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Antagonism by CGS 8216 and Ro 15-1788, Benzodiazepines Antagonists, of the Action of Chlordiazepoxide on a Timing Behavior in Rats

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NAKAMURA, M. AND J. M. CARNEY. *Antagonism by CGS 8216 and Ro 15-1788, benzodiazepine antagonists, of the action of chlordiazepoxide on timing behavior in rats.* PHARMACOL BIOCHEM BEHAV 21(3) 381–385, 1984.—Rats were trained to respond under a differential reinforcement of low rate (DRL) schedule of reinforcement. Pretreatment with relatively low doses of chlordiazepoxide (1–10 mg/kg) produced increases in total DRL responses and decreases in the numbers of reinforced responses. Chlordiazepoxide produced a shift in the interresponse time (IRT) distribution of DRL responses. Low doses of chlordiazepoxide shifted the IRT distribution of DRL responses. Low doses of chlordiazepoxide shifted the IRT distribution from the reinforced to the non-reinforced bins. In addition there was a marked increase in the number of responses that occurred in the earliest IRT bin (0–3.75 sec). The highest dose of chlordiazepoxide (32 mg/kg) produced a decrease in total DRL responses and resulted in an even IRT distribution of responses. Both CGS 8216 and Ro 15-1788 had minimal effect on DRL responding when given alone. Ro 15-1788 had no effect at either 10 or 32 mg/kg, while CGS 8216 produced decreases in DRL responding at 32 and 100 mg/kg. Both Ro 15-1788 and CGS 8216 antagonized the effects of high and low chlordiazepoxide doses on total DRL responding and on the IRT distribution of responding.

Chlordiazepoxide CGS 8216 Ro 15-1788 DRL responding Benzodiazepine antagonism

BENZODIAZEPINES are well known for their anxiolytic, hypnotic and anticonvulsant effects. In addition to their clinical effects, benzodiazepines have been shown to interact with schedule controlled behavior of laboratory animals. For example, Richelle *et al.* [10] have shown that the total response rate is increased at low doses of chlordiazepoxide (CDP), while it was decreased at higher doses in rats performing on fixed-interval (FI) schedule. Similar dose-dependent effects of benzodiazepines have been reported on variable interval (VI), fixed ratio (FR) and differential reinforcement of low rates (DRL) schedules [10, 12, 16, 17]. Sanger *et al.* [11,12] have shown that chlordiazepoxide produced increases in responses during the very short interresponse times bins (IRTs, response bursts) under a DRL schedule much more consistently than did d-amphetamine and caffeine. There are many reports to support that some pharmacological actions of benzodiazepines might be mediated through brain benzodiazepine receptors. Recently, synthetic compounds that antagonize benzodiazepine binding to brain receptors have been discovered [4,5]. Ro 15-1788 and CGS 8216, which both potently inhibited ^{3}H-diazepam binding to rat brain in vitro and in vivo, have been shown to antagonize the action of diazepam in several pharmacological tests and lack characteristic benzodiazepine-like activity [4,5]. Ro 15-1788 was found to be a selective benzodiazepine antagonist [5], while CGS 8216 not only antagonized several effects of diazepam but also of phenobarbital and meprobamate [2]. In this report we examine the effects of GCP on DRL behavior and the antagonist effects of CGS 8216 and Ro 15-1788 on these actions of CDP.

METHOD

Subject

The subject were male Sprague-Dawley rats (Sasco Inc, Omaha, NB) 70 days old and weighing between 250 and 300 g at the beginning of the experiments. Rats were maintained in the OUHSC animal facility under a 12/12 light dark cycle at 24°C. Water was freely available in the home cage. Rats were housed under these conditions for at least 7 days prior to the start of the study. Prior to training each rat was food deprived to 80% of its pre-experimental weights.

Apparatus

Experimental rat chambers (Colburn Instruments Inc.), 20 cm long×23 cm wide×20 cm high, were placed in insu-

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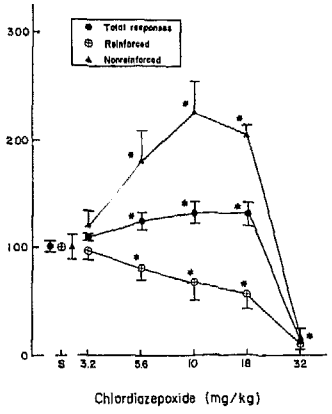

FIG. 1. Mean dose response curves for total DRL responses, reinforced and nonreinforced responding after injection of chlordiazepoxide. All data are expressed as percentages of control performance on the day before a drug day. The point of S show the percentage of control after a saline injection. The vertical lines represent ±S.E. *Significantly different from saline (Paired t-test, $p<0.05$).

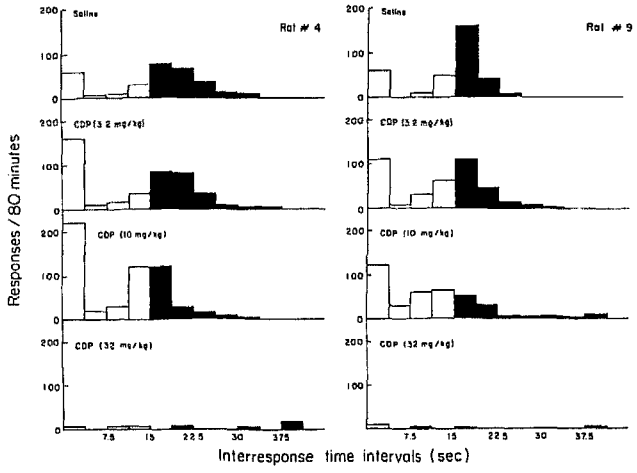

FIG. 2. The effect of saline and doses of chlordiazepoxide on the IRT distributions of two rats. Each bar represents a 3.25 sec interval. Shaded bars represent reinforced IRT's and open bars represent the nonreinforced IRT's.

lated boxes equipped with fans for ventilation and noise attenuation. Each chamber was fitted with two levers but only presses on the right hand lever had any programmed consequences. Pressing the lever (force, 30 g) resulted in the presentation of 45 mg rodent food pellet. The experiment was controlled by an on-line computer system [9] which also recorded total responses, reinforcements and the distribution of responses according to their interresponse time (IRT).

Procedure

After 3 days of acquisition on a FR 1 schedule of food reinforcement, a DRL-15 sec schedule was introduced. This required that responses be spaced at least 15 sec apart in order for reinforcements to be obtained. Responses occurring earlier than the minimum time reset a timer which reinitiated the interval times. Sessions were 80 min in duration and were given daily. When performance on the DRL schedule had stabilized (approximately 50 days), drug treatments were initiated.

Statistics

Response rates were calculated for the entire 80 min session. Efficiency of responding was calculated by dividing the number of reinforced responses by the total number of DRL responses. The response rates after drug injections were converted to a percentage of the response rate on the preceding day. The percentage of bursts was expressed as percent of total responses. The significance of drug pretreatment effects was determined using a Paired t-test.

Drugs

CGS 8216 (CIBA-GEIGY) and Ro 15-1788 (Hoffman-La Roche) were suspended with 0.5% methylcellulose. Chlordiazepoxide HCl (Hoffman-La Roche) was dissolved in 0.9% saline. Doses were given in a mixed order with at least 3 non-drug days separating each drug day. CGS 8216 was injected intraperitoneally 30 min before and Ro 15-1788 was injected at the same time with the intraperitoneal injection of CDP. The session began immediately after the CDP injection.

RESULTS

CDP produced a dose-dependent increase in total response rate and the rate of non-reinforced responding at doses up to 10 mg/kg, while the highest dose (32 mg/kg) reduced them (Fig. 1). Reinforced responding only showed dose-related decrease after chlordiazepoxide. Details of the effects of chlordiazepoxide can be seen in the IRTs frequency distribution in which responses were accumulated into the appropriate of eleven successive 3.75 sec IRT categories (Fig. 2). On the saline days of the IRT distribution shows a peak at approximately the first reinforced IRT. There are also a relatively large number of response of very short (less than 3.75 sec) IRTs which is referred to as bursts by Sanger *et al.* [11,12]. The increase in total nonreinforced response rate and the decrease in total reinforced response rate caused by CDP at lower doses was reflected by a shift in the IRT distribution to predominantly short IRTs and an increase in bursts. The 32 mg/kg dose of CDP decreased total response rate, reinforce response rate and flattened the IRT distribution. CGS 8216 at doses up to 10 mg/kg did not affect the total response rate, and did not change the relative distribution of reinforced and nonreinforced responding. However, 32 and 100 mg/kg CGS 8216 did decrease DRL responding (Fig. 3). The disruption of DRL responding by CGS 8216 was qualitatively different from CDP in that both nonreinforced and reinforced showed the same dose related decreases in responding. No increases in responding were observed at any dose. Ro 15-1788 at doses up to 30 mg/kg produced no significant change in total response rate, and no change in the relative distribution of reinforced and nonreinforced responding (data not shown). The effect of CGS 8216 and Ro 15-1788 on the action of CDP are shown in Fig. 4. CGS 8216 and Ro 15-1788 antagonized the increase in total response rate, the increase in nonreinforced responses and the decrease in reinforced responses induced by CDP at 10 mg/kg. The depressant effects on total response rate

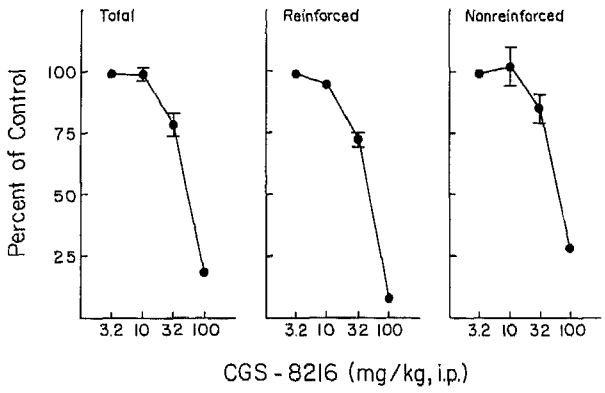

FIG. 3. The effect of CGS 8216 on a DRL 15 sec schedule. CGS 8216 was injected intraperitoneally 30 min before session. See Fig. 1 for further details.

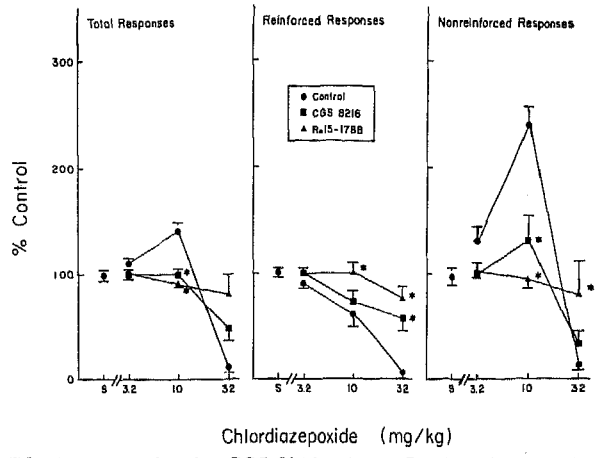

FIG. 4. Antagonism by CGS 8216 (○) and Ro 15-1788 (▲) of the effects of chlordiazepoxide on a DRL 15 sec schedule. CGS 8216 at 10 mg/kg was injected intraperitoneally 30 min before and Ro 15-1788 at 10 mg/kg at the same time with chlordiazepoxide. Control rats (○) were given injection of 0.5% methylcellulose. *Significantly different from control (Paired t-test, $p<0.05$). See Fig. 1 for further details.

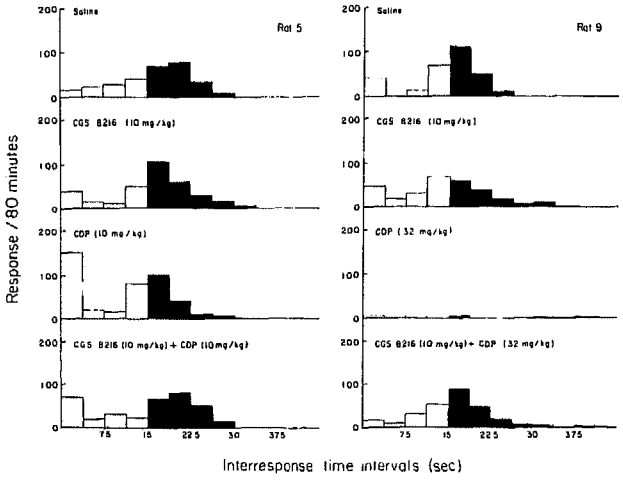

FIG. 5. Antagonism by CGS 8216 of the effect of chlordiazepoxide on the IRT distributions. See Fig. 4 for further details.

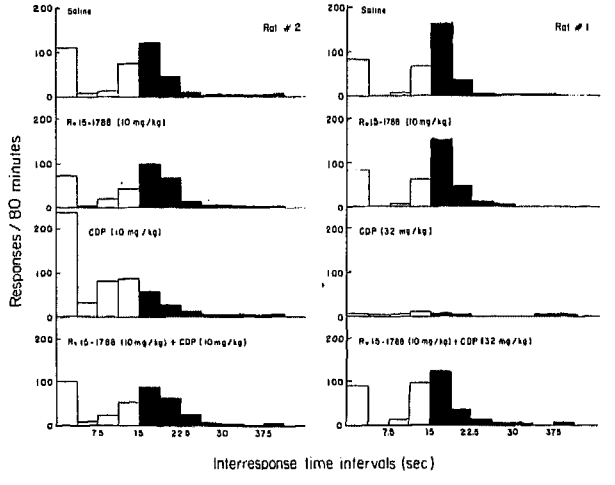

FIG. 6. Antagonism by Ro 15-1788 of the chlordiazepoxide induced changes in the IRT distribution of DRL responding. See Fig. 4 for further details.

produced by a higher dose of CDP (32 mg/kg) also were reversed by the treatment of CGS 8216 and Ro 15-1788. Both CGS 8216 and Ro 15-1788 normalized the disruption of the pattering of DRL response induced by CDP at 10 and 32 mg/kg (Figs. 5 and 6). Table 1 shows that all doses of CDP consistently produced an increase in the percentage of bursts. This occurred regardless of whether total response rate was increased or decreased. CGS 8216 alone did not affect the percentage of bursts at doses up to 10 mg/kg, and only decreased it at a highest dose. Ro 15-1788 at doses up to 30 mg/kg did not affect the percentage of bursts. However, CGS 8216 and Ro 15-1788 at 10 mg/kg blocked the increase in the percentage of bursts induced by CDP.

DISCUSSION

Administration of CDP to rats performing under on a DRL schedule increased total response rate and decreased reinforcement rate at lower doses. The highest dose (32 mg/kg) decreased both response rate and reinforcement rate. Similar effects have been found by other authors [5,13]. Many psychoactive agents have been shown to disrupt performance under DRL schedule. Sanger and Blackman [12] reported that chlorpromazine produced a decrease in both total response rate and reinforcement rate. Tricyclic antidepressants have been shown to decrease total response rate and increase reinforcement rate [7]. Psychostimulants such as amphetamine and caffeine have been reported to increase total response rate and decrease reinforcement rate at low doses, while the higher doses of each drug markedly depressed both rates [1, 6, 14]. Sanger *et al.* [11,13] have reported the detailed examination of the effect of CDP, caffeine and amphetamine on IRTs generated by a DRL

TABLE 1

EFFECTS OF DRUGS ON TIMING BEHAVIOR

Drugs	mg/kg	Percentage of "Bursts"	Total Response Rate (Response/Session)
Saline		18.1 ± 1.3	320 ± 11
Chlordiazepoxide	3.2	25.3 ± 1.8*	370 ± 18
	10	35.5 ± 4.1*	465 ± 39*
	32	29.9 ± 4.1*	18 ± 5*
CGS 8216	10	16.8 ± 2.8	307 ± 12
	32	14.5 ± 2.7	262 ± 11*
	100	16.3 ± 4.5	56 ± 12*
CGS 8216	10		
+ Chlordiazepoxide	10	17.3 ± 1.6†	314 ± 14†
Ro 15-1788	10	20.2 ± 2.6	319 ± 12
	32	17.8 ± 3.1	306 ± 14
Ro 15-1788	10		
+ Chlordiazepoxide	10	22.6 ± 3.5†	300 ± 13†

Each value is the mean ± S.E.
*Significantly different from saline (Paired t-test, $p<0.05$).
†Significantly different from chlordiazepoxide alone (Paired t-test, $p<0.05$).

schedule in rats. CDP was found to reliably increase the percentage of "bursts" while d-amphetamine and caffeine had no consistent effects on this measure. Cannon and Lippa [3] also have reported that d-amphetamine and diazepam reduced the reinforcement rate but only diazepam produced a consistent increase in the nonreinforced "bursts" responding. In general anxiolytics would be expected to decrease reinforcement rate and increase burst responding. The results of the present experiment confirm this suggestion.

It has recently been reported that Ro 15-1788 and CGS 8216 were potent and specific antagonists of benzodiazepine binding both in vitro and in vivo, and that they inhibited many central actions of benzodiazepines such as anticonvulsant, muscle relaxant, hypnotic and anxiolytic actions [2, 4, 5]. CGS 8216 and Ro 15-1788 antagonized the decrease in reinforcement rate and the increase in the percentage of "bursts" induced by CDP. CGS 8216 itself did not affect a DRL-behavior at low doses, while a higher dose range decreased the total response rate, reinforcement rate and the percentage of "bursts." Ro 15-1788 itself at doses up to 32 mg/kg did not show any effect on DRL behavior. These results replicate and extend previous reports that CGS 8216 and Ro 15-1788 were selective antagonists of benzodiazepines and devoid of any benzodiazepine-like activity.

Saturable, high-affinity, and stereospecific sites for benzodiazepines in the central nervous system are thought to mediate many of the pharmacological effects of benzodiazepines [8,15]. A good correlation has been found between the inhibitory potency of active benzodiazepines in ^{3}H-diazepam binding in vitro and their potencies in exciting the central actions [8,15]. Since the increase in the percentage of bursts and the decrease in reinforcement rate produced by CDP was blocked by the pretreatment of CGS 8216 and Ro 15-1788, these actions of CDP on DRL behavior may be included in the various behavioral effects mediated by brain benzodiazepine receptors.

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