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Enhancement of Saline Consumption by Chlordiazepoxide in Thirsty Rats: Antagonism by Ro15-1788

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TURKISH, S. AND S J COOPER *Enhancement of saline consumption by chlordiazepoxide in thirsty rats Antagonism by Ro15-1788* PHARMACOL BIOCHEM BEHAV 20(6) 869–873, 1984.—Water-deprived rats were given access to either water or a highly preferred 0.9% NaCl solution in a 30 min drinking test The animals consumed substantially more saline than water Chlordiazepoxide (2.5–20.0 mg/kg) was administered IP before the drinking test Analysis of the results revealed a significant drug treatment × fluid condition interaction Chlordiazepoxide produced a preferential enhancement of saline intake, achieving a peak effect at 5 0 mg/kg. Consideration of the time-course of drinking showed some complexity in the way in which chlordiazepoxide affected the saline drinking. During the first 6 min of the drinking period, the drug treatments tended to depress consumption, reaching a significant effect at 20 0 mg/kg However, in the following 16 min interval of the drinking test, chlordiazepoxide significantly elevated saline consumption The mechanism for this second effect may have been a retardation in the development of satiety Finally, at the end of the drinking test, when saline consumption had become satiated, chlordiazepoxide exerted no discernible effect The enhancement of saline consumption by chlordiazepoxide appears to have been benzodiazepine-receptor mediated, since the effect was reversed by treatment with Ro15-1788, a benzodiazepine receptor antagonist The implications of a benzodiazepine-induced increase in salt intake are briefly considered The overconsumption of salt is contraindicated in certain clinical conditions Both stress and hypertension are associated with elevated salt appetite Treatment of these conditions using benzodiazepines may require due consideration of the possible stimulant effect of these drugs on salt appetite

Chlordiazepoxide Drinking Ro15-1788 Salt intake Thirst Water

EXPOSURE to stress increases the consumption of sodium chloride (NaCl) solutions in wild rabbits [9] and mice [19]. Endocrinologically, stressors produce concomitant pituitary release of adrenocorticotrophic hormone (ACTH) and β-endorphin [14], and activation of the renin-angiotensin system [18]. Each of these hormonal mechanisms may contribute to the stress-induced salt appetite. ACTH is known to stimulate salt intake in rats [27] and wild rabbits [2], central administration of angiotensin II (AII) increases salt intake in rats [1], and naloxone (an opiate receptor antagonist which blocks the effects of β-endorphin) opposes stress-induced salt appetite in mice [19]. Stress and elevated salt consumption have been implicated as risk-factors in the development of hypertension. Interestingly, spontaneously hypertensive rats (SHR) show an elevated salt intake, which can be blocked with captopril, an angiotensin converting enzyme inhibitor [10].

It is recognised that benzodiazepine treatments afford protection against some effects of stressors [15]. Benzodiazepines also confer beneficial effects in the treatment of hypertension. Daily injections of diazepam (1 mg/kg) delay the development of hypertension in rats which have been chronically exposed to stressful conditions [25]. These data suggest the importance of determining possible effects of benzodiazepine treatments on salt intake. Protection against stress may co-vary with an attenuation of saline acceptability, and therefore we might expect benzodiazepines to reduce saline consumption. On the other hand, the strong evidence that benzodiazepines generally enhance ingestional responses (at least at moderately low dose levels) [4,6] suggests the opposite may be true.

The aim of the first experiment was therefore to examine the effects of chlordiazepoxide (CDP), over the dose-range 2.5–20.0 mg/kg, on the consumption of water or of a 0.9% NaCl solution in thirsty rats. The concentration of maximum saline acceptance is about 0.8–0.9% [13,26], and produces significantly higher levels of intake compared with water [7, 13, 16]. The study was designed with the aim of being able to detect any significant interaction between drug treatments and the levels of water vs. saline consumption.

EXPERIMENT 1

METHOD

Animals

The subjects were 120 male, black hooded rats (General strain) bred in the Psychology Department, University of

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Birmingham. They were housed individually in stainless steel cages, normally with free access to food pellets (modified Diet 41B, Heygate and Sons, U.K.). They were maintained under a 12 hr light–12 hr dark cycle (lights on at 7 a.m.) and the room temperature was kept constant at 21–22°C The animals were adapted over several weeks to a water deprivation schedule which involved removal of water from the home cage for 24 hours on alternate days. Care was taken to familiarise the animals with relevant procedures before running the drug trials They were handled and weighed regularly, and received experience of injections of isotonic saline They weighed 350–450 g at testing

Procedure

Animals were subdivided into two equal groups. The first group was given access to water in a 30 min drinking test, following a 24 hr water-deprivation period. The second was given access to 0.9% saline solution in a 30 min drinking test, following a 24 hr water-deprivation period. During the tests, food was removed from the home-cage for each group. Animals were familiarised with these procedures, before drug trials began

Within each group, animals were allocated to 6 injection conditions (n=10 per condition) in order to examine the effects of chlordiazepoxide hydrochloride (CDP) on fluid consumption. For each group, the injection conditions were 2.5, 5.0, 10.0, 15.0 and 20.0 mg/kg CDP, and an isotonic saline condition. All injections were administered IP 25–30 min before the start of the drinking test. Doses are expressed in terms of salt, and the injection volume was 1 ml/kg.

For the drinking test, the fluids (water or 0 9% saline) were provided in a calibrated cylinder which was clipped to the front of the cage, with the metal spout protruding into the interior Fluid intake was measured at 6 min intervals throughout the test to the nearest 0 5 ml. Hence, the "single-stimulus method" [26] was employed to compare the consumptions of water and 0.9% saline, respectively. The volume of liquid consumed in a single stimulus test is defined as an *acceptance* measure, and should not be confused with a measure of *preference* [11]

Statistical analysis of the data was carried out using a 3-way analysis of variance (ANOVA), to assess the main effects of fluid condition, drug treatments and the time-course of drinking, and any interactions between these factors Individual comparisons against the control vehicle group were made using a Dunnett's *t*-test (one-tailed comparisons).

RESULTS AND DISCUSSION

Water-deprived rats displayed a strong acceptance of the 0.9% NaCl solution. The intake of the salt solution was significantly greater than the intake of water, $F(1,108)=176.21$, $p<0.001$ Figure 1 depicts the 60.6% elevation in fluid intake which occurred over the 30 min drinkng period, in animals given access to the salt consumption, when intakes of water and saline are compared under the control vehicle condition (labelled SAL).

An ANOVA revealed a significant drug × fluid condition interaction, $F(5,108)=2.46$, $p<0.05$. CDP treatments elevated fluid consumption, but there was a particularly marked effect on saline intake (Fig 1). The peak effect of CDP on saline consumption occurred at a dose level of 5 mg/kg (Fig. 1), when there was an 86.5% elevation in saline intake compared with the corresponding level of water intake This result is all the more striking given the already elevated control level consumption of the salt solution The results indicate that the salt concentration and CDP treatments potentiate in their effects to elevate fluid consumption.

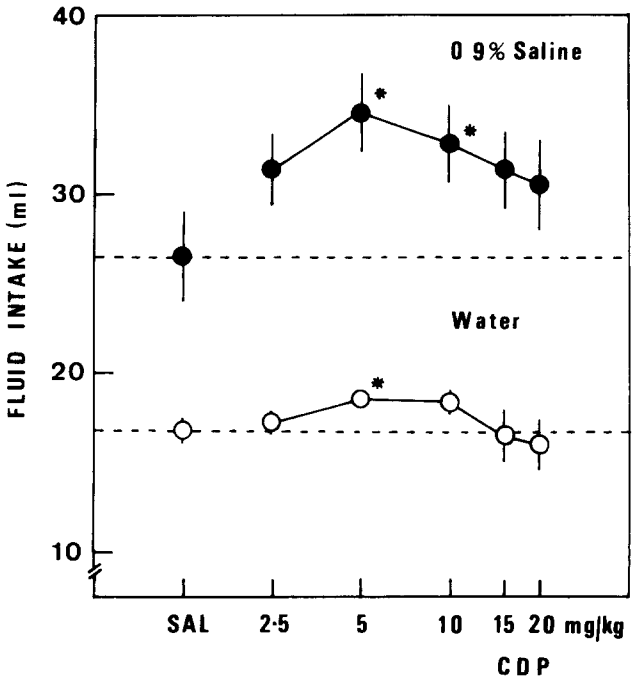

FIG. 1 Effects of CDP (2 5–20 mg/kg) on the intake of a maximally-preferred 0 9% saline solution (●) and of water (○), in water-deprived rats over a 30 min period Each data point represents the mean score for 10 animals Vertical lines indicate SEM Horizontal interrupted lines show baseline levels of consumption following injection of drug vehicle (SAL). *Indicates significant differences from corresponding control group ($p<0$ 05, Dunnett's test)

The ANOVA also showed a highly significant time-course main effect, since the level of fluid consumption declined during the drinking test in each group of animals tested, $F(4,432)=414.0$, $p<0$ 0001 As described previously [7,15], the satiation of drinking was retarded in the animals given 0.9% saline to drink, compared with those given water (significant fluid condition × time interaction, $F(4,432)=11$ 33, $p<0.0001$) Table 1 shows the effect as it occurred in the present experiment There was a significant 3-way interaction term in the ANOVA, $F(20,432)=1$ 77, $p<0.05$ The effects of the CDP treatments on the consumption of the 0.9% saline solution were dependent on the time interval of the test

Figure 2 illustrates representative time-course data. Results for the 5 mg/kg CDP dose (which produced the peak effect on saline acceptance) are shown, together with the results for the 20 mg/kg dose, which produced a significant initial depression in drinking For the experiment as a whole, there was a significant dose × time interaction, $F(20,432)=3$ 08, $p<0.001$ As Fig. 2 shows, there was a triphasic effect of CDP on saline consumption as a function of the time intervals. During the first 6 min period of access to the 0 9% NaCl solution, when control intake was as its highest, CDP treatments appeared to exert a slight depressant effect on consumption This effect was significant at the 20 mg/kg dose level. Since all animals began drinking as soon as

TABLE 1

TIME-COURSE OF DRINKING SHOWING THE SIGNIFICANTLY GREATER CONSUMPTION OF 0 9% SALINE COMPARED WITH WATER BY WATER-DEPRIVED RATS ADMINISTERED A CONTROL INJECTION

Fluid condition	Time 6-min intervals 1	2	3	4	5	Total
Water	11.3 ±0 5	4 0 ±0.4	0.6 ±0 2	0 5 ±0.3	0.4 ±0 2	16 8 ±0.7
0 9% saline	12.6 ±0 8	7 1 ±0 5	2.4 ±0 6	2 6 ±0 9	1 9 ±0.6	26.8 ±2 6

Results are shown in terms of mean ± SEM (n=10 per group)

the drinking tube was clipped into position, the initial CDP-induced depression could not be attributed to longer latencies to begin drinking. Significant elevations in drinking due to the CDP treatments occurred in the following two 6 min intervals (Fig. 2) Finally, at the end of the drinking period, when control animals showed virtually complete satiation of drinking, there were no longer any significant increases in saline drinking following CDP treatments. These results cannot easily be interpreted in terms of baseline-dependency. Clearly, the CDP treatments did not enhance the initial phase of avid saline consumption, but they did retard the development of satiation over the course of the drinking period.

Thus, CDP treatments can enhance saline acceptability, and so produce enhanced consumption of salt solutions. In this experiment, CDP exaggerated the relative magnitude of 0.9% saline drinking compared to that of water drinking. There was not a constant effect of CDP to increase consumption in the two cases, but a greater effect in the case of saline consumption. Falk and Burnidge [12] previously reported that CDP treatments enhanced the consumption of an aversive 1.5% NaCl solution in water-deprived rats. They found that CDP (7.5–30 mg/kg) increased the consumption of 1 5% NaCl solution from <6 ml/100 g body weight to >9 ml/100 g in albino rats (mean weight >350 g). The present data emphasize that CDP can also increase the consumption of a maximally-acceptable saline solution. CDP at 5.0 mg/kg increased the consumption of 0.9% NaCl by 7.6 ml above the control level of intake. At this stage, it is parsimonious to suggest that CDP treatments enhance the intakes of both acceptable and aversive NaCl solutions, and there is as yet no strong evidence that the drug treatments are more effective with regard to one concentration of NaCl solution compared to another. The pattern of results for CDP bear comparison with those reported for phenobarbital. Phenobarbital (40 mg/kg) increased the consumption of acceptable and aversive NaCl solutions in water-deprived rats [24]. The results stand in contrast, however, to those reported for morphine in mice. Morphine increased the preference for an aversive 3% NaCl solution in mice, but had no effect on preferred NaCl solutions [19]. These data suggest that there are interesting pharmacological comparisons to be made with regard to saline acceptability and aversion.

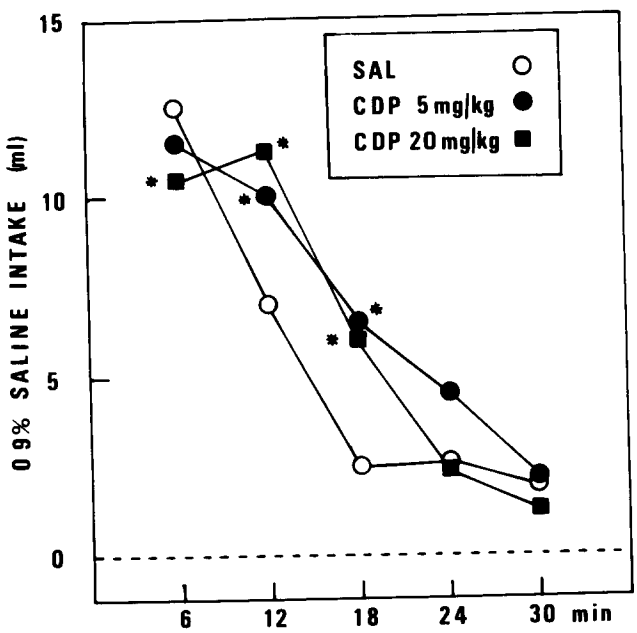

FIG. 2 Effects of CDP at selected doses (5 0 and 20 0 mg/kg) on 0 9% saline intake for consecutive 6 min intervals of a 30 min drinking test Mean scores are for 10 animals (SEMs are omitted for clarity). *Indicates significant differences from baseline level of drinking (○) $p<0.05$, Dunnett's test

EXPERIMENT 2

Ro15-1788 has been developed as a specific benzodiazepine receptor antagonist [3, 5, 8, 15, 17, 20, 21, 22]. The aim of the second experiment was to determine whether or not the CDP-induced enhancement of saline drinking could be reversed by Ro15-1788.

METHOD

Animals

The subjects were 90 additional male, black hooded rats (General strain) which were treated as described in Experiment 1. They were within the same range at testing.

Procedure

The animals were first divided into three equal groups. All were familiarised with drinking a 0.9% saline solution in a 30

min drinking test (food absent), following a 24 hr water-deprivation period. Each animal received two injections prior to the drinking test. For the first injection, the three groups were administered 0, 5 0 or 10.0 mg/kg CDP, respectively, IP, 25–30 min before the start of the drinking test. Within each group, the animals were divided into three equal subgroups, and received 0, 3.0 or 10 mg/kg Ro15-1788, respectively, IP, 15–20 min before the start. CDP was dissolved in isotonic saline, and doses refer to the hydrochloride salt. Ro15-1788 was suspended in distilled water, to which Tween 80 was added (2 drops to 10 ml). Injection volumes were 1 ml/kg. The data were analysed using ANOVA procedures, to detect possible significant main effects of the CDP and Ro15-1788, and importantly whether or not there was a significant drug interaction

RESULTS AND DISCUSSION

As Table 2 shows, CDP (5 0 and 10 mg/kg) significantly enhanced the intake of the palatable 0.9% NaCl solution There was no increase in saline consumption during the first 6 min period of the drinking test (Table 2a). The increase occurred in the following 12 min period, when control animals typically begin to show satiation of the drinking response (Table 2b) The benzodiazepine receptor antagonist, Ro15-1788, displayed no intrinsic effects on saline consumption, but did block CDP's enhancement of saline drinking. Hence the mechanism for the increase in salt appetite produced by CDP appears to be benzodiazepine-receptor mediated.

TABLE 2

INTERACTIONS BETWEEN CDP (5 AND 10 mg/kg) AND THE SPECIFIC BENZODIAZEPINE RECEPTOR ANTAGONIST Ro15-1788 (3 AND 10 mg/kg) IN RELATION TO 0 9% SALINE CONSUMPTION (ml) IN THE WATER-DEPRIVED RAT

		CDP (mg/kg)		
		0	5	10
(a) 0–6 Min Interval				
Ro15-1788 (mg/kg)	0	12 8 ±1 12	11 8 ±1 29	10 1 ±0 62
	3	12 6 ±0 47	12 8 ±0 82	11 1 ±0 99
	10	12 4 ±0 72	12 1 ±0 62	11 5 ±0 77
(b) 6–18 Min Interval				
Ro15-1788 (mg/kg)	0	10 9 ±1 03	15 3* ±0 78	17 5 ±1 12
	3	11 3 ±1 00	13 3 ±1 43	14 6 ±1 68
	10	12 9 ±1 50	13 6 ±1 47	11 5 ±0 79

The data are shown separately for the first 6 min interval and the subsequent 12 min (6–18 min) interval of the 30 min drinking period

Results are shown in terms of mean ± SEM (n=10 per group) ANOVA revealed no significant effects of CDP or Ro15-1788 treatments, or a significant interaction term, for the data of the first 6 min interval There was, however, a significant CDP × Ro15-1788 interaction effect ($p<0.05$) in the data of the 6–18 min interval Planned orthogonal contrasts revealed significant effects of CDP treatments, in the absence of Ro15-1788

*$p<0.005$, one-tailed) No drug-combination condition differed significantly from the control level of intake (Newman-Keuls test)

GENERAL DISCUSSION

The results of the two experiments demonstrated that (1) CDP treatments did not produce equivalent effects on the consumption of 0 9% saline and water, respectively, CDP treatments enhanced the greater acceptability of the salt solution; the peak CDP effect occurred at a dose level of 5.0 mg/kg (2) the CDP-induced increase in saline ingestion was time-dependent, and occurred only during the middle phase of drinking when, under baseline conditions, satiety was in the process of developing, (3) the CDP-induced increase in saline consumption was reversed by Ro15-1788, the benzodiazepine receptor antagonist given alone had no significant effect on saline intake

In the doses used, CDP did not act to attenuate ingestion of a maximally preferred salt solution. Evidence cited in the introduction indicates that salt intake is enhanced by stress, and an increased salt appetite is associated with hypertension. Although benzodiazepine treatments modulate effects of stress, and can reduce hypertension [15, 23, 25], we did not find that CDP treatments counteract salt appetite In fact, the opposite result was obtained CDP treatments appeared to preferentially exaggerate the acceptability of a palatable salt solution. Taken with data from a previous study [12], the present results suggest that CDP may act to increase salt appetite. It remains to be determined whether or not CDP treatments, over a comparable range of doses, successfully enhance stress-induced or hypertension-related salt appetite It would be important to establish effects of benzodiazepine treatments in these instances. There may be a dissociation between effects of benzodiazepines on stress and/or hypertension and their effects on salt appetite, if so, the dissociation would deserve clinical consideration. Finally, our results with CDP and the antagonist Ro15-1788 implicate benzodiazepine receptor mechanism in the neural substrates underlying the control of salt intake

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