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Chronic Anxiolytic Treatment Effects on Conflict Behavior in the Rat

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ELLIS, D. M., D. J. FONTANA, T. C. McCLOSKEY AND R. L. COMMISSARIS. *Chronic anxiolytic treatment effects on conflict behavior in the rat.* PHARMACOL BIOCHEM BEHAV **37**(1) 177–186, 1990.—The present studies examined the effects of chronic posttest treatment with the antipanic agent alprazolam (ALP) or the traditional anxiolytic agents chlordiazepoxide (CDP) and phenobarbital (PhB) on conflict behavior. In daily ten-minute sessions, water-deprived rats were trained to drink from a tube which was occasionally electrified (0.25 or 0.5 mA). Electrification was signalled by a tone. Chronic ALP (10 mg/kg/day), CDP (40 mg/kg/day), PhB (80 mg/kg/day) or vehicle were injected IP *after* conflict testing (in some experiments again 12–16 hours later) for a minimum of 6 weeks. Chronic ALP (but not CDP or PhB) resulted in a time-dependent increase in punished responding, with a latency to onset of 3–4 weeks; this effect was *not* antagonized by the benzodiazepine antagonist Ro15-1788. These data support the hypothesis that conflict paradigms may serve as animal models for the study of antipanic agents. Moreover, these data suggest that not all anxiolytics will exhibit antipanic efficacy.

Alprazolam Antipanic agents Anxiety Chlordiazepoxide Conflict behavior Panic disorder Phenobarbital Ro15-1788

PANIC disorder and Generalized Anxiety Disorder (GAD) are classified as distinct anxiety neuroses by the Diagnostic and Statistical Manual for Psychiatric Disorders (1). Treatment response profiles also support this distinction between panic disorder and GAD. Traditional anxiolytics (e.g., chlordiazepoxide, phenobarbital) are effective in alleviating the symptoms of GAD; moreover, the symptoms of GAD are effectively reduced with a single administration (14,20). In contrast, antidepressant agents have been used frequently to treat patients suffering from panic disorder; clinical improvement with antipanic treatment often exhibits a considerable (up to 3–4 weeks) delay to onset (5, 13, 16–18, 20, 22, 33).

Recent reports have indicated that rat conflict paradigms, perhaps the "gold standard" for assessing anxiolytic drug effects, may be effective models for studying the effects of chronic antipanic drug treatment. For example, chronic treatment with the antipanic agents imipramine (IMI), desipramine (DMI) or phenelzine (PHEN) results in a time-dependent increase in punished responding in the Conditioned Suppression of Drinking (CSD) conflict paradigm (11,12). The 3–4-week delay in onset of the anticonflict effects of these agents is consistent with the time course for the antipanic effects of these agents in man (see above). A similar time-dependent anticonflict effect also has been reported by Bodnoff *et al.* (3) for chronic DMI treatment in the novelty-suppressed feeding task.

Recently, the second generation benzodiazepines alprazolam and clonazepam have been reported to exert antipanic effects in man (2, 4, 6, 24, 26, 27, 29, 32). Whether these agents are unique among benzodiazepines or whether relatively high doses of traditional benzodiazepines might also be effective in the treatment of panic disorder (10,26) remains to be determined. The present study was designed to determine whether chronic posttest treatment with the benzodiazepines would exhibit anticonflict effects in the CSD conflict paradigm. The benzodiazepine agents selected were the second generation antipanic benzodiazepine alprazolam and the traditional benzodiazepine chlordiazepoxide. In addition, the effects of chronic posttest treatment with phenobarbital on CSD behavior were determined.

GENERAL METHOD

Animals

Female Sprague-Dawley rats (Charles River Farms, Cambridge, MA; 250–300 grams at the start of the experiments) were housed in groups of four or five in a climate-controlled room with a 12-hour light:12-hour dark cycle (lights on 0700–1900 hours). Initially, food and water were available continuously. Following a two-week accommodation period and continuing throughout the period of behavioral assessment, all animals were maintained on a

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restricted water schedule (see below). Food continued to be available in the home cage.

Apparatus

Conditioned Suppression testing was conducted in an apparatus similar to that described by Fontana *et al.* (11). The testing chamber was a rectangular box with Plexiglas® sides and a metal floor and top. Protruding from one wall was a metal drinking tube, to which a calibrated (0.5 ml units) length of polyethylene tubing was attached for measuring the volume of water consumed. Programming for the test session was controlled by solid state modular programming equipment (Coulbourn Instruments Co., Lehigh Valley, PA).

General Procedure

For the first few sessions, water-restricted (24-hr deprivation) subjects were placed in the experimental chamber and allowed to consume water freely without the shock contingency. After one week of nonshock sessions, the tone/shock contingency was initiated. The 7-sec tone periods were presented at regular (22-sec ISI) intervals to the subjects. During the last 5 sec of these tone periods, contact between the floor and the metal drinking tube completed a circuit that resulted in the delivery of a shock (0.25 or 0.5 mA) to the rat. Shocks were delivered by a Coulbourn Instruments Shocker (Model No. E13-02).

Initially, the shock inhibited fluid consumption in the test chamber. After several days, however, all subjects learned to consume stable volumes of water during the silent periods and made relatively few and very brief contacts with the tube during the tone. The duration of the shock received was equal to the duration of the tube contact (less than 200 msec).

In all experiments, subjects were tested individually at the same time of day. All subjects achieved stable baselines (day-to-day coefficients of variation of approximately 30% for individual rats) for punished and unpunished responding by the end of the second week of CSD sessions with the alternating tone:no tone periods. Baseline (i.e., nondrug) CSD testing was continued for two additional weeks before drug testing was initiated. For baseline determinations and for at least the first 4 weeks of chronic drug treatment, CSD testing was conducted four days per week (Monday–Thursday) and free access to water was provided on nontest days (Thursday posttest until Sunday a.m.). During the Test Weeks in which acute challenges were conducted, the subjects were tested 6 days per week (Monday–Saturday) and free access to water was available from Saturday posttest until Sunday a.m.

Specific Experiments Conducted

Experiment I: Acute alprazolam and chlordiazepoxide effects: Antagonism by Ro15-1788. The effects of pretreatment with the benzodiazepine antagonist Ro15-1788 on the effects of single doses of alprazolam or chlordiazepoxide were determined using the procedure described by Commissaris *et al.* (7). The benzodiazepine agonist doses (0.6 mg/kg alprazolam; 10 mg/kg chlordiazepoxide) were selected because they produced prominent but not maximal anticonflict effects when administered alone. In these studies, subjects received Ro15-1788 (1.0 mg/kg; 15-minute pretreatment) or its vehicle on both the Friday and Saturday test days of a given Test Week, while the benzodiazepine agonist and its vehicle were administered on alternate days. Thus, for a given Test Week, the "net" effect of alprazolam or chlordiazepoxide ("net" effect = benzodiazepine agonist − vehicle) was determined in animals pretreated with either Ro15-1788 or its vehicle. Pretreatment time was 10 minutes for alprazolam and 30 minutes for chlordiazepoxide. Separate groups of subjects were used for the alprazolam and chlordiazepoxide interaction determinations.

Experiment II: Chronic alprazolam treatment effects. The chronic drug administration studies were conducted using a modification of the procedures described by Fontana *et al.* (11). Subjects were trained for CSD testing as described above (shock intensity = 0.25 mA) and were assigned into two treatment conditions with comparable levels of punished responding over the last two weeks of these control CSD sessions (i.e., baseline). These subjects received chronic treatment with either alprazolam or its vehicle for 8 weeks. The chronic alprazolam treatment consisted of 5 mg/kg alprazolam (once daily at 1600 hours) for 3 weeks, followed by 10 mg/kg (one-third of the dose administered at 0900 hours and the remaining two-thirds of the dose administered at 1600 hours) for 5 weeks. CSD testing (4 days/week at 1400–1600 hours) was continued throughout the period of chronic treatment. To minimize the influence of the acute effects of alprazolam on CSD behavior, CSD test sessions were conducted 24 (Test Weeks 1–3) or 7–9 (Test Weeks 4–8) hours *after* the preceding injection.

On Test Weeks 7 and 8 of these chronic posttest alprazolam or vehicle treatments, the effects of acute pretest challenges with alprazolam or Ro15-1788 were determined. The acute alprazolam challenge (0.6 mg/kg) was conducted on Test Week 7 and the acute challenge with Ro15-1788 (2.0 mg/kg) was conducted on Test Week 8. In these acute challenges, the drug or its vehicle were administered 10 minutes prior to CSD testing using a standard crossover design (19). On Friday of Test Weeks 7 and 8, half the subjects in each chronic treatment condition received an acute challenge with the agent of interest and half received the drug vehicle. These treatments were reversed on the Saturday drug tests. Thus, each animal served as its own control with respect to the effects of pretest alprazolam versus vehicle (Test Week 7) and Ro15-1788 versus vehicle (Test Week 8). Chronic posttest alprazolam and vehicle treatments were maintained during this period, except that the morning injections were not administered on the days of these acute pretest determinations (i.e., Fridays and Saturdays of Test Weeks 7 and 8). Thus, the effects of acute pretest alprazolam or Ro15-1788 administration were determined 21–23 hours after the last injection in the chronic posttest regimen.

Chronic treatment with alprazolam or its vehicle was discontinued after Test Week 8. CSD testing (4 days/week) was continued for three weeks following treatment discontinuation.

Experiment III: Chronic high-dose chlordiazepoxide treatment effects. A second group of subjects was trained for CSD testing and chronic posttest drug treatment as described above (shock intensity = 0.25 mA). The subjects were then assigned into two treatment conditions with comparable levels of punished responding over the last two weeks of these control CSD sessions (baseline). These subjects received chronic treatment with vehicle (distilled water) or a high dose of chlordiazepoxide (40.0 mg/kg, once daily immediately after CSD testing) for 6 weeks. CSD testing (4 days/week) was continued throughout the period of chronic treatment.

After 6 weeks of chronic treatment and CSD testing, all subjects received an acute challenge with Ro15-1788 (2.0 mg/kg; 10-minute pretreatment). The purpose of this challenge was to determine whether the rats which had been chronically treated with chlordiazepoxide had become dependent on chlordiazepoxide. This acute challenge employed a standard "crossover" design as described above and was conducted on Friday and Saturday of Test Week 6.

Experiment IV: Chronic moderate-dose chlordiazepoxide treatment effects. A third group of subjects was trained for CSD testing as described above, except that the shock intensity used was 0.5 mA. Two weeks prior to the initiation of chronic chlordiazepoxide

or distilled water treatments, all subjects in this experiment received an acute challenge (30-minute pretreatment) with 10 mg/kg chlordiazepoxide or vehicle (distilled water). This acute challenge was conducted using a crossover design as described above.

After determination of pretreatment responses to acute chlordiazepoxide, subjects were divided into two treatment conditions and received twice-daily injections of distilled water or chlordiazepoxide (10 mg/kg). The injections were accomplished immediately after CSD testing and again 12 hours later. As with the chronic alprazolam study, this schedule of chronic posttesting drug administration was used to minimize the acute effects of the chronically administered drug on CSD behavior and to enable subsequent pretest challenges with other agents (see below).

Beginning at Test Week 5 of chronic posttest treatment, the effects of acute pretest challenges with chlordiazepoxide were determined. All subjects received a range of chlordiazepoxide doses spaced in logarithmic intervals (5–28.4 mg/kg) over the course of six weeks of testing. The crossover procedure described above was used; chlordiazepoxide was administered using a 30-minute pretreatment.

After 11 weeks of chronic treatment and CSD testing, all subjects received an acute challenge with Ro15-1788 (4.0 mg/kg; 10-minute pretreatment). This acute challenge employed a standard "crossover" design as described above. Chronic chlordiazepoxide or vehicle treatments were discontinued after 11 weeks; CSD testing (4 days/week) was continued for three weeks after discontinuation of these treatments (Test Weeks 12–14).

Experiment V: Chronic phenobarbital treatment effects. A fourth group of subjects was trained for CSD testing as described above (shock intensity = 0.25 mA). The subjects were then assigned to two treatment conditions with comparable levels of punished responding over the last two weeks of these control CSD sessions. These subjects received chronic treatment with vehicle (distilled water) or a high dose of phenobarbital (80 mg/kg, once daily immediately after CSD testing) for 6 weeks. CSD testing (4 days/week) was continued throughout the period of chronic treatment.

Drugs

Chlordiazepoxide hydrochloride and phenobarbital sodium were obtained from Sigma Chemical Company (St. Louis, MO) and were dissolved in distilled water. Alprazolam was received as a gift from the Upjohn Company (Kalamazoo, MI) and was dissolved in 40% propylene glycol and 10% ethanol with 5% sodium benzoate and 5% benzoic acid as buffers. Ro15-1788 was received as a gift from Hoffmann-La Roche (Nutley, NJ) and was suspended in 0.5% methylcellulose. All drugs were administered intraperitoneally in a volume of 1 ml/kg body weight.

Statistical Analyses

For the chronic treatment studies, pretreatment (i.e., baseline) water intake and punished responding were compared using *t*-tests for unpaired values. The effects of chronic drug or vehicle treatment on weekly averages for these parameters were compared using 2 × "X" factorial ANOVA ("X" = the number of Test Weeks + Baseline) with repeated measures (Main Effects: Drug/Vehicle; Baseline and Test Weeks). The effects of the acute challenges with Ro15-1788 on shocks received and water intake were analyzed using 2 × 2 factorial ANOVA with repeated measures (Main Effects: Chronic Drug/Vehicle, Acute Drug/Vehicle). The "net" effects of acute challenges with chlordiazepoxide ("net" effect = acute chlordiazepoxide − acute vehicle) in chronic vehicle- and chlordiazepoxide-treated subjects were analyzed using 2 × 6 factorial ANOVA with repeated measures (Main Effects: Chronic Vehicle/Chlordiazepoxide treatment, Acute Chlordiazepoxide doses). The effects of discontinuation of chronic chlordiazepoxide versus vehicle treatments on CSD behavior (spontaneous withdrawal) were compared using a 2 × 3 factorial ANOVA (Main Effects: Chronic Vehicle/Chlordiazepoxide; Weeks of Withdrawal) with repeated measures. Post hoc comparisons were made using the least significant differences (lsd) test. In all statistical comparisons, $p<0.05$ was used as the criterion for statistical significance (30).

TABLE 1

THE EFFECTS OF ACUTE TREATMENT WITH ALPRAZOLAM OR CHLORDIAZEPOXIDE ON CSD BEHAVIOR: ANTAGONISM BY Ro15-1788

	Change in Shocks Received[a]	Change in Water Consumed (ml)[b]
0.6 mg/kg ALP + Vehicle	+30.8 ± 6.6*	+2.3 ± 0.6*
0.6 mg/kg ALP + 1.0 mg/kg Ro15-1788	+6.0 ± 7.0†	+0.5 ± 0.7†
10 mg/kg CDP + Vehicle	+37.3 ± 14.6*	+0.7 ± 1.3
10 mg/kg CDP + 1.0 mg/kg Ro15-1788	+9.8 ± 5.7†	+0.3 ± 1.5

[a]Values represent the mean ± SEM change in shocks received (drug − vehicle) in CSD sessions following coadministration with either vehicle (methylcellulose) or 1.0 mg/kg Ro15-1788. (See text for further details.)

[b]Values represent the mean ± SEM change in water intake (drug − vehicle) in CSD sessions following coadministration with either vehicle (methylcellulose) or 1.0 mg/kg Ro15-1788. (See text for further details.)

*The indicated treatment significantly different from control, $p<0.05$, *t*-test for paired values.

†Ro15-1788-treated subjects significantly different from vehicle-treated controls, $p<0.05$, factorial ANOVA.

RESULTS

The average baseline (i.e., nondrug) responding for all subjects in the CSD paradigm at the 0.25 mA shock intensity was 32.6 ± 3.4 (mean ± SEM) shocks/session and 10.4 ± 0.3 ml water/session. The average baseline responding for subjects at the 0.5 mA shock intensity was 8.1 ± 1.1 shocks/session and 12.4 ± 0.5 ml water/session. It should be noted that the number of tube contacts during the shock component was insignificant when compared to the number of tube contacts during the unpunished component (2000–3000 per session). Thus, the volume of water consumed accurately reflects unpunished responding in the CSD.

Experiment I: Acute Alprazolam and Chlordiazepoxide Effects: Antagonism by Ro15-1788

Table 1 illustrates the effects of acute treatment with alprazolam or chlordiazepoxide on CSD behavior. As can be seen, acute treatment with either agent alone resulted in a significant increase in shocks received. Ro15-1788 coadministration significantly antagonized this effect for both alprazolam and chlordiazepoxide, as evidenced by a significant Ro15-1788/Vehicle × Drug/Vehicle interaction [for alprazolam: $F(1,21)=7.64$, $p<0.05$; for chlordiazepoxide: $F(1,21)=6.41$, $p<0.05$]. Acute alprazolam or chlordiazepoxide treatment also increased water intake, although the effect of chlordiazepoxide was not statistically significant. The

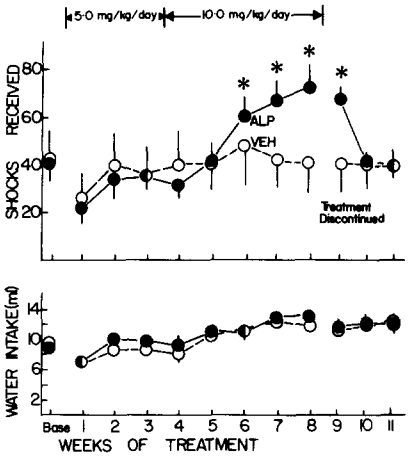

FIG. 1. The effects of chronic administration of alprazolam on CSD behavior. The number of shocks received (upper panel) and the volume of water consumed (lower panel) in CSD sessions during the course of 8 weeks of chronic vehicle [40% propylene glycol and 10% ethanol with 5% sodium benzoate and 5% benzoic acid (VEH): open circles] or alprazolam (ALP: 5 mg/kg/day × 3 weeks, followed by 10 mg/kg/day in divided doses × 5 weeks; filled circles) administration are plotted. Also plotted are the number of shocks received and the volume of water consumed during three weeks following discontinuation of chronic alprazolam or vehicle treatment (Test Weeks 9–11). Each symbol represents the mean ± SEM from 7 (vehicle) or 5 (alprazolam) subjects. *$p<0.05$, alprazolam change from baseline significantly different from vehicle change from baseline at that Test Week, post hoc lsd test following factorial ANOVA.

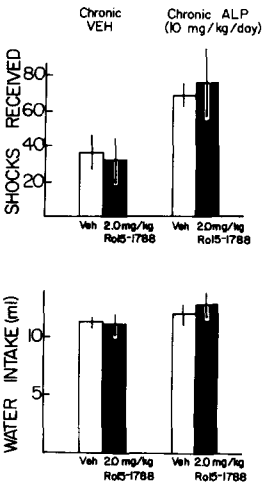

FIG. 2. The effects of acute Ro15-1788 challenge in chronic vehicle- or alprazolam-treated subjects on CSD behavior. The effects of acute vehicle (Veh: open bars) or 2.0 mg/kg Ro15-1788 (shaded bars) administration on the number of shocks received (upper panel) and the volume of water consumed (lower panel) in animals chronically treated with vehicle (VEH: left side) or alprazolam (ALP: right side) are plotted. Data are from Test Week 8 of chronic alprazolam or vehicle treatment. Each symbol represents the mean ± SEM from 7 (vehicle) or 5 (alprazolam) subjects. Ro15-1788 treatment did not alter the increase in punished responding associated with chronic alprazolam treatment. See text for further details.

effect of alprazolam on water intake was also antagonized by Ro15-1788 coadministration as evidenced by a significant Ro15-1788/Vehicle × Alprazolam/Vehicle interaction for this variable, $F(1,21)=4.43$, $p<0.05$.

Experiment II: Chronic Alprazolam Treatment Effects

The upper panel of Fig. 1 illustrates the effects of chronic alprazolam treatment on punished responding in the CSD. The pretreatment baselines for punished responding in the two groups were not statistically different (vehicle: 39.0 ± 7.0; alprazolam: 42.0 ± 11.4; $t=0.22$, n.s.). Both groups exhibited a significant decrease in punished responding in Test Week 1. Although once-daily treatment for 3 weeks with 5 mg/kg alprazolam failed to affect punished responding, increasing the daily dose to 10 mg/kg alprazolam resulted in a gradual and time-dependent increase in punished responding. ANOVA revealed a significant Main Effect for Test Weeks, $F(11,110)=10.12$, $p<0.05$, with no significant Main Effect for Vehicle/Alprazolam treatment, $F(1,10)<1$, n.s. There was a significant Test Week × Vehicle/Alprazolam treatment interaction, $F(11,110)=5.29$, $p<0.05$. Post hoc lsd tests revealed that alprazolam-treated subjects accepted more shocks than vehicle-treated subjects at Test Weeks 6, 7, and 8 of chronic treatment (weeks 3, 4, and 5 after alprazolam dosage adjustment).

The upper panel of Fig. 1 also illustrates the time course for the decline of the alprazolam-induced increase in punished responding. As can be seen, punished responding remained elevated for the first week of CSD testing following discontinuation of chronic alprazolam treatment. By the second week after discontinuation of chronic treatment (Test Week 10), however, there was no difference between chronic alprazolam- and vehicle-treated subjects.

The lower panel of Fig. 1 illustrates the effects of chronic alprazolam or vehicle treatment on water consumption in the CSD. Water intake did not differ between the two groups prior to initiation of chronic Vehicle/Alprazolam treatment (vehicle: 8.8 ± 0.7; alprazolam: 8.0 ± 1.0; $t=0.22$, n.s.). Both chronic vehicle and chronic alprazolam treatment reduced water intake in the first week of chronic treatment. ANOVA revealed a significant Main Effect for Test Weeks, $F(11,110)=34.00$, $p<0.05$, with no Main Effect for Vehicle/Alprazolam treatment, $F(1,10)<1.0$, n.s., and no Test Week × Vehicle/Alprazolam treatment interaction, $F(11,110)<1.0$, n.s. Discontinuation of chronic treatment did not affect water intake in either treatment group (Test Weeks 9–11).

An unpaired t-test comparing the "net" (alprazolam − vehicle) effects of an acute challenge with 0.6 mg/kg alprazolam (10-minute pretreatment; Test Week 7) on punished responding in chronic alprazolam- versus chronic vehicle-treated subjects revealed no difference (chronic vehicle: +69 ± 21 shocks, mean ± SEM; chronic alprazolam: +56 ± 12 shocks, $t=0.83$, n.s.). Similarly, there was no significant difference in the "net" effects of this acute alprazolam challenge on water intake in rats chronically treated with alprazolam or its vehicle (chronic vehicle: 0.0 ± 1.4 ml; chronic alprazolam: −0.2 ± 2.7 ml; $t=0.68$, n.s.). Thus, this chronic alprazolam treatment did not appear to result in tolerance or sensitization to the effects of acute alprazolam treatment on CSD behavior.

The upper panel of Fig. 2 represents the effects of the benzodiazepine antagonist Ro15-1788 on the number of shocks accepted by rats chronically treated with either alprazolam or its vehicle (Test Week 8). As can be seen, treatment with Ro15-1788 failed to antagonize the increase in punished responding produced by chronic alprazolam treatment. Statistically, this was supported by a significant Main Effect for Vehicle/Alprazolam treatment, $F(1,10)=5.35$, $p<0.05$. There was no Main Effect for Ro15-1788/Vehicle treatment, $F(1,10)<1.0$, n.s., nor was there an Vehicle/Alprazolam × Ro15-1788/Vehicle interaction, $F(1,10)<1.0$,

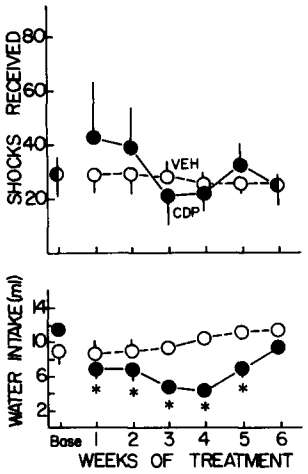

FIG. 3. The effects of chronic administration of a daily high dose of chlordiazepoxide (40 mg/kg; once/day) on CSD behavior. The number of shocks received (upper panel) and the volume of water consumed (lower panel) in CSD sessions during the course of 6 weeks of chronic vehicle [distilled water (VEH): open circles] or chlordiazepoxide (CDP: 40 mg/kg/day, filled circles) administration are plotted. Each symbol represents the mean ± SEM from 8 (vehicle) or 7 (chlordiazepoxide) subjects. *$p<0.05$, chlordiazepoxide change from baseline significantly different from vehicle change from baseline at that Test Week, post hoc lsd test following factorial ANOVA.

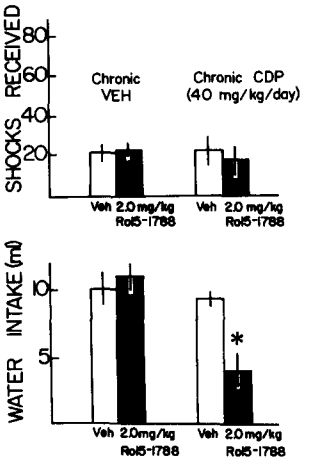

FIG. 4. The effects of acute Ro15-1788 challenge in chronic vehicle- or chlordiazepoxide-treated subjects on CSD behavior. The effects of acute vehicle (Veh: open bars) or 2.0 mg/kg Ro15-1788 (shaded bars) administration on the number of shocks received (upper panel) and the volume of water consumed (lower panel) in animals chronically treated with vehicle [distilled water (VEH): left side] or chlordiazepoxide (CDP: right side) are plotted. Data are from Test Week 6 of chronic chlordiazepoxide or vehicle treatment. Each symbol represents the mean ± SEM from 8 (vehicle) or 7 (chlordiazepoxide) subjects. *$p<0.05$, effect of Ro15-1788 on chronic chlordiazepoxide-treated rats significantly different from effect of Ro15-1788 on chronic vehicle-treated rats, factorial ANOVA.

n.s. Thus, acute challenge with the benzodiazepine antagonist did not alter the effects of chronic alprazolam treatment on punished responding.

The lower panel of Fig. 2 illustrates the effects of this Ro15-1788 challenge on water intake in rats chronically treated with alprazolam or its vehicle. Statistically, there was no Main Effect for Vehicle/Alprazolam treatment, $F(1,10)=1.15$, n.s., or Ro15-1788/Vehicle treatment, $F(1,10)<1.0$, n.s., nor was there a Vehicle/Alprazolam × Ro15-1788/Vehicle interaction, $F(1,10)<1.0$, n.s. Thus, there was no effect of the acute Ro15-1788 challenge on water intake in either group of chronically treated animals.

Experiment III: Chronic High-Dose Chlordiazepoxide Treatment Effects

The upper panel of Fig. 3 illustrates the effects of chronic chlordiazepoxide (40 mg/kg/day) or distilled water treatment on punished responding in the CSD. Pretreatment baselines did not differ between the two groups (chlordiazepoxide: 28.0 ± 8.1 shocks/session; distilled water: 29.6 ± 5.6 shocks/session, $t=0.17$, n.s.). Chronic chlordiazepoxide-treated rats accepted slightly more shocks than vehicle-treated controls for the first two weeks of treatment, but returned to control levels by Test Week 3. Factorial ANOVA revealed a significant Main Effect for Test Weeks, $F(6,78)=2.65$, $p<0.05$. There was no significant Main Effect for Chronic Vehicle/Chlordiazepoxide, $F(1,13)<1$, n.s., nor was there a significant Test Week × Chronic Vehicle/Chlordiazepoxide interaction, $F(6,78)=1.68$, n.s. Thus, chronic chlordiazepoxide treatment did not affect punished responding over the course of six weeks of CSD testing.

The lower panel of Fig. 3 illustrates the effects of chronic chlordiazepoxide or distilled water treatment on water consumption in the CSD. Pretreatment water intake did not differ between the two groups (chlordiazepoxide: 11.0 ± 0.6 ml/session; water: 11.3 ± 0.8 ml/session, $t=0.25$, n.s.). Chronic chlordiazepoxide treatment reduced water intake in the early weeks of CSD testing, but this measure returned to near control levels by Test Week 6. Factorial ANOVA revealed a significant Main Effect for Test Weeks, $F(6,78)=11.54$, $p<0.05$, a significant Main Effect for Chronic Vehicle/Chlordiazepoxide, $F(1,13)=11.93$, $p<0.05$, and a significant Test Week × Chronic Vehicle/Chlordiazepoxide interaction, $F(6,78)=4.11$, $p<0.05$. Post hoc lsd tests comparing the "net" change from baseline (i.e., Test Week "X" − Baseline) revealed that chronic chlordiazepoxide-treated subjects consumed significantly less water than vehicle controls at Test Weeks 1–5.

The upper panel of Fig. 4 illustrates the effects of an acute challenge with Ro15-1788 on the number of shocks accepted by rats chronically treated with chlordiazepoxide or vehicle. As can be seen, treatment with Ro15-1788 did not affect punished responding in either chronic vehicle or chronic chlordiazepoxide-treated rats. Statistically, the Main Effects for Vehicle/Chlordiazepoxide treatment, $F(1,10)=5.35$, $p<0.05$, or Ro15-1788/Vehicle treatment, $F(1,10)<1.0$, n.s., were not significant. The Vehicle/Chlordiazepoxide × Ro15-1788/Vehicle interaction also was not significant, $F(1,10)<1.0$, n.s.

The lower panel of Fig. 4 illustrates the effects of this Ro15-1788 challenge on water intake in rats chronically treated with chlordiazepoxide or vehicle. As can be seen, Ro15-1788 dramatically reduced water intake in rats chronically treated with chlordiazepoxide, but not vehicle. The Main Effects for Vehicle/Chlordiazepoxide, $F(1,13)=8.76$, $p<0.05$, and Ro15-1788/Vehicle, $F(1,13)=11.20$, $p<0.05$, were significant. Most important, there was also a significant Vehicle/Chlordiazepoxide × Ro15-1788/Vehicle interaction, $F(1,13)=70.85$, $p<0.05$.

Experiment IV: Chronic, Moderate-Dose Chlordiazepoxide Treatment Effects

Prior to the initiation of chronic chlordiazepoxide or distilled

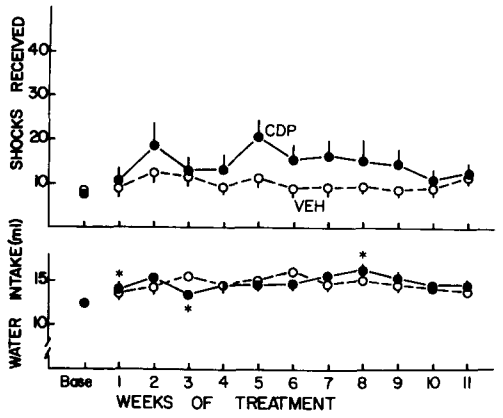

FIG. 5. The effects of chronic administration of a moderate daily dose of chlordiazepoxide on CSD behavior. The number of shocks received (upper panel) and the volume of water consumed (lower panel) in CSD sessions during the course of 11 weeks of chronic vehicle [distilled water (VEH): open circles] or chlordiazepoxide (CDP: 10 mg/kg, b.i.d.; filled circles) administration are plotted. Each symbol represents the mean ± SEM from 9 (vehicle) or 10 (chlordiazepoxide) subjects. *$p<0.05$, chlordiazepoxide change from baseline significantly different from vehicle change from baseline at that Test Week, post hoc lsd test following factorial ANOVA.

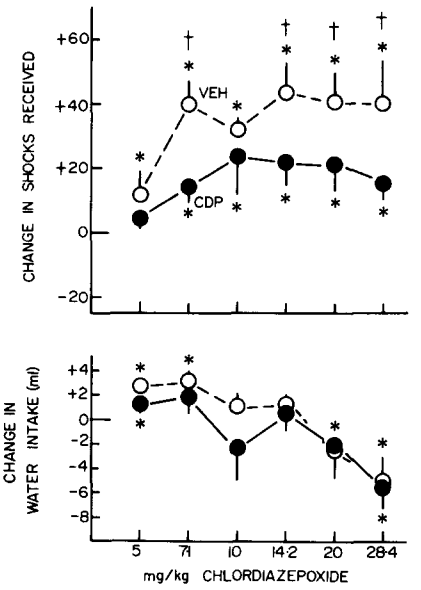

FIG. 6. Acute chlordiazepoxide effects on CSD behavior in rats treated chronically with chlordiazepoxide or vehicle. Plotted are the net change (chlordiazepoxide − vehicle) in the number of shocks received (top panel) and the volume of water consumed (bottom panel) following acute chlordiazepoxide treatment in rats chronically treated with vehicle [distilled water (VEH): open symbols] or chlordiazepoxide (CDP: 10 mg/kg, b.i.d., for 5–10 weeks; filled symbols). Each symbol and vertical line represents the mean ± SEM obtained from 9 (vehicle) or 10 (chlordiazepoxide) subjects. *$p<0.05$, the indicated acute chlordiazepoxide dose significantly different from acute vehicle treatment, paired t-test. †$p<0.05$, acute chlordiazepoxide effect in chronic chlordiazepoxide-treated subjects significantly different from acute chlordiazepoxide effect in chronic vehicle-treated controls at the indicated chlordiazepoxide dose, post hoc lsd test following factorial ANOVA.

water treatments, the effects of an acute challenge with a single dose (10 mg/kg) of chlordiazepoxide were comparable in the two groups, as evidenced by similar "net" effects for both the change in shocks received [vehicle: +37.1 ± 6.3 (mean ± SEM); chlordiazepoxide: 34.1 ± 5.6; $t=0.35$, n.s.] and the change in water intake (vehicle: +2.9 ± 0.7; chlordiazepoxide: +2.6 ± 0.7, $t=0.32$, n.s.).

The upper panel of Fig. 5 illustrates the effects of chronic post-test treatment with a moderate dose (10 mg/kg, b.i.d.) of chlordiazepoxide on punished responding in the CSD (shock intensity = 0.5 mA). Pretreatment baselines were comparable in the two groups [vehicle: 8.5 ± 1.9 (mean ± SEM) shocks/session; chlordiazepoxide: 7.7 ± 1.2 shocks/session, $t=0.37$, n.s.]. The subjects receiving chronic chlordiazepoxide treatment exhibited a mild tendency to accept more shocks than chronic vehicle-treated rats, but this was not statistically significant. Both groups accepted more shocks over the course of the 11 weeks of chronic chlordiazepoxide or vehicle treatment. This was supported statistically by a significant Main Effect for Test Weeks, $F(11,187)=2.77$, $p<0.05$. There was no Main Effect for Chronic Vehicle/Chlordiazepoxide treatment, $F(1,17)=1.15$, n.s., nor was there a significant Chronic Vehicle/Chlordiazepoxide × Test Week interaction, $F(11,187)=1.28$, n.s.

The lower panel of Fig. 5 illustrates the effects of chronic posttest treatment with this moderate dose of chlordiazepoxide on water intake. Pretreatment baselines were comparable in the two groups (vehicle: 12.5 ± 0.9 ml/session; chlordiazepoxide: 12.4 ± 0.7 ml/session; $t=0.12$, n.s.). Both vehicle- and chlordiazepoxide-treated subjects consumed greater volumes of water as a function of Test Weeks. This was supported statistically by a significant Main Effect for Test Weeks, $F(11,187)=7.63$, $p<0.05$. There was no influence of this chronic chlordiazepoxide treatment on water intake, as evidenced by the lack of a significant Main Effect for Chronic Vehicle/Chlordiazepoxide treatment, $F(1,17)<1$, n.s. There was a significant Chronic Vehicle/Chlordiazepoxide × Test Weeks interaction, $F(11,187)=2.63$, $p<0.05$. Post hoc lsd comparisons of the "net" change in water intake from baseline revealed that chronic chlordiazepoxide-treated subjects consumed greater volumes than vehicle-treated rats at Test Weeks 1 and 8, but less than vehicle-treated rats at Test Week 3.

The top panel of Fig. 6 illustrates the effects of acute chlordiazepoxide administration during the course of these chronic distilled water or chlordiazepoxide treatments (Test Weeks 5–10). Acute chlordiazepoxide treatment resulted in an increase in punished responding which was dose-related; this was supported statistically by a significant Main Effect for Chlordiazepoxide Dose, $F(5,75)=3.12$, $p<0.05$. Significant tolerance to the anti-conflict actions of acute chlordiazepoxide treatment was present; this was supported statistically by a significant Main Effect for Chronic Vehicle/Chlordiazepoxide, $F(1,15)=6.47$, $p<0.05$. The Chronic Vehicle/Chlordiazepoxide × Chlordiazepoxide Dose interaction was not significant, $F(5,75)<1$, n.s. Post hoc lsd analyses indicated that chronic chlordiazepoxide-treated subjects were less responsive than vehicle-treated controls at several (7.1, 14.2, 20 and 28.4 mg/kg) chlordiazepoxide doses.

The lower panel of Fig. 6 illustrates the effects of acute challenges with chlordiazepoxide on water intake in subjects treated chronically with vehicle or chlordiazepoxide. In general, lower doses of chlordiazepoxide administered acutely increased water intake, whereas the highest dose dramatically decreased water intake. Statistically, this was supported by a significant Main Effect for Chlordiazepoxide Dose, $F(5,75)=9.22$, $p<0.05$. There was no Main Effect for Chronic Vehicle/Chlordiazepoxide treatment, $F(1,15)=1.48$, n.s., nor was there a significant Chronic Vehicle/Chlordiazepoxide × Chlordiazepoxide Dose interaction,

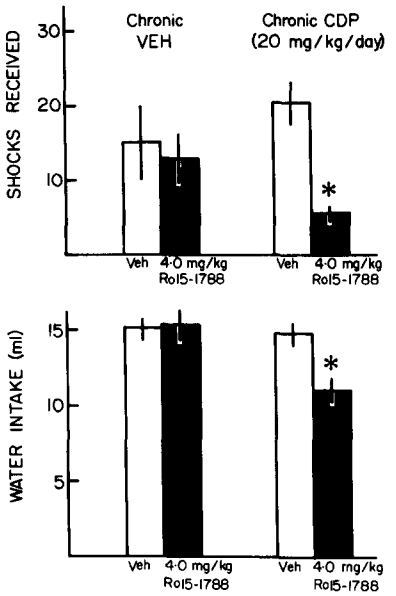

FIG. 7. The effects of acute Ro15-1788 challenge in chronic vehicle- or chlordiazepoxide-treated subjects on CSD behavior. The effects of acute vehicle (Veh: open bars) or 4.0 mg/kg Ro15-1788 (shaded bars) administration on the number of shocks received (upper panel) and the volume of water consumed (lower panel) in animals chronically treated with vehicle [distilled water (VEH: left side)] or chlordiazepoxide (CDP: right side) are plotted. Data are from Test Week 11 of chronic chlordiazepoxide or vehicle treatment. Each symbol represents the mean ± SEM from 9 (vehicle) or 10 (chlordiazepoxide) subjects. *$p<0.05$, effect of Ro15-1788 on chronic chlordiazepoxide-treated rats significantly different from effect of Ro15-1788 on chronic vehicle-treated rats, factorial ANOVA.

$F(5,75)<1$, n.s. Thus, with respect to either the increase in water intake associated with low doses or the decrease in water intake associated with higher doses, there was no evidence of tolerance to the acute effects of chlordiazepoxide treatment on this measure in animals previously treated with chlordiazepoxide.

The upper panel of Fig. 7 illustrates the effects of an acute challenge with 4.0 mg/kg Ro15-1788 on the number of shocks accepted by rats chronically treated with chlordiazepoxide or vehicle. As can be seen, treatment with Ro15-1788 in this experiment decreased punished responding in chlordiazepoxide-treated but not vehicle-treated rats. The Main Effect for Vehicle/Chlordiazepoxide treatment, $F(1,16)<1$, n.s., was not significant. The Main Effect for Ro15-1788/Vehicle treatment, $F(1,16)=11.90$, $p<0.05$, and the Vehicle/Chlordiazepoxide × Ro15-1788/Vehicle interaction, $F(1,16)=5.98$, $p<0.05$, were significant.

The lower panel of Fig. 7 illustrates the effects of this Ro15-1788 challenge on water intake. As was observed in Experiment III, Ro15-1788 treatment dramatically reduced water intake in rats chronically treated with chlordiazepoxide, but not vehicle. The Main Effects for Vehicle/Chlordiazepoxide, $F(1,16)=9.33$, $p<0.05$, and Ro15-1788/Vehicle, $F(1,16)=24.96$, $p<0.05$, were significant. Most important, there was also a significant Vehicle/Chlordiazepoxide × Ro15-1788/Vehicle interaction, $F(1,16)=20.05$, $p<0.05$.

Table 2 summarizes the effects of discontinuation of these chronic chlordiazepoxide or vehicle treatments on CSD behavior. There was no effect of this abrupt treatment discontinuation on punished responding in either treatment group, as evidenced by the lack of significant Main Effects for either Chronic Vehicle/Chlordiazepoxide, $F(1,17)<1$, n.s., or Test Weeks, $F(2,34)<1$, n.s. The Chronic Vehicle/Chlordiazepoxide × Test Weeks interaction also was not significant, $F(2,34)<1$, n.s. In contrast, chronic chlordiazepoxide-treated subjects consumed significantly less water than did their vehicle-treated counterparts for the first week following treatment discontinuation. Water intake returned to control values by the second following treatment discontinuation. Statistically, there was no Main Effect for Chronic Vehicle/Chlordiazepoxide, $F(1,17)<1$, n.s., or Test Weeks, $F(2,34)<1.0$, n.s. There was, however, a significant Chronic Vehicle/Chlordiazepoxide × Test Weeks interaction, $F(2,34)=4.79$. $p<0.05$. Post hoc lsd tests revealed that chronic chlordiazepoxide-treated subjects consumed significantly less water than chronic vehicle-treated subjects in the first Test Week of this spontaneous withdrawal period.

TABLE 2

CSD BEHAVIOR DURING WITHDRAWAL FROM CHRONIC MODERATE-DOSE CHLORDIAZEPOXIDE (10 mg/kg, b.i.d. FOR 11 WEEKS) OR VEHICLE TREATMENTS

Week of Withdrawal	Chronic Vehicle	Chronic Chlordiazepoxide
	Shocks Received	
1 (Test Week 12)	14.3 ± 3.6[a]	11.4 ± 1.9
2 (Test Week 13)	14.3 ± 2.9	13.5 ± 2.1
3 (Test Week 14)	13.2 ± 3.6	12.8 ± 2.2
	Water Consumed	
1 (Test Week 12)	15.6 ± 0.8[b]	13.2 ± 0.4*
2 (Test Week 13)	15.1 ± 0.9	14.5 ± 0.6
3 (Test Week 14)	14.8 ± 0.9	14.7 ± 0.6

[a]Values represent the mean ± SEM number of shocks accepted in CSD sessions during the indicated week after discontinuation of chronic chlordiazepoxide or vehicle treatment. (See text for further details.)

[b]Values represent the mean ± SEM volume of water consumed (ml) in CSD sessions during the indicated week after discontinuation of chronic chlordiazepoxide or vehicle treatment.

*Water intake in chlordiazepoxide-treated subjects significantly different from vehicle-treated subjects at the indicated Test Week, $p<0.05$, post hoc lsd test following 2 × 3 factorial ANOVA.

Experiment V: Chronic Phenobarbital Treatment Effects

The upper panel of Fig. 8 illustrates the effects of chronic phenobarbital or distilled water treatment on punished responding in the CSD. Pretreatment baselines did not differ between the two groups (phenobarbital: 29.8 ± 2.2 shocks/session; vehicle: 29.3 ± 9.8 shocks/session; $t=0.04$, n.s.). Chronic phenobarbital treatment increased punished responding for the first few weeks of treatment, but punished responding returned to baseline levels by the end of 6 weeks of chronic treatment. Factorial ANOVA revealed a significant Main Effect for Test Weeks, $F(6,66)=8.70$, $p<0.05$, with no significant Main Effect for Chronic Vehicle/Phenobarbital, $F(1,11)=2.52$, n.s. ANOVA also revealed a significant Test Week × Chronic Vehicle/Phenobarbital interaction, $F(6,66)=4.81$, $p<0.05$. Post hoc lsd tests comparing the "net" change from baseline revealed that chronic phenobarbital-treated subjects accepted significantly more shocks than vehicle-treated controls at Test Weeks 1 and 2.

The lower panel of Fig. 8 illustrates the effects of chronic phenobarbital or distilled water treatment on water consumption in

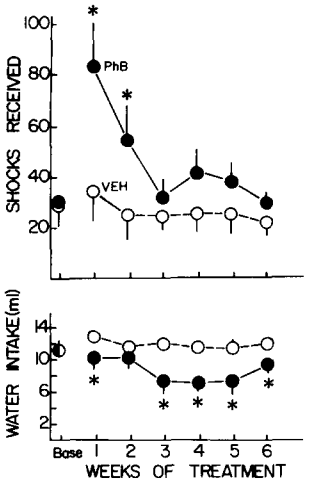

FIG. 8. The effects of chronic administration of phenobarbital on CSD behavior. The number of shocks received (upper panel) and the volume of water consumed (lower panel) in CSD sessions during the course of 6 weeks of chronic vehicle [distilled water (VEH): open circles] or phenobarbital (PhB: 80 mg/kg/day; filled circles) administration are plotted. Each symbol represents the mean ± SEM from 8 (vehicle) or 5 (phenobarbital) subjects. *$p<0.05$, phenobarbital change from baseline significantly different from vehicle change from baseline at that Test Week, post hoc lsd test following factorial ANOVA.

the CSD. Pretreatment water intake did not differ between the two groups (phenobarbital: 10.0 ± 0.9 ml/session; vehicle: 10.8 ± 0.6 ml/session; $t=0.47$, n.s.). Chronic phenobarbital-treated subjects consumed less water than vehicle-treated controls across all Test Weeks. Factorial ANOVA revealed significant Main Effects for Test Weeks, $F(6,66)=4.24$, $p<0.05$, and Chronic Vehicle/Phenobarbital, $F(1,11)=5.38$, $p<0.05$, as well as a significant Chronic Vehicle/Phenobarbital × Test Weeks interaction, $F(6,66)=3.42$, $p<0.05$. Post hoc lsd tests comparing the "net" change from baseline revealed that chronic phenobarbital-treated subjects consumed significantly less water than vehicle-treated controls at Test Weeks 1, 3, 4, 5, and 6.

GENERAL DISCUSSION

Similar to the typical antidepressant/antipanic agents imipramine, desipramine and phenelzine (11,12), chronic administration of the clinically effective antipanic benzodiazepine alprazolam produced a time-dependent increase in punished responding in the CSD. This increase in punished responding was statistically significant at Test Weeks 6, 7 and 8 of chronic treatment (weeks 3, 4, and 5 after dosage adjustment). Although this latency to "onset" after dosage adjustment is somewhat longer than that reported in man for the antipanic effect of alprazolam (4–6, 26, 27, 32), these data provide further evidence that the CSD paradigm might serve as an "animal model" for the study of panic disorder and potential antipanic agents.

The increase in punished responding produced by chronic alprazolam treatment was unaffected by an acute challenge with the benzodiazepine antagonist Ro15-1788. Ro15-1788 treatment effectively antagonized the acute anticonflict effects of alprazolam or chlordiazepoxide. Together, these data suggest that while *acute* alprazolam exerts an anticonflict effect through benzodiazepine receptor activation, the time-dependent anticonflict effect of alprazolam following this chronic posttest administration is *not* related to alprazolam "on board" at the time of CSD testing. Moreover, these data suggest that the time-dependent anticonflict effect associated with chronic alprazolam treatment is not mediated through benzodiazepine receptors. Bodnoff *et al.* have shown that the time-dependent anticonflict effect of chronic desipramine treatment in the NSF task is not antagonized by treatment with Ro15-1788 (3).

Although only a single acute dose of alprazolam was examined, there was no evidence for tolerance or sensitization to the acute anticonflict effects of this agent following chronic alprazolam administration. For the first week following treatment discontinuation (Test Week 9), subjects previously treated with alprazolam continued to accept significantly more shocks than controls. Punished responding in these chronic alprazolam-treated subjects returned to control levels by the second week following discontinuation of alprazolam treatment, indicating that the increase in punished responding in the chronic alprazolam-treated rats was not an irreversible effect of alprazolam treatment.

Although chlordiazepoxide and other traditional benzodiazepines have been regarded as relatively ineffective agents in the management of panic disorder, it has been proposed that alprazolam and clonazepam may not be unique in their efficacy in the treatment of panic disorder and that all benzodiazepines, if administered at sufficiently high doses, may exert clinical antipanic effects (10,26). In contrast to the effects observed with alprazolam, chronic chlordiazepoxide (up to 40 mg/kg/day) treatment did not produce a time-dependent increase in punished responding. These data are not consistent with the hypothesis that all benzodiazepines are effective antipanic agents when administered at high enough doses.

It should be noted that the dose of chlordiazepoxide employed in the high-dose study (40 mg/kg/day) was sufficient to produce mild to moderate sedation throughout the day, as evidenced by a decrease in water intake at many Test Weeks throughout the study. (Note that CSD testing was conducted approximately 24 hours after drug administration in Experiment III.) Further, an acute challenge with the benzodiazepine antagonist Ro15-1788 significantly decreased water intake in chronic chlordiazepoxide-treated subjects, but not vehicle-treated controls, suggesting that the chlordiazepoxide-treated subjects had become dependent on chlordiazepoxide as a result of the chronic treatment. Therefore, the lack of anticonflict effect observed with chronic chlordiazepoxide treatment cannot be attributed to insufficient dosing.

Following several weeks of chronic treatment with a lower dose of chlordiazepoxide (10 mg/kg, twice/daily; Experiment IV), significant tolerance to the acute anticonflict effects of this agent was observed. It should be noted, however, that there was no evidence of tolerance to the effects of acute chlordiazepoxide on unpunished responding (water intake) in this study. Acute challenge with Ro15-1788 significantly reduced both punished and unpunished responding in subjects which had received this chronic chlordiazepoxide treatment; this acute Ro15-1788 treatment was without effect in chronic vehicle-treated subjects. Similar to the effects of an acute challenge with Ro15-1788, chronic chlordiazepoxide-treated subjects consumed significantly less water than vehicle-treated controls during the first week of CSD testing in the absence of chronic treatment (spontaneous withdrawal). As was observed in the Ro15-1788-elicited withdrawal experiments, no gross signs of benzodiazepine withdrawal (body weight loss, hyperactivity, convulsions) were observed in these subjects during this period of chlordiazepoxide abstinence. These data suggest that the spontaneously occurring or Ro15-1788-elicited decreases in water intake may be a sensitive bioassay for detecting benzodiazepine dependence. Based on the lack of effect exerted by acute treatment with Ro15-1788, there was no evidence of benzodiazepine dependence in the chronic alprazolam-treated subjects. It is possible, however, that higher doses or more frequent administration of alprazolam would result in an Ro15-1788-elicited withdrawal in chronic alprazolam-treated subjects.

Similar to chlordiazepoxide and unlike alprazolam, phenobarbital (80 mg/kg/day) treatment also did not produce a time-dependent increase in punished responding. Indeed, these animals exhibited a significant increase relative to vehicle-treated rats at Test Weeks 1 and 2, with a gradual decline in punished responding across Test Weeks 1–4. As was observed in the chronic chlordiazepoxide treatment study, these animals also exhibited mild to moderate sedation throughout the day, as evidenced by the decrease in water intake throughout the course of chronic treatment for most Test Weeks. It is possible that some portion of the tolerance to the anticonflict effects of phenobarbital was pharmacokinetic in nature, relating to the induction of hepatic microsomal drug metabolism. However, since there was little if any tolerance to the effects of phenobarbital to decrease water intake, it appears likely that the tolerance to the anticonflict effects of chronic phenobarbital treatment might also be pharmacodynamic in nature. Studies using chronic treatment with the nonmetabolized barbiturate barbital are planned to resolve this question.

The observation that chronic treatment with phenobarbital and chlordiazepoxide was associated with tolerance to the anticonflict effects is somewhat surprising, since it has been reported frequently that there is a lack of tolerance to, or even an increase in, the anticonflict effects of these agents associated with their repeated administration (9, 15, 21, 23, 25). One possible explanation for this discrepancy relates to the "intensity" of chronic treatment. In many of the studies where tolerance to the anticonflict effects was not observed, the duration of the "chronic" treatment was relatively short (i.e., less than one week), the doses administered were relatively low and/or the frequency of repeated drug administration was insufficient to facilitate the development of tolerance. In the present study, as well as in the studies reported by Soderpalm (28), Stephens and Schneider (31) and Vellucci and File (34), antianxiety agents were administered chronically at relatively high doses for greater than one week. In these studies, animals treated chronically with anxiolytics did indeed develop tolerance to the anxiolytic effects. Finally, it should be noted that the anxiolytic efficacy of benzodiazepines in man decreases over periods of repeated administration (8).

In summary, chronic treatment with the clinically effective antipanic benzodiazepine alprazolam resulted in a time-dependent anticonflict effect on behavior in the CSD paradigm. These data support further the hypothesis that conflict paradigms such as the CSD may be useful "animal models" for the study of panic disorder and potential antipanic agents. In contrast, chronic treatment with chlordiazepoxide or phenobarbital did not result in a time-dependent anticonflict effect. These findings are not consistent with the hypothesis that all anxiolytic agents possess antipanic activity.

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