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Differential Effects of Clozapine and Pimozide on Fixed-Ratio Responding During Repeated Dosing

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WILEY, J. L., A. D. COMPTON AND J. H. PORTER. *Differential effects of clozapine and pimozide on fixed-ratio responding during repeated dosing.* PHARMACOL BIOCHEM BEHAV **48**(1) 253–257, 1994.—Previous research has shown that the differential development of tolerance to the disruption of operant responding produced by repeated dosing with pimozide (PMZ) or clozapine (CLZ) can distinguish these two drugs. In the present study, the effects of PMZ (1 mg/kg) and CLZ (10 mg/kg) on response rate and response duration in rats lever pressing for food reward under a fixed-ratio 30 (FR-30) operant schedule were examined. PMZ suppressed response rates across all 10 days of drug dosing; CLZ produced an initial response rate decrease, with partial recovery (50%) occurring within the 10 day period. Similarly, PMZ produced an increase in response duration that persisted into the postdrug vehicle-injection period, while CLZ did not significantly change response duration. The prolonged suppression of FR responding produced by PMZ is similar to the lack of tolerance to this drug in other types of operant schedules. In contrast, CLZ's effects on response rate are schedule dependent. These results suggest that changes in response duration with repeated dosing may more reliably differentiate typical and atypical neuroleptics than do changes in response rate under FR schedules.

Clozapine Pimozide Tolerance Duration Fixed ratio Rats

THE lack of preclinical methods for differentiation of neuroleptics that produce extrapyramidal motor side effects (typical neuroleptics) and those that do not (atypical neuroleptics) has hindered development of new clinically effective atypical neuroleptics. Because neuroleptics require chronic administration for production of symptomatic relief, repeated dosing regimens may produce the clearest distinction between the two types of neuroleptics. Preclinical research predominantly has employed acute dosing paradigms. The effects of neuroleptics often differ, dependent upon whether they are administered acutely or chronically. With acute administration, high doses of both typical and atypical neuroleptics produce suppression of learned schedule-controlled behavior [e.g., (12,13,15)]; however, with repeated dosing, differential effects are seen with typical and atypical neuroleptics. Under a fixed interval schedule of reinforcement, the typical neuroleptic pimozide (PMZ) produced sustained response rate suppression for 38 days (18). In contrast, complete tolerance developed to the atypical neuroleptic clozapine's (CLZ) initial disruption of response rates by the seventh day of the chronic dosing regimen (18). Villanueva and Porter (25) reported similar results with CLZ and PMZ using a multiple random interval schedule.

The present study examined the effects of PMZ (1 mg/kg) and CLZ (10 mg/kg) on responding during repeated dosing with a fixed ratio schedule. With this type of schedule, any disruption in responding is more directly related to reinforcement loss, a factor that can markedly alter behavioral sensitivity to drug treatments [e.g., (3,22)]. Thus, attenuation of the response-rate decreasing effects of drugs might be expected to develop more easily under a fixed ratio schedule than under a fixed or random interval schedule. To characterize further the nature of the effects of repeated administration of PMZ and CLZ, the effects of these drugs on response duration during repeated dosing also was measured. Response duration has been shown to provide a measure of motor effects that simple response rates cannot (8–10,13,15,27).

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METHOD

Subjects

Twelve adult male Sprague-Dawley rats (280–320 g), obtained from Dominion Breeders (Dublin, VA), were used as subjects. All rats had been previously trained to lever press for food reinforcement in other procedures. Eight rats were 5 months old at the beginning of the present study and were drug naive. Four rats were 7 months old and had received several doses of chlordiazepoxide. Prior to the beginning of the present study, all rats had been reduced to 80% of their free-feeding body weights and were maintained at this level by restriction of daily food intake. When they were not being tested, animals were housed individually in wire cages in a temperature-controlled (22°C) animal colony room with a 12 L : 12 D cycle (lights on at 0600 h). Water was available ad lib in the home cages.

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 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. Solid-state programming equipment that controlled the experimental contingencies and recorded lever presses was located in an adjacent room. Response duration (to the nearest tenth of a second) was measured from the output of the switch input that was activated whenever the lever was depressed.

Procedure

Initially, all rats were trained to lever press according to a fixed ratio (FR) 1 schedule of food reinforcement in daily 15 min sessions. This ratio was gradually increased over eight sessions to a terminal FR-30 schedule. Subsequently, daily session length was increased to 30 min.

After responding stabilized on the FR-30 schedule (approximately 50 sessions), the first repeated dosing regimen was instituted. During the first 5 days, the rats received vehicle injections (predrug baseline); then, over the next 10 days, the rats received CLZ (10 mg/kg) injections (drug sessions); and, finally, during the following 5 days, the rats again received vehicle injections (postdrug baseline). During a 3 week washout period between the two dosing regimens, daily operant sessions were continued, but the rats did not receive any injections. The second dosing regimen with PMZ (1 mg/kg) was identical to that for CLZ, except that the rats received 3 days of predrug baseline vehicle injections. One rat died before completing the repeated dosing procedure with PMZ; thus, a total of 12 rats were tested during the CLZ dosing regimen and 11 rats were tested during the PMZ dosing regimen. A single dose of each drug was chosen based on previous research (18,25,26), which indicated that acute administration of each of these doses suppressed responding in other types of schedules.

Drugs

CLZ (Sandoz Pharmaceuticals, Hanover, NJ) was prepared in a vehicle solution of lactic acid (10–15 drops) and distilled water. PMZ (Janssen Pharmaceutica, Beerse, Belgium) was dissolved in a vehicle solution of Tween 80 (0.5 ml), lactic acid (10–15 drops), and distilled water. Both drugs and their vehicles were administered intraperitoneally (IP) at a volume of 1 ml/kg of body weight. CLZ and its vehicle were injected 1 h presession; PMZ and its vehicle were injected 4 h presession. Doses of each drug refer to the free base.

Statistical Analysis

The number of responses and the total duration (s) of responding were recorded during each session. The number of responses was converted to response rate (resp/min). Response duration (s/resp) was calculated by dividing total duration of responding by total number of responses. The response rate data for each dosing regimen were analyzed separately with two repeated measures analyses of variance (ANOVA). One ANOVA compared mean response rate during the entire predrug vehicle baseline period to mean response rate during each of the 10 sessions of repeated dosing. The second ANOVA compared mean response rate during the predrug vehicle sessions to mean response rate during each of the 5 days of the postdrug baseline period. Because response duration could not be calculated when a rat did not respond, number of observations across drug sessions was unequal for this measure; hence, a general linear model procedure (SAS Institute, Cary, NC) was used to compare mean response duration during predrug vehicle and drug sessions and during predrug vehicle and postdrug vehicle sessions. Duncan post hoc tests ($\alpha = 0.05$) were used to specify differences from the predrug baseline revealed by significant ANOVAs and GLMs (1).

RESULTS

Response Rate

Both CLZ (10 mg/kg) and PMZ (1 mg/kg) suppressed responding for the entire 10 days of repeated dosing, $F(10, 110) = 4.90, p < 0.001$, and $F(10, 100) = 11.87, p < 0.001$, respectively (Fig. 1, top panel). During the CLZ dosing regimen, responding was severely decreased on the first drug day, but significantly increased on the second drug day. Responding remained at approximately 50% of the predrug level for the remainder of the drug period. Complete recovery to the predrug vehicle level occurred on the first day of postdrug vehicle injections.

During the PMZ dosing procedure, responding was suppressed by more than 65% across all 10 drug days. Although responding increased on the first day of the postdrug period, full recovery to predrug vehicle levels did not occur until the fourth day of postdrug vehicle injections.

Response Duration

CLZ (10 mg/kg) did not significantly change response duration during the entire dosing period (Fig. 1, bottom panel), although there was a trend for CLZ to increase response duration during the first drug session, $F(10, 103) = 1.79$, $p = 0.07$. On drug days 2–10 and during the postdrug vehicle sessions, response duration was not different from the predrug level. In contrast, PMZ (1 mg/kg) increased response duration across the 10 days of repeated dosing, $F(10, 98) = 5.21$, $p = 0.0001$ (Fig. 1, bottom panel). During the PMZ dosing regimen, increased response durations were observed on drug day 5 and drug days 8–10. Response duration was elevated compared to predrug baseline through the first postdrug vehicle session, $F(5, 50) = 2.54, p = 0.0001$.

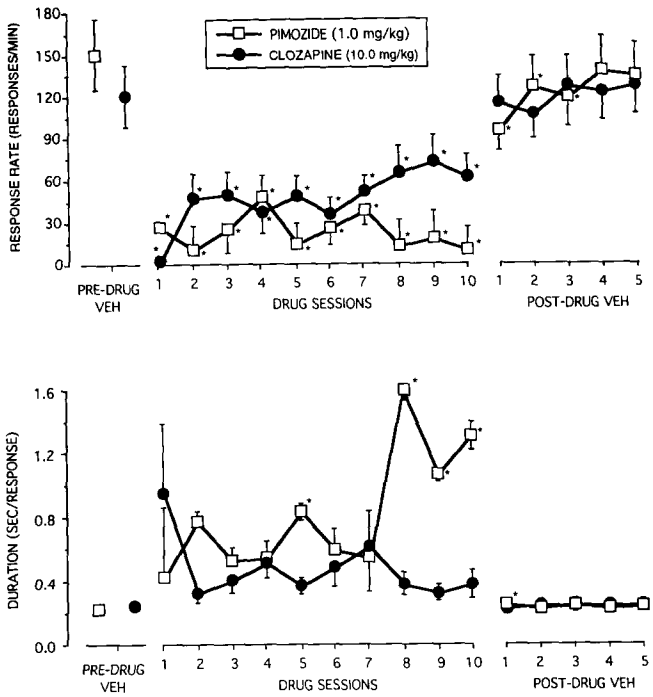

FIG. 1. Mean response rate (resp/min) (top panel) and response duration (sec/resp) (bottom panel) during 10 consecutive sessions of repeated dosing with CLZ (10 mg/kg) and with PMZ (1 mg/kg) and during five postdrug sessions of vehicle injections. Asterisks indicate significant ($p < 0.05$) differences from the mean response rate or duration during the predrug vehicle period. [For response rates: n = 12 for CLZ and n = 11 for PMZ. For response durations: n = 12 for CLZ, except during drug session 1, n = 8, and during drug sessions 4–6, n = 11; n = 11 for PMZ, except during drug sessions 9–10, n = 10.]

DISCUSSION

Repeated dosing with CLZ (10 mg/kg) and with PMZ (1 mg/kg) produced different patterns of effects on response rate under a FR-30 schedule of food reinforcement. CLZ decreased response rate initially; however, on days 2–10, partial (50%) recovery to pre-CLZ vehicle levels was observed. Response rates on the first day of post-CLZ vehicle injection were similar to those during the pre-CLZ baseline period. In contrast, PMZ produced a sustained suppression (>65%) of response rate across 10 days of repeated dosing, with full recovery to pre-PMZ vehicle baseline level not occurring until the fourth day of post-PMZ vehicle injection. Because repeated dosing with CLZ was completed in all rats prior to repeated dosing with PMZ, it is possible that carry-over effects of CLZ contributed to the PMZ results. Although this possibility cannot be ruled out, several lines of evidence argue against it. First, a 3 week wash-out period occurred between dosing regimens. During this period, rats did not receive any injections. Second, response rates and response durations during the pre-PMZ vehicle period had recovered to pre-CLZ baseline levels. Finally, the PMZ results are consistent with those of earlier studies from this lab in which fixed-interval (FI) or random-interval (RI) schedules of reinforcement were used. In these studies, 1 mg/kg PMZ decreased FI response rate for 38 days (18) and suppressed multiple RI responding for the 10 day dosing regimen (25).

Unlike PMZ, CLZ's effects on operant responding appear to be schedule dependent. Whereas complete tolerance has been shown to CLZ's disruption of FI responding (18) and to RI responding (25), only partial attenuation of the rate-disrupting effects of 10 mg/kg CLZ on FR responding occurred in the present study. This schedule-dependent effect of attenuation of the rate suppressant effect of 10 mg/kg CLZ also has been shown when rats were tested with both FI and FR schedules using a multiple FI-FR (MULT FI-FR) schedule (26).

The effects of repeated dosing with 10 mg/kg CLZ on schedule-controlled response rate were rather surprising. The reinforcement density hypothesis (22) would predict that tolerance to the rate-decreasing effects of a drug would develop more readily in situations in which the effect of the drug resulted in a loss of reinforcers. Because impairment of responding is more directly related to a decrease in the number of reinforcers with a FR schedule than with a FI schedule, the theory would predict that tolerance would be more likely to occur with a FR schedule than with a FI schedule. Repeated dosing with 10 mg/kg CLZ produced less tolerance under a FR schedule than it did under a FI schedule (18) during the FI component of a MULT FI-FR schedule (26) or during a RI schedule (25). While it is possible that greater tolerance to CLZ's rate decreasing effects under a FR schedule may have developed if testing had been continued for more than 10 days [e.g., see (10)], the slower rate of tolerance development under a FR schedule suggests that loss of reinforcers is an insufficient explanation for the different degree of tolerance to CLZ's rate-decreasing effects under FI and FR schedules. Indeed, previous studies have shown that factors such as duration of testing (5), individual differences (5), and work requirement (14,23) affect the rate and degree of tolerance development. Thus, it is likely that one of these other factors is responsible for the fact that repeated dosing produced only partial tolerance to CLZ's rate-decreasing effects with a FR schedule in the present study.

Although repeated dosing with 10 mg/kg CLZ produced only partial tolerance to its rate-decreasing effects, complete tolerance developed to CLZ's effect on response duration. By the second day of repeated dosing, recovery to pre-CLZ vehicle levels had occurred and the trend for an initial increase in response duration was no longer evident. Unlike CLZ, 1 mg/kg PMZ did not affect response duration initially, but produced an increase in response duration during the latter half of the dosing regimen. Villanueva et al. (26) reported similar results for both FR and FI components of a MULT FI-FR schedule. Thus, CLZ and PMZ are more clearly differentiated by their effects on response duration than by their effects on response rate under FR schedules. These results suggest that the effects of repeated dosing with PMZ and CLZ on response duration (if not on response rate) are consistent across different types of schedule requirements (i.e., FI, RI, and FR).

Faustman and Fowler (8,9) and Walker et al. (27) argue that response duration and response rate provide independent measures of the effects of drugs on responding and that increases in response duration primarily reflect drug-induced motor deficits. The fact that PMZ produced increases in duration only during the latter half of the regimen suggests that the animals became more sensitive to PMZ-induced motor deficits with repeated dosing. This suggestion is consistent with the observed lack of tolerance to PMZ's rate-decreasing effects. On the other hand, Wise (30) has proposed that typical neuroleptics produce decreases in the reward value of reinforcers that could also contribute to their effects on rate. Indeed, in procedures specifically designed to measure the

anhedonic effects of neuroleptics, typical neuroleptics such as haloperidol and PMZ produced anhedonic effects (7,16, 21,29). Thus, the lack of tolerance to the rate-decreasing effects of PMZ may reflect a combination of motor and anhedonic effects. In contrast, partial tolerance to the rate-decreasing effects of CLZ are more difficult to explain. CLZ does not produce anhedonic effects (28) and does not show the typical neuroleptic profile of motor effects in nonoperant procedures (2,4). In addition, the fact that CLZ produced minimal changes in response duration across 10 days of repeated dosing suggests that motor deficits cannot entirely account for the lack of tolerance to this drug's rate-decreasing effects. Thus, neither motor nor anhedonic effects readily explain CLZ's differential effects on FI and FR responding.

Neuroleptic-induced motor and anhedonic effects are presumed to be mediated via dopaminergic mechanisms (11,30). Whereas PMZ is a relatively selective dopamine antagonist at D_2 receptors, CLZ acts as an antagonist at a variety of receptors, including acetylcholine, serotonin, histamine, and several subtypes of dopamine receptors (17); hence, it is likely that some of CLZ's behavioral effects are mediated by nondopaminergic mechanisms. Previous studies suggest that the anticholinergic action of CLZ may contribute to its effects on schedule-controlled responding (19). In addition, several authors have suggested that CLZ's effects may be mediated by dopamine D_1 (6,20) or D_4 receptors (24). These actions on other receptors may modulate expression of CLZ's antidopaminergic action at D_2 receptors and could help to explain differences between the behavioral effects of CLZ and typical neuroleptics such as PMZ.

In summary, the results of the present study emphasize the importance of operant context in determining the effects of repeated dosing with typical and atypical neuroleptics on operant responding. Whereas the typical neuroleptic PMZ (1 mg/kg) produced prolonged suppression of FR response rate similar to its suppression of FI response rate, the effects of the atypical neuroleptic CLZ (10 mg/kg) are schedule-dependent. In contrast, the effects of both drugs on response duration appears to be consistent across both FI and FR schedules. In the present study, repeated dosing with PMZ resulted in sensitization to the duration-increasing effects of this drug, whereas repeated dosing with CLZ produced little change in response duration. These results suggest that changes in response duration with repeated dosing may more readily differentiate typical and atypical neuroleptics than do changes in response rate under FR schedules. At minimum, a different pattern of changes in these measures between drugs and between schedules emphasizes the need to include more than one measure of operant responding in screening procedures using repeated dosing regimens.

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