29).

Although the mechanism for CLZ's antipunishment effects cannot be determined based upon the results of the present study, it is possible that CLZ's strong antiserotonergic proper-

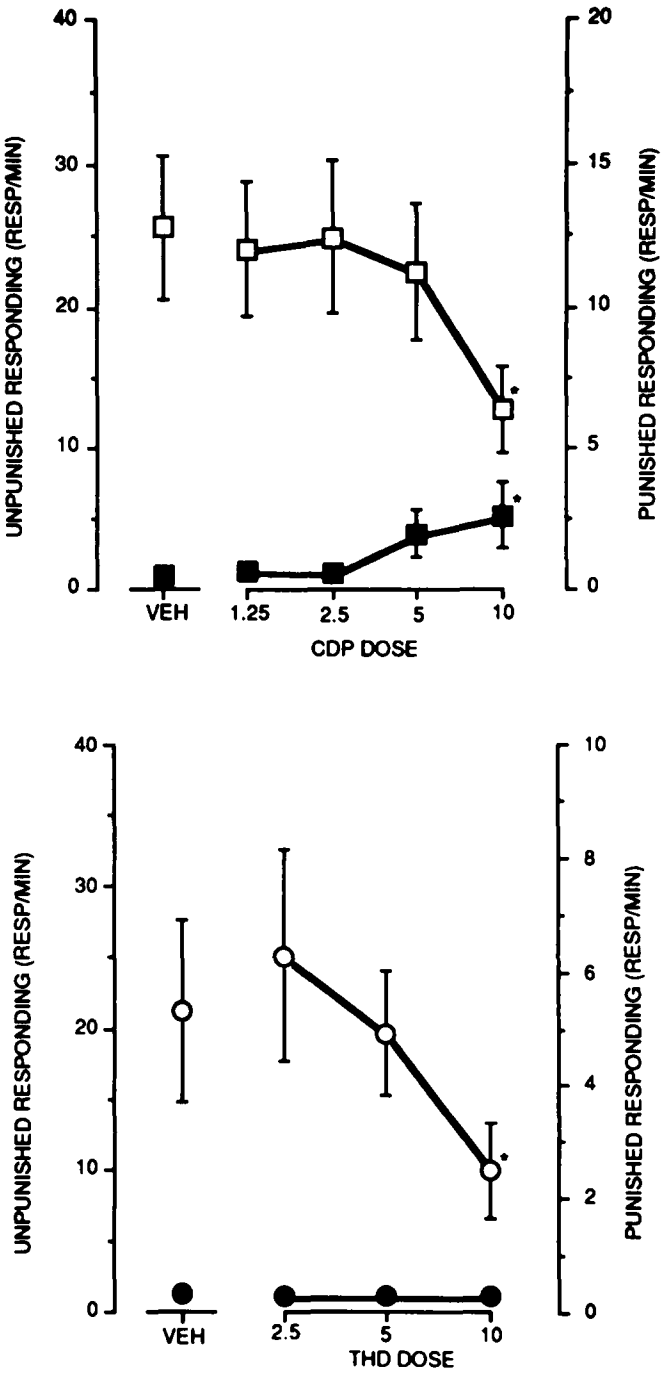

FIG. 2. Mean response rates for CDP (n = 7) tests (upper panel) and during THD (n = 7) tests (lower panel). Open symbols indicate unpunished responding. Filled symbols indicate punished responding. Asterisks indicate significant differences ($p < 0.05$) from the corresponding vehicle tests.

ties (13) may play a role in mediating these effects. Previous research has suggested that antagonism at serotonin neuroreceptors is a potential mediator of the antipunishment effects of other drugs (11,21,30). Several findings offer support for this interpretation of CLZ's effects. First, other drugs acting on serotonin neurons have been shown to have antipunishment effects (11,17,20,30); however, it should be noted that CPZ and THD also bind to serotonin receptors (13) but were inactive in this procedure. More convincingly, within the dose range that produced antipunishment effects in the present study CLZ increases the levels of the serotonin metabolite 5-hydroxyindoleacetic acid (5-HIAA) without affecting the levels of metabolites of dopamine or norepinephrine (21).

In contrast to CLZ's effects, HAL and CPZ, the two typical antipsychotics tested in the present study, did not affect punished responding. CPZ has been listed as inactive in several conflict models in a number of previous reports (7,16,18,22). Previous studies with HAL have shown less consistent results. Similar to the results of the present study, most researchers have found that HAL does not have anticonflict effects (7,18,21); however, one study (23) reported that HAL produced a small increase in punished responding over a narrow dose range in rats. The low doses of HAL used by Pich and Samanin (23) did not overlap with the doses used in the present study. These factors (small magnitude and narrow dose range) may limit the generality of these findings. It seems likely that HAL produces anticonflict effects only in restricted circumstances and that, in most procedures, HAL's effect on punished responding will be similar to that observed in the present study and in most previous reports (7,18,21). This suggests that, under most conditions, HAL's effects on punished responding resemble those of CPZ rather than those of CLZ.

Thioridazine also did not increase punished responding in the present study. This finding is consistent with a previous report (18); thus, in conflict procedures THD produces behavioral effects that are similar to those seen with the typical antipsychotics, CPZ and HAL, rather than to those produced by the atypical antipsychotic CLZ. Compton and Porter (6) found that the effects of THD also differed from those of CLZ in a tolerance study with a multiple operant schedule of food reinforcement in rats. In this study, repeated dosing with THD produced a pattern of behavioral disruption like that of the typical antipsychotic pimozide and unlike that of CLZ (14). In addition, the results of drug discrimination studies show that THD does not substitute fully for CLZ in rats trained to discriminate CLZ from vehicle (2,32) or in pigeons (12), suggesting that THD and CLZ do not share discriminative stimulus properties. Thus, while THD and CLZ produce similar atypical effects in some behavioral tests of neuroleptic action (19,26,31) the two drugs do not display the same profile in all operant procedures.

In summary, typical antipsychotics such as HAL and CPZ do not produce consistent increases in punished responding in the conflict procedure, whereas the atypical antipsychotic CLZ produces consistent but modest increases in several species [(21,27,28) and the present study]. THD, another "atypical" antipsychotic, did not increase punished responding in the present study. While the results of the present study suggest that differences in chemical structure may be more predictive of the effects of these drugs in the conflict procedure than are their binding profiles or their effects in traditional neuroleptic screening procedures, recent work by Hoenicke, Vanecek, and Woods (12) suggests that the antiserotonergic effects [5-hydroxytryptamine$_{1C}$ (5-HT$_{1C}$) and 5-HT$_2$] of CLZ are particularly important for the discriminative cue properties of CLZ [see also (34)] and may represent an important aspect of clozapine's atypical antipsychotic properties (4). Given the important role that serotonergic activity may play in many of the behavioral effects of CLZ and the importance

of serotonin in behavioral tests such as the conflict procedure [see (17,20)], further research on the serotonergic action of CLZ may yield important information for the development of other atypical antipsychotic drugs.

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