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Chlordiazepoxide and the Drinking of Water by Rats: Effects of Shock and Other Suppressive Measures

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SHEPHARD, R. A. *Chlordiazepoxide and the drinking of water by rats: Effects of shock and other suppressive measures.* PHARMACOL BIOCHEM BEHAV 31(2) 313–316, 1988.—The effects of chlordiazepoxide (5 and 10 mg/kg) on fluid consumption in water deprived rats were assessed. Drinking was inhibited to approximately equal extents by a water preload, by d-amphetamine (1.5 mg/kg), by neophobia and by shock at mild (0.3 mA) or moderate (0.5 mA) intensities, the latter condition having an enhanced level of deprivation also. At both doses chlordiazepoxide significantly enhanced drinking in the neophobia, mild shock and, especially, the moderate shock condition but failed to increase drinking suppressed by preload or d-amphetamine. It is concluded that the increases in drinking suppressed by neophobia or shock which chlordiazepoxide induces may be due to anxiolytic actions of the drug or to enhanced palatability since they cannot be explained in terms of nonspecific enhancement of fluid consumption.

Chlordiazepoxide Drinking Conflict Neophobia Anxiolysis Amphetamine Rats

THE behavioral effects of anxiolytic drugs are commonly assessed in what are known as "conflict tests." In such tests, behavior is suppressed by aversive events such as electric shock or unfamiliarity and anxiolytic drugs facilitate behavior. One of the most extensively used conflict tests is the punished drinking procedure (23), in which thirsty rats are given the opportunity to drink but are punished for so doing, say, by a mild electric shock for a given amount of cumulated drinking time. Initially described as a procedure for naive subjects, it has been shown to be even more sensitive and reliable when used with test-sophisticated rats (13).

For some time, however, it has been evident that benzodiazepines not only enhance drinking which has been suppressed by electric shock, but increase fluid consumption in other circumstances too (4). In addition to electric shock, conditions which favor increased drinking with benzodiazepines include prior deprivation (7, 19, 22) and offering flavored or calorific solutions, even when such solutions are familiar to, and preferred by, the subjects (4,8). Such behavioral actions of benzodiazepines cannot readily be explained by reference to effects on anxiety systems and it therefore seems that these drugs have dipsogenic actions and the capacity to enhance fluid palatability (1). This opens the possibility that enhancement of punished drinking by benzodiazepines may be mediated by dipsogenesis or other processes independent of any "anxiety" or "conflict" mechanism. If the punishment contingency is indeed irrelevant to drug action on drinking, there would be no reason for retaining it in experimental studies since punishment complicates their conduct and interpretation (21) and unnecessary use of shock would also be indefensible ethically.

Separating the relative role of dipsogenic and anxiolytic actions of such drugs as the benzodiazepines in determining the increased punished drinking which they induce is therefore an important question. One way of addressing this is to compare the magnitude of drug action on punished drinking with that on unpunished. If these were equal, there would seem no reason to suppose anxiety to be involved in the punished drinking procedure. Superficial examination of the literature does not support this equality; for example, a number of anxiolytic drugs increase punished drinking to approximately 4000% of control (13), whilst such drugs typically elevate unpunished drinking to about 150% or 200% of control (4,8). Such comparisons are, however, invalid for at least two reasons. Firstly, studies of punished drinking tend to report time spent drinking or some direct function of it as the main or sole measure, whereas studies of unpunished drinking generally report the amount drunk as the chief dependent variable. Secondly, and probably more importantly, studies of punished drinking usually have a much lower control baseline of behavior on which benzodiazepines may act. This circumstance obviously favors larger increases in punished drinking with benzodiazepines, particularly when such increases are expressed as percentages of control (21).

Since published studies of punished and unpunished drinking are not directly comparable, the present investiga-

TABLE 1
EFFECTS OF CHLORDIAZEPOXIDE ON FLUID INTAKE (ml) IN 30 MIN

Chlordiazepoxide Dose (mg/kg)	Condition				
	Preload	d-Amphetamine	Neophobia	Weak Conflict	Strong Conflict
0	7.69 ± 1.20	7.00 ± 2.64	8.63 ± 1.36	8.13 ± 2.10	6.82 ± 2.55
5	7.61 ± 1.79	7.14 ± 0.55	11.50 ± 1.91*	12.44 ± 4.35†	15.61 ± 1.86†
10	8.28 ± 2.02	9.03 ± 2.83	12.94 ± 2.20†	16.07 ± 1.89†	19.48 ± 2.63†

Figures shown are means (n=10) ± S.D.
*Significant at 1% level; †significant at 0.1% level.

tions were carried out. The fluid consumption of water deprived rats was suppressed to about 50% of control by each of the following manipulations: Water preload, d-amphetamine (1.5 mg/kg), neophobia (unfamiliar taste), as well as "weak" (standard deprivation, low shock) and "strong" (enhanced deprivation, moderate shock) "conflict" conditions. The effects of chlordiazepoxide (5 mg/kg or 10 mg/kg) on these behaviors were evaluated.

METHOD

Subjects

Subjects were 50 adult male Wistar rats, bred in our laboratory, caged individually and weighing 350–450 g throughout the study. They were maintained on a 12 hr light/dark cycle at 22–25°C. The rats were randomly allocated to five groups of ten, three of which were used for the neophobia part of the study, one for the preload and d-amphetamine parts and one in the shock conditions. Apart from the neophobia experiments, fully randomised repeated measures designs were used and appropriate statistical analyses (analysis of variance, *t*-tests) used.

Apparatus and Procedure

General. Except as detailed in the following sections, a general protocol was adopted. Rats were thoroughly acclimatised (at least 20 days) to presentation of tap water for only 1 hr per day (11.00 to 12.00 G.M.T. except on test days when they were given water for 1 hr after testing) and to all apsects of the experimental apparatus and procedure (including administration of IP injections). They had free access to water at this time and to food at all times except during actual experimental observations. Tests were conducted in the home cage for the preload, d-amphetamine and neophobia conditions, and in an "Anxiometer" (Columbus Instruments Inc.) for the shock conditions. The latter differed from the home cages in several respects, but all subjects were given at least eight exposures to it with the shock contingency described for weak conflict below before any data reported here were gathered. Fluid intake was measured by attaching pipettes securely to the drinking tubes and recording the level (to an accuracy of 0.1 ml) every 6 min for the 30 min period of the test, though for clarity, only the total drunk in the whole test is reported. The "Anxiometer" also gave a measure of "number of licks" (a direct function of the cumulated time that rats were in contact with the drinking tube). The control (i.e., with no drugs or suppressive treatments) level of drinking under these circumstances in 30 min was 16.28±1.46 (S.D.) ml. Extensive pilot work established parameters which would reduce fluid consumption to about (within one S.D.) 50% of this level and these parameters were used as described below.

Chlordiazepoxide hydrochloride (Roche Products Ltd.) and d-amphetamine sulfate (Sigma Ltd.) were dissolved in 0.85% NaCl and given IP 30 min prior to test, all doses being expressed as salt. Vehicle controls were used throughout, and all tests were conducted between 12.00 and 16.00 G.M.T. Data reported were from tests separated by at least 72 hours.

Preload condition. Water consumption in this group was inhibited by giving the rats access to 10 ml of water six hours prior to test. Rats generally consumed this in 10–20 min.

d-Amphetamine condition. Suppression of drinking in this group was induced by IP injection of d-amphetamine (1.5 mg/kg), which has hypodipsic effects (20). Where both d-amphetamine and chlordiazepoxide were given, this was as a single injection.

Neophobia condition. Suppression of drinking was achieved by offering an unfamiliar solution (containing both 2 mM sodium saccharin and 1 mM citric acid) to the subjects. Data reported are for subjects' only exposure to this solution.

Weak conflict condition. For this condition, rats had access to water in the Anxiometer, but after every 2.86 sec of cumulated contact with the drinking spout, a shock of 0.3 mA intensity was delivered between it and the cage floor. The duration of the shock was preset at 2 sec but the shock was only experienced by the subjects as long as they remained in contact with the tube. In practice, withdrawal seemed extremely rapid and certainly well within 0.5 sec.

Strong conflict condition. Rats were transferred to this condition only after completion of the weak conflict studies since the effects of enhanced shock levels can be prolonged. This strong conflict condition differed from the weak only in that the daily watering was omitted on the day prior to the test and that the shock intensity was set at 0.5 mA.

RESULTS

The effects of chlordiazepoxide on the volume of fluid consumed in the five conditions are presented as Table 1. The amount of drinking occurring in the various conditions without chlordiazepoxide was roughly constant. However, chlordiazepoxide differentially affected drinking, being without effect in the preload and d-amphetamine conditions, but producing highly significant increases in consumption suppressed by neophobia or shock. Such increases occurred at both 5 and 10 mg/kg, the latter dose being somewhat more

effective. For the conflict studies, drinking was approximately returned to control (i.e., unshocked) levels by chlordiazepoxide.

Although some data for the time subjects spent in contact with the drinking tube or "number of licks" were gathered, these are not reported here since this measure seemed directly proportional to the amount of fluid drunk. Thus, in both the conflict conditions described and also in pilot work for other conditions the amount drunk divided by the number of licks was essentially a constant and not significantly affected by any of the suppressive events or by chlordiazepoxide.

DISCUSSION

In these studies, chlordiazepoxide did not significantly enhance water consumption which was suppressed by a preload. Although previous studies have shown a number of benzodiazepines to increase deprivation-induced drinking (3, 4, 8, 19) their effects on drinking in satiated rats are more equivocal. Whilst moderate increases in such drinking have been reported for chlordiazepoxide (11,25), negative findings have been reported for diazepam (24) and for valproate, which shows may behavioral effects of benzodiazepines (16). The methodology most comparable with the present work is a study in which deprived rats were given 5 min access to water shortly before testing. Under these circumstances chlordiazepoxide, as well as clonazepam, midazolam and RO 15-1788 did not significantly increase drinking (4).

Similarly, the present studies did not show significant enhancement of d-amphetamine-suppressed drinking with chlordiazepoxide. Although benzodiazepines antagonise the effects of amphetamines on other behaviors such as feeding (5), conditioned taste aversion (2) and hyponeophagia (17), actions of this combination on drinking have not previously been reported.

By contrast, chlordiazepoxide at both doses produced large and highly significant increases in drinking suppressed by shock. This finding is generally consistent with previous ones (7, 10, 11, 13, 23) except that the parameters used here induced only a mild degree of suppression and a baseline of drinking in the control subjects similar to the other conditions. Therefore it cannot be maintained that the efficacy of chlordiazepoxide in enhancing shock-suppressed drinking is merely a function of the baseline level of drinking (7,21). Alternative explanations must be considered of which most obvious is reversion to the original interpretation of this procedure as an anxiety model (23). Chlordiazepoxide is more effective in the "strong" conflict phase of this study than in the "weak"; this difference is significant (5% level or beyond) at both dose levels. This finding is analogous to other conflict tests such as punished operant behavior and hyponeophagia in both of which a strong degree of apparent conflict maximises the behavioral effects of anxiolytic drugs (15,18). Drug effects on amount and duration of drinking in these studies were essentially parallel. However, it would seem prudent to measure both of these in future work in view of the reported dissociation of the effects of chlordiazepoxide on them (10) and other possible effects on response topographies (4).

Chlordiazepoxide significantly increased drinking suppressed by novel taste at both doses, in line with its effects on food and apparatus neophobia (15,18). Chlordiazepoxide appears to overcome this neophobia and these data would seem to ally this procedure with shock-suppressed drinking as a model of anxiety. For the practical purpose of selecting anxiety models for experimental work however, it should be noted that some of the advantages of neophobia procedures have to be set against the requirement for large numbers of naive subjects per experiment (15). This is amply illustrated in the present work where 60% of the subjects yielded only 20% of the data.

It is important to note that the sodium saccharin/citric acid combination solution used for the neophobia part of this study does not have an aversive taste. That its consumption is depressed relative to that of water in undrugged rats is therefore presumably due to its novelty. However, since benzodiazepines are known selectively to increase ingestive responses to both palatable fluids (1) and foods (26), it may be that chlordiazepoxide effects on palatability influence these results. Moreover, it is also possible that preload and d-amphetamine reduce fluid palatability, as the latter certainly does in conditioned taste aversion studies (2). Electric shock, however, seems to lack effect on palatability (1). Therefore, the present results could theoretically be accounted for in terms of a selective effect of chlordiazepoxide in situations of relatively high fluid palatability, that is, the neophobia and shock studies, but further work would be required to substantiate this hypothesis.

The possible anxiogenic effects of drugs have been assessed by determining their effects on unpunished drinking and on drinking punished by shock of low intensity which produces no behavioral suppression. Selective reduction of mildly punished drinking is considered evidence of anxiogenic effects since the behavioral baseline is identical. Such selectivity has been reported for picrotoxin and bicuculline (6,12), for pentylenetetrazole (6,14) and for CGS 8216 (9,12), though the latter has been disputed (14). These present studies are analogous in that the behavioral baseline of drinking is controlled and that the changes induced by the drug are selective. Chlordiazepoxide-induced increases are confined to those paradigms which would seem to have a component of anxiety, that is, neophobia and shock-suppressed drinking and the increases cannot be artifacts of the behavioral baseline of drinking.

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