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Effects of Benzodiazepines on Taste Aversions in a Two-Bottle Choice Paradigm[1]

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ROACHE, J. D. AND J. E. ZABIK. *Effects of benzodiazepines on taste aversions in a two-bottle choice paradigm.* PHARMACOL BIOCHEM BEHAV **25**(2) 431–437, 1986.—Diazepam (DZ) and chlordiazepoxide (CDP) were tested for their ability to antagonize LiCl-established conditioned taste aversions (CTAs) to saccharin in a two-bottle free-choice paradigm. CTAs to saccharin were established in male Sprague-Dawley rats on a chronic fluid-deprivation schedule by the administration of LiCl (3 mEq/kg, IP) following a forced-choice exposure to a novel saccharin solution (0.1%, w/v). Three days later, rats were provided with a two-bottle choice presentation of saccharin and distilled water. Conditioned rats drank distilled water almost exclusively while unconditioned animals preferred saccharin. Pretreatment with DZ (6, 9, 12 mg/kg, IP) and CDP (12 mg/kg, IP) significantly increased the saccharin intake of conditioned rats indicating an attenuation of the manifestation of the CTA. While these results are consistent with the known disinhibitory effects of benzodiazepines, alternative mechanisms involving polydipsia or interactions with the taste characteristics of saccharin could not be excluded. Both hypertonic saline (16%, w/v NaCl) and Barbital Sodium (100 mg/kg) produced polydipsia without attenuating CTAs suggesting that the two-bottle procedure is capable of distinguishing between polydipsic effects and anti-aversion effects for these drugs.

Benzodiazepines Diazepam Chlordiazepoxide Barbital Conditioned taste aversion (CTA) Polydipsia Lithium chloride Saccharin Saccharin preference Free-choice Hypertonic saline Fluid-deprivation

CONDITIONED taste aversions (CTAs) presumably reflect a learned avoidance of the ingestion of a distinctively-tasting substance which has previously been associated with an aversive enteroceptive stimulus. A common method of establishing CTAs is to administer a drug such as LiCl following the ingestion of a novel saccharin solution in fluid-deprived rats [1, 14, 23, 28, 29]. In conditioned animals, reduced saccharin ingestion on subsequent re-exposure to the fluid is thought to be due to a conditioned association between the novel taste of saccharin acting as a conditioned stimulus (CS) and the LiCl-induced enteroceptive stimulation acting as an unconditioned stimulus (UCS) or punishment. The CTA paradigm has been described as a conflict procedure in that following the establishment of a CTA with LiCl, fluid-deprived rats given a forced exposure to the CS displayed a conditioned elevation of corticosterone and a reduced intake of the fluid CS [18].

In a variety of different behavioral procedures, anxiolytic benzodiazepines are known for their ability to disinhibit behavior which has been suppressed by punishment, fear, or conflict. Anti-aversion or anti-conflict paradigms are commonly used to screen for anxiolytic drug activity [10, 12, 27]. Using the CTA paradigm as a conflict procedure, several anxiolytic barbiturates [14,28] and chlordiazepoxide (CDP) [1,2] have been shown to antagonize the taste avoidance behavior of rats with a previously established CTA. However, in each of these studies, rats were given a forced exposure to a single bottle containing a saccharin solution. Under these conditions, a drug might simply act to stimulate fluid consumption (polydipsia) yielding a "false positive" and be inaccurately determined to have anti-aversion activity. In shock-conflict procedures using fluid intake as the dependent measure, false positives have been shown to occur with drugs which produce polydipsia [17,24]. In forced-choice, CTA procedures, manipulations which enhance thirst or drinking also antagonize CTAs [6,11]. Since both the barbiturates and the benzodiazepines stimulate fluid-deprivation-induced drinking [4, 20, 21], the antagonism of CTAs in

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forced-choice procedures could be due to polydipsic effects of these drugs rather than anxiolytic or anti-conflict effects.

In an attempt to control for possible polydipsic effects of CDP, one study [25] employed a two-bottle choice procedure in which a single 5 mg/kg dose of CDP was administered prior to a two-bottle choice exposure to saccharin and water. The effect of CDP on saccharin intake in the two-bottle choice test was compared to the saccharin intake of the same rats given a forced-choice exposure to saccharin five days earlier under non-drug conditions. The results showed that the saccharin intake of rats in the two-bottle choice test (following CDP treatment) was not significantly greater than the intake of the same rats forced to drink saccharin five days earlier. On the basis of these results, the authors concluded that CDP did not antagonize CTAs in two-bottle choice procedures and that earlier reports [1,2] of CDP-induced CTA antagonism were probably due to polydipsic rather than anxiolytic mechanisms. Unfortunately, conclusions from this study [25] are limited by the fact that only one dose of CDP was examined and the saccharin intake under two-bottle choice conditions was compared with saccharin intake under forced-choice conditions five days earlier; this may not be an appropriate comparison. Rats in a forced-choice procedure would be motivated by thirst to drink at least some of the saccharin solution [7, 18, 25] while in a choice procedure, conditioned rats might be expected to almost completely avoid drinking the saccharin solution as has been shown in other studies employing two-bottle procedures [7,9]. Since the former report [25] did not include a non-drug control group for comparison with the CDP-treated group, it is difficult to conclude what effect CDP had on the CTA to saccharin (or fluid intake in general) under the two-bottle choice conditions.

The present study represents an attempt to more clearly evaluate the effects of anxiolytic benzodiazepines such as diazepam (DZ) and CDP in a two-bottle, free-choice CTA paradigm. The rationale for this study was that with appropriate controls, such procedures might be able to distinguish between the drug-induced polydipsia and the anti-aversion or anxiolytic effects of benzodiazepines. Both DZ and CDP were administered in a range of doses (3–12 mg/kg) to both conditioned and unconditioned animals in a two-bottle, saccharin/water choice paradigm and statistical comparisons were made to corresponding vehicle control groups. To examine the possibility of obtaining "false positives" in this procedure, the effects of Barbital Sodium (BARB) and hypertonic saline (HS) were also examined since both of these agents are known to produce polydipsia [13, 14, 28] but are generally not considered to have anxiolytic activity.

METHOD

Animals

These experiments utilized a total of 188 adult male Sprague-Dawley rats, weighing between 225–275 g, obtained from Murphy Breeding Laboratories, Plainfield, IN. Upon receipt, rats were housed in groups of 8–10 in stainless steel cages (57×40×27 cm) with free access to tap water and Wayne Lab Blox®. Environmental conditions were maintained constant with temperatures of 21–24°C and a 14/10 hr light/dark cycle beginning at 0600 hr.

Materials

All solutions and suspensions were prepared using distilled, deionized water. The experimental drinking fluids were a saccharin solution (0.1%, w/v) and/or distilled, deionized water. Both DZ and CDP were generously supplied by Hoffman-LaRoche and were administered in a 2 ml/kg volume. DZ was prepared as a suspension in 0.25% (w/v) methylcellulose and CDP was prepared as a solution; the vehicle control injections were comparable volumes of the methylcellulose vehicle or physiological saline (NaCl, 0.9%, w/v) for DZ and CDP respectively. Both BARB and HS were administered as solutions in a 1 ml/kg volume; the vehicle control injection for these agents was a comparable volume of physiological saline. Lithium chloride (LiCl) (3 mEq/kg) was administered as a solution in a 2 ml/kg volume; the control injection for the LiCl was a comparable volume of physiological saline. All drugs were administered intraperitoneally as described below.

Procedures

The present study was performed as three separate experiments, each designed to examine the effects of different treatments on previously established CTAs. After an initial four day acclimation period, each experiment began by restricting the rats to a one-hour, daily fluid access. All experiments were conducted between 1200 and 1700 hr and all rats were provided their daily fluid access at a constant time each day. At the designated time, animals were placed into individualized drinking cages (20×17×19 cm) for one hour. Each cage was equipped with two 100 ml calibrated Richter tubes, attached externally with the drinking spouts protruding approximately one inch into the cage. At the end of the drinking session, the amount of fluid consumed from both Richter tubes was recorded to the nearest 1.0 ml. Food was continuously available during the 23 hours of fluid deprivation but not during the one-hour access to fluid. Following the drinking session, chunks of Wayne Lab Blox® were distributed about the home cage floor so that each rat could eat upon return to the home cage without having to compete at the cage feeder.

Rats were given distilled water in both drinking tubes for the first six days to allow them to adjust to the deprivation schedule. On the seventh day (day 7), a novel saccharin solution (0.1%, w/v) was substituted for distilled water (in both tubes). Immediately following the drinking session on this day, all rats received intraperitoneal injections as described below. In each of the different experiments, rats were divided into two groups; one group received a conditioning injection of LiCl (3 mEq/kg) to establish a CTA to saccharin; the other group received a control injection of NaCl (0.9%, w/v). On the next two days (days 8–9), distilled water access was again provided in both drinking tubes; these two days were provided to allow the rats to recover from any noxious effects of the LiCl injection. On the tenth day (day 10), all animals were subjected to a two-bottle choice test (Test Day) between saccharin and distilled water; the position of the saccharin tube was counter-balanced across animals. Prior to the Test Day drinking session, all rats received intraperitoneal injections as described below. In the first experiment, rats (N=80) received either vehicle (methylcellulose, 0.25%, w/v) or one of four DZ doses (3, 6, 9, or 12 mg/kg) 30 min prior to testing. In the second experiment, rats (N=60) received one of four CDP doses (3, 6, 9, or 12 mg/kg) or physiological saline (NaCl, 0.9%, w/v) 30 min prior to testing. In the third experiment, rats (N=48) received either HS (NaCl, 16%, w/v), one of two doses of

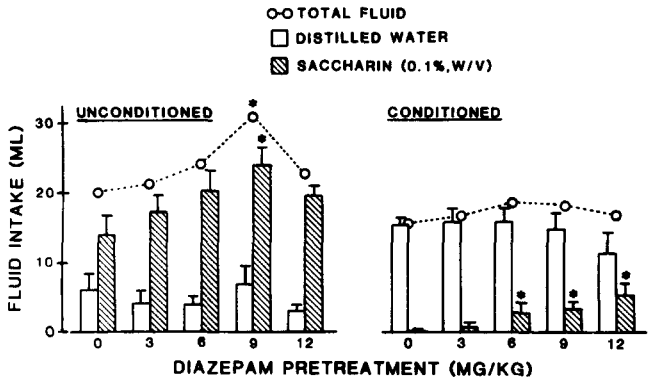

FIG. 1. Effects of diazepam on saccharin, water, and total (saccharin + water) fluid intake. Data are means of 8 rats per dosage group; vertical bars indicate the S.E.M. Asterisks indicate significant differences ($p<0.05$) from corresponding vehicle control group (0) using Dunnett's Test.

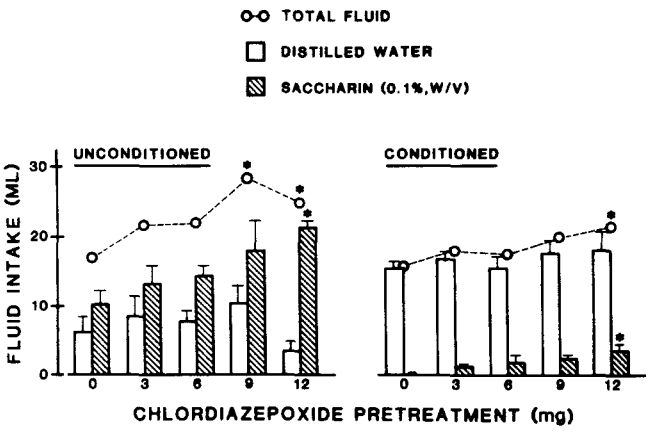

FIG. 2. Effects of chlordiazepoxide on saccharin, water, and total (saccharin + water) fluid intake. Data are means of 6 rats per dosage group; vertical bars indicate the S.E.M. Asterisks indicate significant differences ($p<0.05$) from corresponding vehicle control group (0) using Dunnett's Test.

BARB (100 or 150 mg/kg), or physiological saline (NaCl, 0.9%, w/v) 15 min prior to testing.

The data from each experiment were statistically analyzed by means of a two-way analysis of variance (ANOVA) which examined the effects of DOSE (i.e., vehicle control + various doses of the drug) and TREATMENT (i.e., conditioned verses unconditioned treatment groups) on the intake of water, saccharin, and total fluid (water + saccharin), and the saccharin preference ratio (saccharin/total). The effects of each dose of a drug treatment were compared with the corresponding vehicle control group by use of the Dunnett's Multiple Comparisons with a Control procedure [8]; this test employed the error term appropriate for the TREATMENT × DOSE interaction F-test although it does not require a significant F-value in order to compare individual dosage groups to the corresponding vehicle control group. All F-tests were conducted at probability levels ranging from $p<0.05$ to $p<0.001$; the Dunnetts' Tests were conducted at a probability level of $p<0.05$ only.

RESULTS

Figure 1 presents the Test Day fluid intake of the rats pretreated with DZ. Clearly, prior treatment with LiCl established a CTA to saccharin; while the unconditioned rats preferred saccharin, the conditioned rats clearly preferred water. The effect of conditioning (TREATMENT) was highly significant on water intake, $F(1,70)=64.50$, $p<0.001$, and saccharin intake, $F(1,70)=250.03$, $p<0.001$. The effect of DZ dose (DOSE) was significant on saccharin intake, $F(4,70)=7.63$, $p<0.001$, but not on water intake. There were no significant TREATMENT × DOSE interactions on these measures. In the unconditioned rats, saccharin intake tended to be increased by 6 and 12 mg/kg DZ, however, only the 9 mg/kg dose was significantly increased compared to vehicle. In the conditioned rats, DZ significantly attenuated the CTAs to saccharin in that rats treated with either 6, 9, or 12 mg/kg of DZ drank significantly more than did the corresponding vehicle control group. Although not shown in the figure, similar results were obtained when saccharin preference ratios were examined. With the preference ratios, significant effects of TREATMENT, $F(1,70)=182.04$, $p<0.001$, DOSE, $F(4,70)=6.22$, $p<0.001$, and a TREATMENT × DOSE interaction, $F(4,70)=2.80$, $p<0.05$, were observed. DZ doses of 6, 9, and 12 mg/kg significantly increased ($p<0.05$) the preference ratios of conditioned rats whereas the preference ratios of unconditioned rats were not significantly altered.

The polydipsic effects of DZ can also be seen in Fig. 1. A significant effect of TREATMENT, $F(1,70)=29.63$, $p<0.001$, on total fluid intake was observed; compared to the unconditioned rats, the conditioned rats generally drank less total fluid volume across all DZ dose levels. The effect of DOSE was also significant, $F(4,70)=3.49$, $p<0.05$. Although the TREATMENT × DOSE interaction was not significant, significant DZ-induced increases in total fluid intake were only observed in the unconditioned rats treated with 9 mg/kg. The lack of DZ-induced polydipsia in the conditioned rats indicates that the LiCl-establishment of a CTA to saccharin may have antagonized the polydipsic effects of DZ.

Figure 2 presents the Test Day fluid intake of rats pretreated with CDP. As can be seen, CDP treatment resulted in qualitatively similar results as those obtained with DZ. As before, LiCl conditioning resulted in highly significant TREATMENT effects on water, $F(1,50)=52.50$, $p<0.001$, and saccharin, $F(1,50)=182.09$, $p<0.001$, intake due to the establishment of a CTA to saccharin. There was a significant effect of DOSE on saccharin, $F(4,50)=5.32$, $p<0.05$, but not water intake. With both the conditioned and unconditioned groups, all doses of CDP tended to produce some increase in saccharin intake, however, in both groups, only the 12 mg/kg dose achieved significance. Thus, as with DZ, CDP pretreatment attenuated the LiCl-established CTA to saccharin but CDP was less potent than DZ since higher doses were required (12 versus 6 mg/kg respectively). When saccharin preference ratios were examined (data not shown), the ANOVA detected only a significant effect of TREATMENT, $F(1,50)=155.13$, $p<0.001$. Compared to the corresponding vehicle control groups, CDP did not significantly increase saccharin preference ratios in the unconditioned animals, however, 12 mg/kg CDP did significantly increase the preference ratios of conditioned rats.

Also shown in Fig. 2 are the polydipsic effects of CDP. As with DZ, a significant TREATMENT effect, $F(1,50)=17.04$,

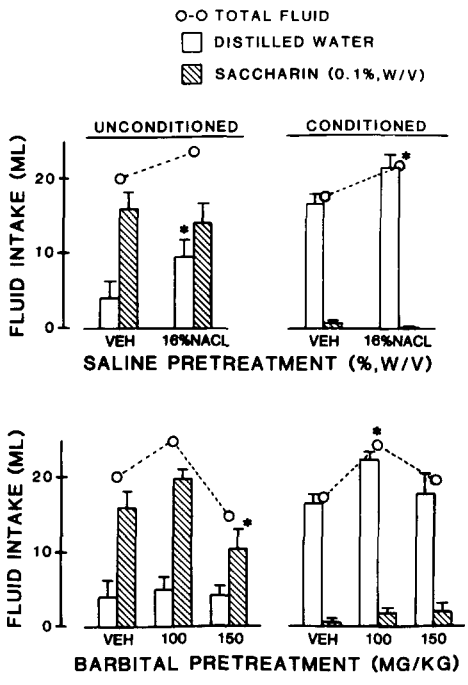

FIG. 3. Effects of hypertonic saline (16% NaCl) and barbital on saccharin, water, and total (saccharin + water) fluid intake. Data are means of 6 rats per dosage group; vertical bars indicate the S.E.M. Asterisks indicate significant differences ($p<0.05$) from corresponding vehicle control group (VEH) using Dunnett's Test.

$p<0.001$, was due to the fact that the conditioned rats generally drank less total fluid volume than the unconditioned rats. The effect of DOSE was significant, $F(4,50)=7.16$, $p<0.001$, but the TREATMENT × DOSE interaction was not. Total fluid intake was significantly increased by 9 and 12 mg/kg CDP in the unconditioned rats and by 12 mg/kg CDP in the conditioned animals. Thus, in contrast to DZ, CDP did produce polydipsia in both the conditioned and unconditioned animals. However, the polydipsic effect of CDP in the conditioned rats was less than in the unconditioned animals in that a higher dose was required (12 versus 9 mg/kg respectively) and total fluid volumes were less stimulated by CDP.

In an effort to test whether the two-bottle free-choice procedure was capable of distinguishing polydipsic drug effects from anti-aversion effects, two other agents which are known to produce polydipsia were tested for their effects in this paradigm. Figure 3 shows the effects of HS and BARB on Test Day fluid intake; the effects of HS and BARB were analyzed separately.

The upper portion of Fig. 3 presents the effects of HS. Significant effects of TREATMENT were observed with the intake of saccharin, $F(1,20)=159.14$, $p<0.001$, and water, $F(1,20)=27.70$, $p<0.001$. Although there was a tendency for the conditioned rats to drink less total fluid volume (as observed formerly in Figs. 1 and 2), the effect of TREATMENT on total fluid intake was not significant. The effect of HS pretreatment (DOSE) was significant on the intake of water, $F(1,20)=4.63$, $p<0.05$, and total fluid, $F(1,20)=7.36$, $p<0.05$, but not on saccharin intake. Compared to the vehicle group, the only significant effects were an increased water intake of the unconditioned rats and an increased total fluid intake of the conditioned animals. With the saccharin preference ratios of these animals (data not shown), significant effects of TREATMENT, $F(1,20)=139.77$, $p<0.001$, and DOSE, $F(1,20)=6.15$, $p<0.05$, were obtained; the effect of HS pretreatment (DOSE) was predominately due to a decrease in the preference ratio of unconditioned rats. Taken together, these data indicate that HS produced a polydipsic effect in the conditioned rats without increasing saccharin intake, however, the increased water intake (and reduced saccharin preference) of the unconditioned rats indicates that HS may selectively increase water preference.

The lower portion of Fig. 3 presents the effects of BARB in this paradigm. Significant effects of TREATMENT were observed with the intake of saccharin, $F(1,30)=115.37$, $p<0.001$, and water, $F(1,30)=95.82$, $p<0.001$, but not total fluid. Significant effects of DOSE were observed with saccharin, $F(2,30)=4.33$, $p<0.05$, and total fluid, $F(2,30)=7.63$, $p<0.01$, intake but not with water intake. There were no significant TREATMENT × DOSE interactions on these measures. Compared to the appropriate vehicle control groups, the only significant effects were a decrease in the saccharin intake of unconditioned rats at the 150 mg/kg dose and an increase in the total fluid intake of conditioned rats at the 100 mg/kg dose of BARB. Although other comparisons were not significant, certain trends in the effects of BARB are clear. In the unconditioned rats, total fluid intake tended to be increased by 100 mg/kg and decreased by 150 mg/kg of BARB. These changes in total fluid intake predominately reflect parallel changes in saccharin intake. In the conditioned rats, 100 mg/kg BARB produced a significant increase in total fluid intake (polydipsia) largely due to increases in water intake. Slight increases in saccharin intake were observed in the conditioned rats with both BARB doses, but these were not significant. When the saccharin preference ratios were examined, the only significant effect was the main effect of TREATMENT, $F(1,30)=202.50$, $p<0.001$; the preference ratios of the conditioned animals treated with BARB were not significantly different than their respective vehicle control group. The unconditioned rats treated with 150 mg/kg of BARB showed a high degree of sedation which may account for the reductions in fluid intake in these animals.

In the data presented in Fig. 3, there was a great deal of variability in the saccharin intakes of the conditioned rats. In the vehicle group, one rat drank 2.0 ml, three drank 1.0 ml, and two did not drink any detectable volume of saccharin. All six rats treated with HS failed to drink any detectable volume of saccharin. With BARB pretreatment, two rats from each dosage group drank at least 2.0 ml of saccharin; the volumes consumed by these rats were 4–5 ml and 2–7 ml in the 100 and 150 mg/kg groups respectively. These data indicate that BARB may have some efficacy in attenuating CTAs.

DISCUSSION

These experiments have shown that in a two-bottle, free-choice paradigm, DZ and CDP attenuated the manifestation of a LiCl-established CTA to saccharin by increasing the amount of saccharin consumed by conditioned rats. Previous investigations have shown that LiCl is a powerful UCS [23] and this agent is often used as the prototypic UCS in CTA studies [1, 14, 23, 28, 29]. Other studies have shown that CDP [1,2] and several barbiturates [14,28] antagonize the manifestation of previously established CTAs in single-

bottle, forced-choice paradigms. Previous studies have also shown that two-bottle, free-choice methodologies are more sensitive techniques for measuring CTAs than are single-bottle, forced-choice methods [7, 9]. Therefore, to the extent that the CTAs manifested in a two-bottle choice procedure represent "punishment-inhibited behavior," the results of the present study are in empirical agreement with other reports of disinhibitory effects of benzodiazepine anxiolytics on suppressed behavior such as has been observed in the former CTA studies [1,2] and in a wide variety of other paradigms and procedures [3, 10, 12, 14, 26].

The present results are in apparent contrast to a previous report [25] which suggested that CDP did not antagonize CTAs in a two-bottle choice CTA paradigm. In that report, amphetamine (5 mg/kg) was used to establish a CTA to saccharin (0.1%, w/v) in fluid-deprived rats following a forced exposure to the saccharin solution. Two days later, rats were given a forced exposure to saccharin and the amount consumed was recorded. Five days after that forced-choice exposure, a single 5 mg/kg dose of CDP was administered prior to a two-bottle, free-choice exposure to saccharin and water. The effect of CDP on saccharin intake in the two-bottle choice test was compared to the saccharin intake of the same rats given a forced exposure to saccharin five days earlier. Under those conditions, the CDP-induced saccharin intake in the two-bottle test was not significantly different than the saccharin intake of the same rats forced to drink saccharin five days earlier. Because of this apparent negative result, the authors concluded that earlier reports [1,2] of a CDP-induced antagonism of CTAs in forced-choice procedures may in fact have been due to the polydipsic effects of CDP and not anxiolytic or disinhibitory effects. However, conclusions from that study [25] are limited by the fact that only a single dose of CDP was tested and the effects of CDP were not compared to a non-drug control group under the two-bottle choice conditions. Comparisons of saccharin intake under free-choice conditions with saccharin intake under forced-choice conditions may not be appropriate. Whereas rats in a forced-choice procedure would be motivated by thirst to drink at least some of the saccharin solution [7, 18, 25], the present results, in agreement with previous studies [9], have shown that in a two-bottle, free-choice paradigm, conditioned rats not treated with any drug almost completely avoided drinking the saccharin solution.

In the present study, DZ and CDP were shown to have attenuated the manifestation of LiCl-established CTAs to saccharin in that conditioned rats treated with these drugs consumed significantly more saccharin than non-drugged control animals. This effect was modest in degree in that normal saccharin preference was not restored and conditioned rats were induced to drink only small volumes of saccharin. However, compared to the almost zero level of saccharin intake in the conditioned, vehicle-treated rats, the modest drug-induced increases were significant. Whereas these observations are consistent with the purported disinhibitory or anti-aversion activity of benzodiazepines, alternative mechanisms involving polydipsia or interactions with the taste characteristics of the saccharin solution must be considered.

With the DZ-treated animals, there was some evidence that the observed CTA antagonism might be distinguishable from polydipsic mechanisms. DZ (6–12 mg/kg) was shown to increase the saccharin intake of conditioned animals without producing polydipsia in those same animals. This CTA attenuation occurred at doses which did not produce polydipsia in unconditioned animals (i.e., only the 9 mg/kg dose produced significant increases in saccharin or total fluid intake). In addition to the above observations, the finding of DZ-induced (6–12 mg/kg) increases in the saccharin preference ratios of the conditioned rats in the absence of such changes in the unconditioned rats, also argues for a selective CTA antagonism. However, in contrast to these observations with DZ, CDP only attenuated the manifestation of the CTA at a dose which also produced polydipsia in the conditioned rats (i.e., 12 mg/kg) and an even lower dose (i.e., 9 mg/kg) was found to produce polydipsia in the unconditioned animals. These observations suggest that polydipsic mechanisms may have been responsible for the effects of CDP on fluid intake observed in the present study. It is possible that the disinhibitory and polydipsic actions of benzodiazepines may be separate but related phenomena such that DZ may have a greater potency to produce disinhibitory effects over polydipsic actions while the reverse may be true for CDP (i.e., a greater potency in producing polydipsia than in producing disinhibitory effects). Evidence for such a proposition comes from the fact that DZ is consistently found to be more potent that CDP in producing disinhibitory and anti-conflict effects [3,26] but CDP is often found to be more potent than DZ in producing polydipsia in fluid deprived rats [17,20].

Other than general polydipsic effects of enhanced fluid consumption, it is also possible that some of the observed benzodiazepine effects on fluid intake may have been due to interactions with the taste characteristics of the dilute 0.1% saccharin solution. For example, both DZ and CDP tended to selectively increase the saccharin but not the water intake of unconditioned rats. Since previous studies have shown that the polydipsic effects of benzodiazepines may depend on the taste characteristics of the fluid [21] and that benzodiazepines increase food intake [5], it is possible that DZ and CDP may have simply enhanced the natural preference for a sapid fluid like the saccharin solution. The present results can not exclude this as a possible mechanism for the observed CTA attenuation. The only data which suggest that this may not be the case are the observations that the saccharin preference ratios of unconditioned animals were not significantly effected and that DZ significantly attenuated the CTA of conditioned animals at doses which did not significantly increase saccharin intake in the unconditioned rats.

An expected finding of the present study was the observation of a reduced polydipsic effect of DZ and CDP in the conditioned rats. Unpublished observations in our laboratory suggest that this reduced polydipsic effect of benzodiazepines in conditioned animals may have been due to an associative effect of LiCl (i.e., due to CTA conditioning) and not due to a pharmacological interaction between LiCl and the benzodiazepines. When LiCl was administered following a water drinking session rather than following novel saccharin exposure (i.e., LiCl administered non-associatively), DZ did produce significant polydipsia in a subsequent two-bottle choice between saccharin and distilled water. Such observations may support the suggestion that the effects of DZ and CDP observed in the present study were in part due to selective influences on saccharin intake such that polydipsic effects in the conditioned animals were limited by the conditioned avoidance of saccharin intake.

Both HS and BARB were employed in the present study in an attempt to partly distinguish polydipsic actions from anti-aversion activity in the two-bottle choice procedure. Whereas these agents are presumably devoid of or have very

little benzodiazepine-like anxiolytic activity, both HS [13] and BARB [14,28] have been reported to produce polydipsia. Although polydipsia was not observed in unconditioned rats, both HS and BARB produced polydipsia in conditioned animals without significantly increasing saccharin intake. These results demonstrate that, at least with some drugs, it may be possible to distinguish between polydipsia and CTA antagonism using a two-bottle choice procedure and that agents which produce polydipsia will not of necessity produce false positives on the test for CTA antagonism in conditioned animals. There was some indication that BARB may have efficacy in attenuating CTAs in this paradigm, however, the effect was not significant and was not as pronounced as seen with DZ and CDP. The lack of anti-aversion effect of BARB is consistent with the known pharmacology of this drug. Although barbital has been reported to reduce shock-induced emotional suppression [16] it is not generally recognized as an anxiolytic barbiturate [19]. Purported disinhibitory effects of barbital have been variable even in forced-choice CTA paradigms. Using fluid-deprived rats given a forced exposure to saccharin, one study [14] concluded that barbital was ineffective in antagonizing LiCl-established CTAs, while another study [28] cited unpublished data to support observations of an insignificant effect of barbital to attenuate LiCl-established CTAs to saccharin.

The two-bottle choice paradigm employed in the present study was not sensitive to low dose effects of DZ and CDP; this was apparent in the moderate degree of CTA attenuation which occurred and in the high doses of DZ and CDP required to produce CTA attenuation and polydipsic effects. The reason(s) for this are not clear. Both DZ and CDP have been shown to produce polydipsia in lower doses in studies employing within-subject comparisons of fluid intake measured to the nearest 0.1 ml [20]. The insensitivity of the present procedure to measure polydipsia may be due in part to comparisons between groups of animals and the measurement of fluid intake as the sum of two measures recorded to the nearest 1.0 ml. With regard to the high doses required for the attenuation of CTAs, different procedures are known to detect anxiolytic effects with differential dose sensitivities [22] and in shock procedures examining conflict and conditioned suppression, high doses comparable to those used in the present study were found to be more effective than the lower doses [15]. It is possible that the CTA procedure employed in this study involved a more robust avoidance behavior which was less sensitive to benzodiazepine anxiolytic effects and therefore, higher doses were required. If this were the case, then experiments using a less strong UCS and therefore, less robust CTAs, might show greater effects of DZ and CDP at lower doses.

In summary, the present results have shown that DZ and CDP attenuated the manifestation of LiCl-established CTAs to saccharin in a two-bottle choice procedure. While these empirical results are consistent with reported disinhibitory anxiolytic effects of benzodiazepines, alternative explanations involving polydipsic mechanisms and interactions with the taste characteristics of saccharin can not be eliminated. Future research needs to determine the mechanism(s) of the benzodiazepine-attenuation of CTAs and to determine whether a more complete antagonism of CTAs is possible under different experimental conditions.

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