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Chlordiazepoxide's Interaction with Ethanol Intake in the Rat: Relation to Ethanol Exposure Paradigms[1]

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ROEHRS, T., O YANG AND H SAMSON *Chlordiazepoxide's interaction with ethanol intake in the rat Relation to ethanol exposure paradigms* PHARMACOL BIOCHEM BEHAV **20**(6) 849–853, 1984.—Chlordiazepoxide's interaction with ethanol (5% v/v) intake was assessed in rats on a feeding regimen producing high daily quantities of ethanol intake (schedule-induction procedure with intermittent feeding), more moderate amounts of ethanol intake (a single daily feeding), and small amounts of ethanol intake (free feeding). Six days of twice daily sham injections (IP) were followed by 12 days of 0 (vehicle), 5, 10, or 15 mg/kg (twice daily) chlordiazepoxide, and finally six days of the saline (vehicle) injections Rats in the intermittent feeding daily consumed 9 9–12 3 g/kg (80–95 ml) of ethanol on baseline which was reduced 15 to 33% by the drug In the single feed condition most rats were drinking 70 to 85 ml (8.8–10 3 g/kg) of ethanol and this was reduced 15–40% by the drug During the six days after drug, intake in both of these feeding regimens returned to the baseline level Ethanol intake of rats under the free feeding condition (48 ml, 3.5 g/kg on average) was not affected by the drug, nor was water intake under any of the three feeding regimens

Schedule induction Ethanol intake Chlordiazepoxide Rats

A large number of studies done in humans and animals have shown that benzodiazepines interact with ethanol to affect various behaviors. For example, diazepam and ethanol produced greater decrements in human psychomotor performance than either alone [6]. Oxazepam or diazepam in combination with ethanol produced a more marked disruption of human perceptual function than that obtained with drug and delayed ethanol administration [8]. In a conditioned suppression experiment diazepam and ethanol had an antagonistic effect on responding of pigeons during the shock-warning stimulus, but a synergistic effect on safe-period responding [2]. While there are many studies of the combined effects of ethanol and benzodiazepines on a variety of behaviors, there is little information regarding the interactions of benzodiazepines with ethanol drinking.

Several different patterns of ethanol drinking have been produced in the rat [12]. These drinking patterns differ in the g/kg of ethanol consumed per day, in the temporal distribution of drinking throughout the day, and in the potential to lead to physical dependence [13]. It is well established that rats under free-feeding conditions (FF) will drink ethanol concentrations of 4–6 % (v/v) or lower when ethanol is the only available drinking fluid [9]. Studies in our laboratory have found daily intake of 5% ethanol was similar in volume of water intake, yielding approximately 4 g/kg of ethanol consumed per day [10]. These moderate intake levels do not produce detectable blood ethanol elevations for any substantial period within a given day. Under a limited feeding regimen in which a single food ration (SF) is given each day rats drank 11.7 g/kg of ethanol per day with a single peak in blood ethanol level reaching 150 mg/100 ml [13]. However, signs of physical dependence were not observed in these rats even after prolonged daily ethanol drinking. One method which produced excessive and intoxicating ethanol intake leading to physical dependence used the schedule-induction procedure [3] in which animals are fed small portions of food intermittently (IF). In this procedure, rats were exposed to a 24-hour feeding schedule consisting of six one-hour food delivery periods separated by three-hour intervals. During the one hour food delivery periods a 45 mg pellet was delivered every two minutes. Under these conditions rats ingested a daily average of 13.1 g/kg of ethanol and maintained blood ethanol levels above 100 mg/100 ml throughout most of the day [3,4]. After three months of exposure to this procedure removal of ethanol produced symptoms of physical dependence including death from tonic-clonic seizures.

One of the few reported studies of a benzodiazepine's effect on ethanol drinking found a dose-dependent and

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ethanol-specific effect. The acute effects of chlordiazepoxide (CDP) on schedule-induced water or 3% ethanol (v/v) drinking was examined in three monkeys [1]. All doses tested enhanced schedule-induced water intake while 1 and 3 mg/kg enhanced and 10 and 17 mg/kg suppressed ethanol intake These results suggest that there is a specific drug interaction apart from an effect on drinking behavior per se Thus it is important to compare a drug's effect on ethanol drinking with its effect on water drinking.

This study assessed the dose-related effects of CDP on ethanol or water intake. Drug effects were examined in rats under feeding regimens previously shown to induce high daily quantities of ethanol intake with a single blood ethanol peak, high intakes with elevated blood ethanol levels throughout the day, and small daily amounts of ethanol intake with no blood ethanol elevations. These effects were compared to effects on water intake under the same feeding regimens

METHOD

Animals

Thirty-six Long Evans strain male rats, bred in the Psychology Department at the University of Washington and 90 days old at the start of the experiment, were randomly assigned to one of three experimental conditions They ranged in body weight from 307 to 487 g and there were no difference among the three groups in mean body weight. Within each experimental condition the twelve were assigned randomly to receive one of four CDP doses: 0 (vehicle only), 5, 10, and 15 mg/kg.

Experimental Environments

All rats were housed individually. Artificial lighting was regulated on a 12 hr light and 12 hr dark cycle. Room temperature was maintained at 78°F. Rats maintained on the intermittent feeding condition (IF) were housed in 12×11×10 inch Plexiglas chambers with stainless steel bar floors. On the front wall of each chamber was a food cup with a stainless steel, ball-point drinking tube with a 250 ml graduated cylinder mounted on the side wall. Food pellets (45 mg Noyes, Formula A) were delivered automatically to each cage by a pellet dispenser (Gerbrands). The single feed condition (SF) and the free feed condition (FF) rats were housed in standard, stainless steel, hanging rodent cages. Drinking tubes and fluid reservoirs, the same as those of the IF rats, were mounted on the front wall of the cage. Food was standard Purina Laboratory chow placed on the cage floor.

Procedure

Rats assigned to the IF and SF conditions were gradually reduced to 80% of free feeding body weight by limiting daily food intake. After body weights had stabilized at the 80% level the IF rats were placed on the same intermittent feeding schedule described by Falk *et al* [3]. On this schedule a food pellet (45 mg) was delivered every two minutes during six one-hour periods every 24 hr (180 pellets totaling 8.1 g per day). Each one-hour delivery period was separated by a three-hour period of no food. At 0930 hr each day after being weighed, if necessary, a food supplement was given to maintain each rat at the 80±2% level. The SF rats were fed their food ration (8.1 g as the IF rats and any supplement necessary to maintain at 80±2% level) as a single meal at the same time each day (0930 hr), which remained on the cage floor until consumed By daily adjusting the food supplement each rat could be maintained at 80±2% of free feeding body weight. The FF rats had continuous access to food

The drinking fluid, continuously available, was either water or 5% (v/v) ethanol During the first phase of the experiment water was the only available fluid and during the second phase ethanol was the available fluid. A two week adaptation to the feeding regimen and drinking fluid was given before the injections were begun in each of the two phases of the experiment.

All injections were given IP twice daily between 0630–0730 and 1830–1930 hr. To adapt the rats to the injection procedure, for six days the rats were handled and injected without delivering the fluid (sham injections) Then drug injections were given for twelve days CDP (Hoffman La Roche) stored in the dark and prepared fresh for each injection, was dissolved in isotonic saline (10 mg/1 ml) Doses of CDP were 0 (vehicle only), 5, 10, and 15 mg/kg given at each injection. These doses were chosen because they previously had been shown by other studies to affect water intake [7,14] and by preliminary tests in our laboratory to affect ethanol intake. Of the 12 rats in each experimental feeding condition, 3 rats each, randomly assigned, received a given CDP dose or control saline injection (0.5 ml in volume) The appropriate injection volume for each animal was calculated daily based on its daily body weight measurement. After the twelve days of drug injections, saline injections, the same volume as received during the drug injections, were given for six days. Following the injections water remained as the available fluid for two more weeks before ethanol was introduced

The second phase of the experiment was conducted as the first phase with the exception that ethanol (5% v/v) was the only available drinking fluid. Before beginning the second injection regimen, a two week period of adaptation to the ethanol was given Thus between the two series of drug injections there was a total of six weeks without drug administration, which was considered sufficient time for all CDP metabolites to be excreted [5]. During the second phase of the experiment each animal received the same CDP dose or control saline injection as it received in the first phase

Throughout the experiment at the same time each day, fluid intakes (to the nearest ml) and body weights were recorded, fluid reservoirs were replenished, and general maintenance was done Food supplements (Purina chow) were given to the IF and SF rats to maintain them at 80% free-feeding body weight

Mean daily intakes in ml and g/kg based on daily body weight measures were calculated over three-day blocks for two blocks of sham injections at baseline, four blocks of drug administration, and two blocks of recovery. Analyses of single day intakes did not differ from the mean data taken over three-day blocks. Mean fluid intakes then were compared using two factor mixed design ANOVAs and Tukey post hoc comparisons. The between groups factor was dose and the within factor three-day blocks A separate analysis of ethanol and water intake was done for each of the three feeding conditions

RESULTS

Ethanol intake of rats in the IF condition is presented in Fig 1. Baseline intake of ethanol during sham injections (blocks 1 and 2) varied from a mean of 80–95 ml among the four groups. This represents a mean of 9 9–12.3 g/kg of ethanol per day among the groups (see Table 1) With administration of CDP (blocks 3–6) ethanol intake (ml) was re-

TABLE 1

MEAN g/kg ETHANOL INTAKE OVER THREE-DAY BLOCKS

	Sham		Drug				Recovery	
	1	2	3	4	5	6	7	8
Intermittent Feed Condition								
00 mg	12.1	12.3	11 8	12 5	11.0	11 6	12.5	12 5
05 mg	9 8	10.0	7 8	7 6	6.7	7 7	9 1	9 2
10 mg	12 3	12.3	9 8	9 9	9.4	10 8	12.3	11 9
15 mg	11 0	11.1	8.8	8.9	7 7	8 7	11 0	10 8
Single Feed Condition								
00 mg	10 4	10 3	10 4	10 4	10 7	10 8	10 5	9.8
05 mg	12 4	12.8	11 2	10.4	11 0	11 0	12 6	12 4
10 mg	9 7	9 5	8 8	9 5.	8 5	8 9	10 5	8 4
15 mg	8.4	9 2	5 6	7 1	7.0	7 1	8.8	8 3
Free Feed Condition								
00 mg	3 3	3 2	3 1	3 3	3 2	3 6	3.6	3 1
05 mg	3 6	3 4	3 5	3 8	3 6	3.6	3 2	3 3
10 mg	4.0	3 9	3 8	4 2	3.9	3 9	3 7	4.0
15 mg	3.6	3 5	3.5	3 5	3 3	3 4	3 0	3 1

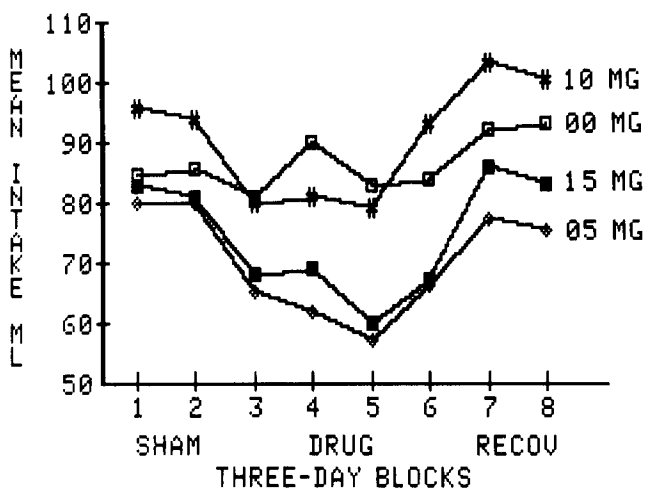

FIG 1 Mean ethanol intake (ml) of rats in the intermittent feeding condition (IF) over three day blocks for each dose of chlordiazepoxide

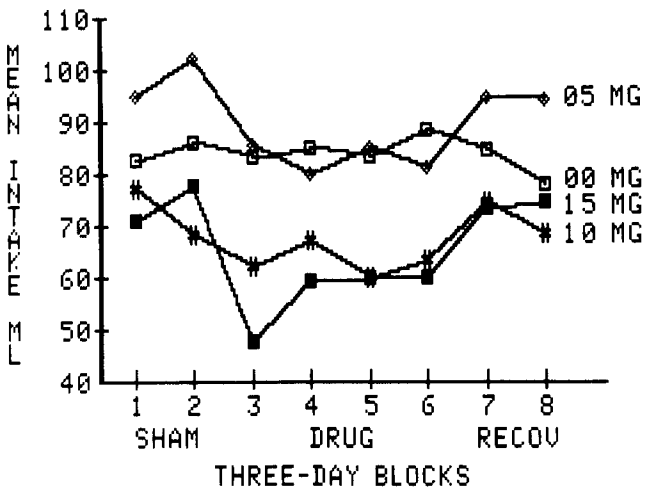

FIG. 2 Mean ethanol intake (ml) of rats in the single feeding condition (SF) over three day blocks for each dose of chlordiazepoxide.

duced to between 67% and 85% of this baseline level. The analysis revealed a significant day effect ($F=9.72$, $p<0.001$) with each three-day block differing from baseline ($p<0.05$). During the recovery (blocks 7 and 8) intake again returned to the baseline level (varying from 94% to 108% of baseline). The drug effects on mean g/kg intake were similar (see Table 1). The main effect of dose on intake (ml) and the interaction of dose with days was not significant. As seen in Fig. 1 mean intake (ml) during the treatment period in the placebo group varied between 95% and 106% of baseline. There were no significant group differences in intake which would indicate systematic effects as a function of dose.

The ethanol intake of animals in the SF condition is presented in Fig. 2. They drank on the average per group between 70 and 85 ml on baseline except for one group which drank a mean of 99 ml. The mean g/kg intake among the groups varied from 8.8 to 10.3 and for the three divergent animals (the 5 mg group) it was 12.6. Administration of CDP significantly reduced ethanol intake, again, to between 60% and 85% of baseline. Each three-day block differed significantly from the sham injection baseline ($F=6.89$, $p<0.01$). There also were significant between group differences ($F=7.23$, $p<0.01$) with 10 and 15 mg doses differing from 5 mg, but not placebo (00 mg). However, these differences do

TABLE 2
MEAN WATER INTAKE (ml) OVER THREE-DAY BLOCKS

	Sham		Drug				Recovery	
	1	2	3	4	5	6	7	8
Intermittent Feed Condition								
00 mg	56 3	69 3	67 0	65 3	66.3	68 7	63 0	62 3
05 mg	31 3	30 7	32 3	39 3	34 0	35 0	26 7	35 0
10 mg	81 7	91 3	63 3	56 7	59 0	55 0	47 0	47 7
15 mg	43 0	41 7	42 0	49 0	44 3	46 3	42 3	46 7
Single Feed Condition								
00 mg	50 7	50 7	54.3	48 3	49 7	47 3	46 0	41 0
05 mg	41 3	44 7	37 7	47 7	50 7	43 0	45 0	39 7
10 mg	38 7	35 7	41 0	42 3	40 0	42 0	40 3	34 7
15 mg	44 0	41.7	40 0	48 3	45 3	46 0	52 3	41 3
Free Feed Condition								
00 mg	49.0	47 0	48.0	45 0	47 0	46 3	52 3	50 3
05 mg	51 0	49 3	55 3	50 0	53 7	51 3	50 7	52 7
10 mg	54 7	44 3	49 7	44 0	50 3	50 3	51 7	48 7
15 mg	47 3	49 0	55 7	52 3	61 7	61 7	60 7	59 3

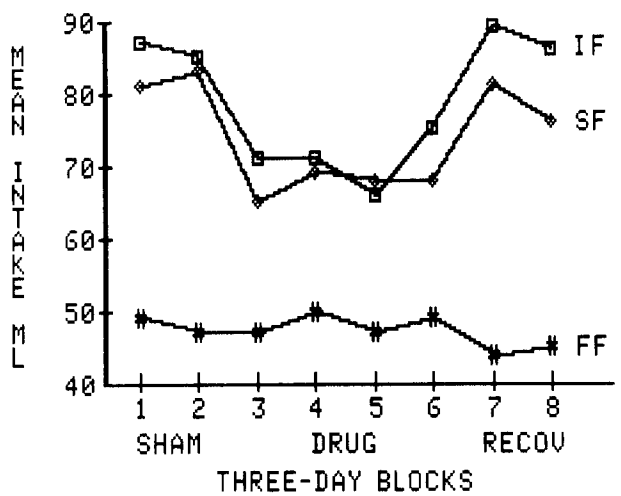

FIG 3 Mean ethanol intake of rats in each feeding condition over all active chlordiazepoxide doses

not reflect a systematic dose-related effect, but rather differences in initial baseline intakes among the groups. During the post drug period ethanol intake once again returned to the previous baseline levels. Intake expressed as mean g/kg changed in a similar fashion (see Table 1).

On baseline the animals in the FF condition drank on the average 48 ml of ethanol (3.5 g/kg). Administration of CDP had no effect on their intake. Figure 3 presents the mean intake (over all active doses) of the FF animals and compares it to the mean intake (over all active doses) of animals in the SF and IF conditions. Intake of the FF animals did not vary by more than 5% from baseline during the period of drug administration. This is in contrast to the reduction of intake seen for animals in the SF and IF conditions.

Water intake among animals in the three different feeding conditions for each dose is presented in Table 2. Administration of CDP had no effect on the intake of animals in the FF or the SF condition. Intake changed significantly among animals in the IF condition (days by dose interaction, $F=5.18$, $p<0.01$) However, post hoc testing indicated this change was due only to the high intake on baseline of the three animals at the 10 mg dose.

DISCUSSION

The ethanol intake levels under the three different feeding regimens seen on baseline with sham injections, in general, were similar to those reported previously [5, 6, 7]. The rats in the FF condition drank small amounts of ethanol, rats in the SF condition some what higher amounts, and rats in the IF condition the highest amount of ethanol. While blood ethanol concentration was not evaluated, it is expected that these rats maintained the previously reported characteristic blood ethanol patterns for that particular feeding regimen. Of note is the variability at baseline among rats within a given feeding regimen. Three rats in the SF condition (the 10 mg group) had an unusually high intake and three rats in the IF condition (the 5 mg group) had an unusually low intake. This variability at baseline was probably an artifact of the sham injection procedure.

The results showed a drug effect which was specific to the intake of ethanol. Intake of water, except for three rats, was not affected by CDP. The high water intake of these rats at baseline was reduced during drug administration. However, after drug was removed intake did not return to the baseline, suggesting that the effect was due to factors other than CDP administration. Thus CDP did not appear to affect drinking per se, but rather the drinking of ethanol

Of further interest is the fact that CDP affected only rats in the two feeding conditions in which high levels of ethanol

were consumed. Sufficient ethanol was ingested under these two conditions to produce ethanol intoxication. However, in addition to its pharmacologic effect ethanol also has caloric value. It is noted that rats in these conditions were partially food deprived. Intake in these conditions was reduced despite the fact that animals lost calories associated with drinking the ethanol. As a result daily food supplements necessary to maintain animals at 80% levels had to be increased. This suggests that there was a specific interaction of CDP with ethanol's pharmacologic properties. Of course, volume of ethanol intake (ml) may also be an important consideration. We did not attempt to separate these factors in the present experiment. We have recently shown that rats under an IF condition drank two times the volume of 5% ethanol than of 10% ethanol, but ingested a similar g/kg of ethanol per day [11]. A study of CDP's effect on intake of 5% or 10% ethanol would be interesting. If in fact there is a specific interaction of CDP with ethanol's pharmacological effect, one would expect that intake of 5% and 10% ethanol would be reduced by a similar g/kg ethanol.

Finally, of note, no dose-related effects on ethanol intake were found. A number of factors could explain the absence of a dose effect. First the variability among rats at baseline could have made it difficult to demonstrate a dose effect. A second factor is that measurement of the dependent variable (ml intake over 24 hr) was too gross, which made it difficult to detect significant differences. However, a more likely explanation is that accumulation of active metabolites occurred and or relatively high daily doses (10–30 mg/day) were used in fasted animals [15].

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