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Variable-Interval Schedules of Timeout From Avoidance: Effects of Anxiolytic and Antipsychotic Drugs in Rats

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GALIZIO, M., J. W. JOURNEY, S. A. ROYAL AND J. A. WELKER. *Variable-interval schedules of timeout from avoidance: Effects of anxiolytic and antipsychotic drugs in rats.* PHARMACOL BIOCHEM BEHAV **37**(2) 235–238, 1990.—Concurrent performances were studied in rats under conditions where responses on one lever postponed shock on a Sidman avoidance schedule and responses on another lever produced periods of signaled timeout from avoidance on a variable-interval schedule. Chlorpromazine decreased rates of responding on both the timeout and avoidance levers to about the same extent. The effects of chlordiazepoxide and CGS 9896 depended upon the event maintaining responding. Both drugs increased responding on the timeout lever at doses that concurrently decreased responding on the avoidance lever. Thus, the novel anxiolytic CGS 9896 produced effects that closely resembled those of the benzodiazepine anxiolytic, chlordiazepoxide. Like chlorpromazine, buspirone decreased both avoidance and timeout responding. Despite the documented anxiolytic properties of buspirone, its actions here were unlike those of the other anxiolytic drugs tested. Nonetheless, the differentiation between drugs obtained with the timeout from avoidance procedure indicates its utility for behavioral pharmacology.

Buspirone CGS 9896 Chlordiazepoxide Chlorpromazine Anxiolytic Antipsychotic Timeout from avoidance Rats

MANY studies have shown that various types of tranquilizing drugs generally decrease behavior maintained by negative reinforcement (8,17). Thus, a recent finding (9) that chlordiazepoxide increased response rates maintained by a type of negative reinforcement is of some interest. Galizio and Perone (9) trained rats on concurrent schedules where one response postponed shock (Sidman avoidance) and a second response intermittently produced periods of timeout from avoidance. The effects of chlordiazepoxide appeared to depend on the nature of the maintaining event. At doses that depressed or had no effect on avoidance responding, chlordiazepoxide increased rates of responding on the timeout lever. Ethanol produced similar results with this procedure (10). Galizio and Perone (9) also found that the effects of morphine depended on the type of negative reinforcement maintaining the behavior, but morphine decreased responding maintained by timeout at doses that increased or had no effect on avoidance (9). However, not all drugs produce event-dependent effects with this procedure. Dalrymple and Stretch (7) studied timeout from avoidance in squirrel monkeys and found that chlorpromazine decreased both avoidance and timeout responses to approximately the same degree.

The present study was an attempt to determine whether novel anxiolytic drugs CGS 9896 and buspirone would act similarly to chlordiazepoxide in the timeout from avoidance procedure. CGS 9896 is a pyrazoloquinoline compound that is thought to derive its putative anxiolytic properties from activity at the benzodiazepine receptor, where it has been classified as a mixed agonist-antagonist (6). Although CGS 9896 has been shown to share with benzodiazepines the capacity to elevate punished responding (16,21), it also has some unique properties. For example, CGS 9896 shows little evidence of sedation or muscle relaxation across the effective dose range (5), does not produce benzodiazepine-like effects on schedule-controlled behavior (16,19), and does not generalize to a lorazepam cue (1). Buspirone is a clinically effective anxiolytic drug in humans that is inactive at the benzodiazepine receptor, but shows activity at dopamine and 5-HT_{1A} receptors (3,20). Buspirone elevates punished responding in pigeons, but does not produce robust antipunishment effects in other species (3, 12, 15, 18). In drug discrimination procedures, responses trained to buspirone do not generalize to benzodiazepines (3,13). However, buspirone has been shown to decrease behavior maintained by negative reinforcement (11), an effect it shares with antipsychotic and anxiolytic drugs.

In the present study the effects of chlordiazepoxide on timeout from avoidance were replicated and contrasted with CGS 9896 and buspirone. If the selective increases in timeout responding produced by chlordiazepoxide were also produced by novel anxiolytic drugs, the timeout procedure would be of value as a screening

tool. Finally, in order to permit contrast with an antipsychotic drug, the present study also evaluated the effects of chlorpromazine. The effects of chlorpromazine in rats were expected to be similar to those seen in previous research with monkeys using the timeout from avoidance procedure, where decreases in both avoidance and timeout responding were observed (7).

METHOD

Subjects

Sixteen Holtzman specific-pathogen (mycoplasm) free male rats (purchased from Holtzman Laboratory Animals, Madison, WI) completed the behavioral training procedures and provided data on drug effects. Rats were individually housed with ad lib access to food and water. Most had previous exposure to drugs (either amphetamine or pentobarbital). They began avoidance training when 80–120 days old.

Apparatus

Training took place in commercially constructed operant chambers (Gerbrands G7400 series) approximately 28 cm long, 26 cm wide, and 28 cm high, enclosed in sound-attenuated, ventilated chests. The chambers were equipped with two retractable levers centered 12 cm apart on the stainless steel front wall, 7.5 cm above the floor. Both levers required a force of approximately 0.3 N to operate. A 28-V houselight located at the top of the chamber provided illumination through the clear Plexiglas top and sides. White noise (78 dB) was provided through a speaker located behind the front wall. The floor was constructed of 0.2-cm diameter stainless steel rods spaced 1.3 cm apart through which shock (1 mA, 0.5 sec) was delivered by a constant-current shock generator and scrambler (Lafayette 82400-SS and 58020). Events in the chambers were controlled and recorded by microcomputer (Tandy, TRS-80 Model 4) interfaced to the chambers using software described elsewhere (14).

Drug Preparation

Chlorpromazine hydrochloride, chlordiazepoxide hydrochloride (Sigma), and buspirone hydrochloride (Bristol-Myers) were dissolved in isotonic (0.9%) sodium chloride solution, and intraperitoneal injections were administered in a volume of 1 ml/kg. Doses are expressed in terms of the total salt. CGS 9896 (CIBA-GEIGY) was added to a vehicle of 1 drop Tween 20 per ml of isotonic saline and placed in suspension by ultrasonic mixing. Tween vehicle was used as the injection control in the CGS 9896 study.

Procedure

Preliminary training. Rats were trained to press the right (avoidance) lever to postpone shock on a free-operant avoidance schedule where each response produced a brief (0.5 sec) termination of the white noise (response feedback) and postponed the shock for 30 sec (response-shock interval). In the absence of responding, shocks were presented every 5 sec (shock-shock interval). White noise and chamber illumination accompanied the initiation of the session, and were terminated at the end of each 2-hr session. Training under these conditions continued until animals avoided at least 80% of the shocks programmed by the response-shock interval for 10 consecutive sessions with a minimum of 10 sessions and a maximum of 35. Two rats failed to reach criterion within 35 sessions and were discarded from the study.

In order to establish a discrimination between stimuli signalling periods of avoidance and timeout from avoidance, the next phase was a multiple schedule with 10-min avoidance components (houselight and white noise on, shock-postponement schedule operative) alternated with 10-min timeout components (houselight and white noise off, no shock programmed). Training on the multiple schedule continued until virtually no responding occurred during timeout components (2–25 sessions).

Concurrent schedule training. To begin this phase, the left lever was inserted for the first time and each response on it produced a 5-min timeout signalled by the retraction of the left (timeout) lever, removal of the houselight and white noise, and suspension of the shock postponement schedule. When responding was consistent the duration of timeout was reduced to 2-min and the schedule gradually thinned to variable-interval (VI) 45 sec (i.e., on the average 45 sec had to elapse before a response on the timeout lever produced timeout). Thus, the final baseline parameters were Sidman avoidance (response-shock interval 30 sec; shock-shock interval 5 sec) on the avoidance lever and VI 45 sec on the timeout lever. Session duration was 2 hr, and data were collected separately for the first 20 min of each session (warm-up), and the final 100 min (main session). Because avoidance was generally variable during warm-up, only the main session data are presented in the analyses. Training on these terminal parameters continued 5 days/week until responding stabilized on both levers. The stability criterion was based on the most recent 10 sessions and required that the difference between the means of the first and last five sessions be within 15% of the grand mean. After reaching stable levels of performance (11–41 sessions), the drug probes were introduced.

Drug administration. Drugs were administered twice per week (Tuesday and Friday for some rats, and Wednesday and Friday for others) and sessions were conducted under baseline conditions the other 3 days. One or 2 baseline sessions were always conducted between drug administration sessions. Baseline sessions that were preceded by another baseline session (either Tuesday or Thursday) were used to calculate percent control data. On drug administration days, subjects received intraperitoneal injections 15 min prior to session onset. Session duration was 2 hr unless an animal was so impaired that it failed to respond for 250 sec, in which case the session was automatically terminated. The schedule of drug conditions was randomly generated with the constraint that no dose was administered on successive drug days, and that the end of each cycle (one exposure to each dose) of the drug regimen was completed before beginning the next cycle. A minimum of 2 determinations of each dose were made at each dose of chlordiazepoxide (0, 2.5, and 25 mg/kg) and CGS 9896 (0, 10, and 40 mg/kg) for each of the 6 subjects tested with these drugs. Doses of chlordiazepoxide were chosen to reflect the range of effective doses previously determined with the procedure (9), and 2 comparable doses of CGS 9896 were selected [based on (16)]. Since no previous studies have examined the effects of chlorpromazine or buspirone in rats under schedules of timeout from avoidance, three different doses of each drug were selected. Eight rats were studied under chlorpromazine and at least 3 replications were obtained at each chlorpromazine dose (0, 0.5, 1.0, and 2.0 mg/kg). Six rats were tested with buspirone (0, 0.5, 1.0, and 2.0 mg/kg). Two or more determinations of each dose were made for 5 of the rats. The sixth animal was not tested at the 2 mg/kg dose. Some of the rats were tested with more than one drug.

RESULTS

Table 1 shows the average baseline data for the 4 studies (standard deviations in parentheses). Steady rates of responding on both the avoidance and timeout levers were maintained for all rats.

TABLE 1

MEAN DATA FOR BASELINE CONDITIONS

Drug Condition	Number of Subjects	Avoidance R/Min (SD)	Timeout R/Min (SD)	Avoidance % (SD)
Chlorpromazine	8	7.0 (1.8)	3.7 (1.5)	95.1 (4.1)
Buspirone	6	6.0 (1.9)	4.9 (2.3)	90.7 (17.1)
Chlordiazepoxide	6	6.2 (2.1)	4.5 (2.6)	97.3 (9.2)
CGS 9896	6	7.2 (2.4)	4.8 (2.3)	97.3 (9.1)

Response rates on the avoidance lever were somewhat higher than those on the timeout lever for most rats. Table 1 also shows percent of the programmed shocks successfully avoided (calculations based on shocks scheduled by the response-shock interval), and reveals that proficient avoidance was generally maintained (range 59–100%).

The effects of the various drugs studied are presented in Fig. 1 (response rates as a percent of baseline) and Fig. 2 (percent shocks avoided). The leftmost panels of the figures show the effects of chlorpromazine. Figure 1 shows that response rates on both the avoidance lever (black circles) and timeout lever (white triangles) declined in a dose-dependent fashion with chlorpromazine. Decreased timeout responding was apparent even at the 0.5 mg/kg dose and was most marked at the 2.0 mg/kg dose. The rate-decreasing effects of chlorpromazine on avoidance responding were not clear until the 1.0 mg/kg dose was reached, and were also most marked at the 2.0 mg/kg dose. A 4×2 factorial ANOVA with Dose and Response Type (Timeout vs. Avoidance) as main factors revealed a significant main effect for Chlorpromazine Dose, $F(3,49)=25.24$, $p<0.05$. Neither the Dose × Response Type interaction nor the main effect for Response Type was significant ($p>0.05$ in both cases). All eight subjects showed decreased avoidance response rates at both the 1.0 and 2.0 mg/kg doses, while 7/8 showed decreased rates on the timeout lever at these doses. The leftmost panel of Fig. 2 shows that the decreased avoidance response rates produced by chlorpromazine were associated with clear decreases in percent shocks avoided, $F(3,21)=19.64$, $p<0.05$.

The effects of buspirone are shown in the second panels of each figure. Figure 1 shows that buspirone produced dose-dependent decreases in both avoidance and timeout response rates. The reliability of these effects was confirmed by ANOVA which revealed a significant main effect for Buspirone Dose, $F(3,33)=22.84$, $p<0.05$. No effects involving Response Type were significant ($F<1$ in both cases). As might be expected given the decrease in avoidance response rates, buspirone sharply decreased percent avoidance (shown in Fig. 2), $F(3,14)=13.81$, $p<0.05$. Decreased responding on the avoidance lever was observed in all 6 rats even at the lowest (0.5 mg/kg) dose of buspirone, and decreases in responding on the timeout lever were present in 5/6 rats at the 0.5 mg/kg dose, and in all subjects at the 1.0 mg/kg dose.

The third panel from the left of Fig. 1 shows the effects of chlordiazepoxide. Unlike buspirone or chlorpromazine, the effects of chlordiazepoxide differed for the two responses. Responding on the timeout lever tended to increase with dose of chlordiazepoxide, while avoidance rates increased slightly at the 2.5 mg/kg dose, but decreased at the 25 mg/kg dose. A 2×3 factorial ANOVA confirmed the observation that the effects of chlordiazepoxide depended on the response with a significant Dose × Response Type interaction, $F(2,35)=4.56$, $p<0.05$. No significant main

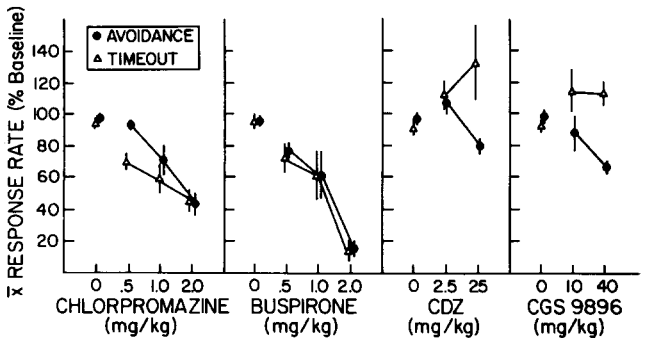

FIG. 1. Mean ± S.E.M. responses per minute on the avoidance lever (dark circles) and timeout lever (white triangles) expressed as percent of baseline.

effects were obtained ($p>0.05$ in both cases). Five of the 6 rats studied showed increased timeout responding and decreased avoidance responding at the 25 mg/kg dose while the sixth rat showed increased timeout responding only at the 2.5 mg/kg dose. Avoidance proficiency (Fig. 2) was largely unaffected even at the higher dose of chlordiazepoxide, and the single-factor ANOVA was not significant ($p>0.05$).

Finally, the rightmost panel of Fig. 1 shows the effects of CGS 9896 on response rates. Like chlordiazepoxide, CGS 9896 affected avoidance and timeout responding differently. At both doses studied, timeout responding was elevated relative to vehicle control, but avoidance was decreased. The differential action of CGS 9896 was confirmed by a significant Dose × Response Type interaction, $F(2,35)=6.34$, $p<0.05$. There was also a significant main effect for Response Type, $F(1,35)=8.41$, $p<0.05$, but not for Dose ($F<1$). Avoidance proficiency (Fig. 2) was not significantly affected by CGS 9896 ($p>0.05$).

DISCUSSION

Chlordiazepoxide increased rates of responding on the timeout lever at doses that decreased responding on the avoidance lever, a finding that replicated previous studies from our laboratory (9). Chlorpromazine decreased response rates on both timeout and avoidance levers and reduced avoidance proficiency in the present study. The chlorpromazine effect seen here in rats was consistent with the results of Dalrymple and Stretch (7) using a similar procedure with squirrel monkeys, but was in contrast with the effects of chlordiazepoxide. CGS 9896, a novel ligand of the benzodiazepine receptor, produced effects that were quite similar to those of chlordiazepoxide, but the atypical anxiolytic agent, buspirone, did not. Instead, buspirone's effects resembled those of chlorpromazine, decreasing both avoidance and timeout responding at all doses tested. Buspirone also reduced avoidance profi-

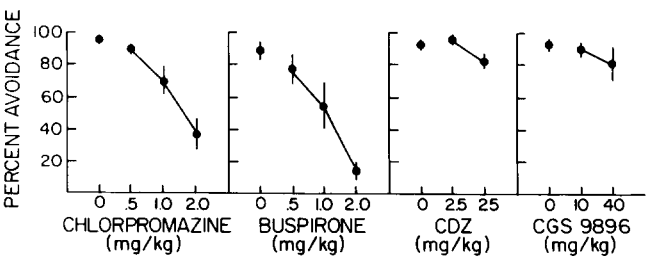

FIG. 2. Mean ± S.E.M. percent shocks avoided based on the number of shocks scheduled by the response-shock interval.

ciency, an effect it shared with chlorpromazine, but not with chlordiazepoxide or CGS 9896.

The present results were thus consistent with those of several studies that have shown differences between the behavioral effects of buspirone and those of traditional anxiolytic drugs (3, 15, 18) in rats. Thus, the timeout procedure was not capable of detecting the common anxiolytic actions of 5-HT_{1A} agonist buspirone and ligands of the benzodiazepine receptor. It may be that the dopaminergic actions of buspirone, properties shared with chlorpromazine, were responsible for the rate-decreasing effects seen here. Subsequent studies of buspirone analogs such as gepirone might prove of interest here, because gepirone is thought to lack activity at the dopamine receptor.

The present results were consistent with studies that have shown similar behavioral effects of CGS 9896 and benzodiazepines (6). Both increase rates of punished responding (16,21), an effect shared with some other depressant drugs (barbiturates, ethanol), but not generally produced by chlorpromazine or other antipsychotic drugs (15). The antipunishment actions of anxiolytic drugs such as chlordiazepoxide and CGS 9896 may provide an account of their unusual actions in the present study. Schedules of avoidance and timeout, such as those studied here, share some properties with punishment paradigms. For example, if responding on the timeout lever in the present study persisted for 30 sec without a response on the avoidance lever, a shock would be delivered. Thus, the timeout response could, in effect, be punished, and such a contingency might limit the rate of response on the timeout lever. Viewed in this way, the increases in timeout responding produced by chlordiazepoxide and CGS 9896 in the present study could have been produced through their anticonflict actions. Consistent with such an interpretation, ethanol has also been shown to increase timeout responding under conditions similar to those studied here (10). Also consistent with an anticonflict interpretation is the observation that drugs that do not generally produce antipunishment effects, such as chlorpromazine (present study) and morphine (9), decreased timeout responding. Support for such an account might be developed by testing other compounds with known actions in other "conflict" paradigms to determine whether similar actions occur with the timeout procedure.

There are other possible explanations for the differential effects of chlordiazepoxide and CGS 9896 on responding maintained by production of timeout vs. shock postponement. These behaviors were maintained by different schedules (Sidman avoidance vs. variable-interval), at different rates (baseline timeout response rates were lower than avoidance rates in 10 of the 16 rats tested), as well as by different events. Thus, event-, schedule-, or rate-dependency could account for the differential drug effects observed in the present study [see (2)]. Further research that assesses drug effects with the timeout response maintained under different schedules and at different rates will be necessary to evaluate the viability of such explanations. Regardless of the theoretical explanation, the timeout from avoidance procedure appears to have potential as a baseline for behavioral pharmacology due to its sensitivity to different drug actions.

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