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Chlordiazepoxide Injection Elevates the NaCl Solution Acceptance-Rejection Function[1]

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FALK, J. L. AND M. TANG. *Chlordiazepoxide injection elevates the NaCl solution acceptance-rejection function.* PHARMACOL BIOCHEM BEHAV 21(3) 449–451, 1984.—Injection (SC) of chlordiazepoxide (2–8 mg/kg) increased the intake of NaCl solution (0.5–3.0% NaCl) as well as water in water-deprived rats when these fluids were made available singly during daily 1-hr rehydration periods. The marked enhancement of this drug effect when NaCl solution was the available fluid does not appear to be due to the induction of a sodium appetite or the mimicking of an increase in water deprivation. The exaggeration of the drug effect when an NaCl solution is the drinking fluid, as opposed to water, may be useful as a sensitive index of action for punishment-attenuating (anxiolytic) drugs.

Chlordiazepoxide Benzodiazepine NaCl intake Anxiolytic agents Fluid intake Punishment attenuation

INCREASED acceptance of hypertonic NaCl and other noxious-tasting solutions (citric acid, tartaric acid) occurs in rehydrating rats injected with various punishment-attenuating drugs [5, 9, 18, 19, 21]. It has been suggested [5] that the reason animals increase their intake of such solutions is that a noxious taste can function much like response-contingent electric shock, the effect of which is attenuated by various anxiolytic agents [6,7]. In both cases the administration of such an agent has a permissive effect on either operant [6,7] or consummatory behavior [5, 8, 10] allowing the positive-reinforcement component of the situation to have free play since the punishing component has been attenuated.

The present experiment was designed to study the effect of chlordiazepoxide on the acceptance of various concentrations of NaCl solution that ranged from highly acceptable to those that are relatively rejected by the rehydrating rat. If punishment attenuation is the mechanism underlying the drug-induced increase in NaCl intake, then the increase should be maximal at those concentrations falling within the rejection limb of the typical NaCl acceptance-rejection function [12,22].

METHOD

Animals

Twenty adult, male, albino Holtzman (Madison, WI) rats with an initial body weight of 332 g (range: 329–381 g) were used. They were housed individually in standard Acme stainless-steel cages in a temperature-controlled room with a 12-hr light-dark cycle (lights on 0700–1400 hr).

Procedure

All animals were maintained on a 23-hr fluid deprivation schedule with a 1-hr no-food drinking session. Daily drinking sessions began immediately after body weight determinations and removal of food (Purina Laboratory Chow, pelleted) from the home cages. At the end of 1 hr, intakes were recorded, the drinking tubes were removed and food replaced. Thus, for each 24-hr period, a fluid was available for 1 hr and food was freely available except during the 1-hr drinking session. All fluids were available from 100-ml Richter-type drinking tubes mounted at the front of the cage.

Animals were randomly divided into 4 equal groups (N=5 each) differing in the dose of chlordiazepoxide given (0, 2, 4 or 8 mg/kg, SC). Distilled water was the fluid available during the 1-hr drinking session except on injection days when a NaCl solution (0.0, 0.1, 0.5, 0.9, 1.5, 2.0 or 3.0%) was given instead. The concentrations were presented in a random order and the entire sequence of presentation was repeated a second time. Chlordiazepoxide was dissolved in distilled water and was always prepared immediately before an injection. Injections were given every 3–4 days and were administered subcutaneously into the loose skin behind the neck.

RESULTS

Figure 1 shows the group mean NaCl solution intakes of

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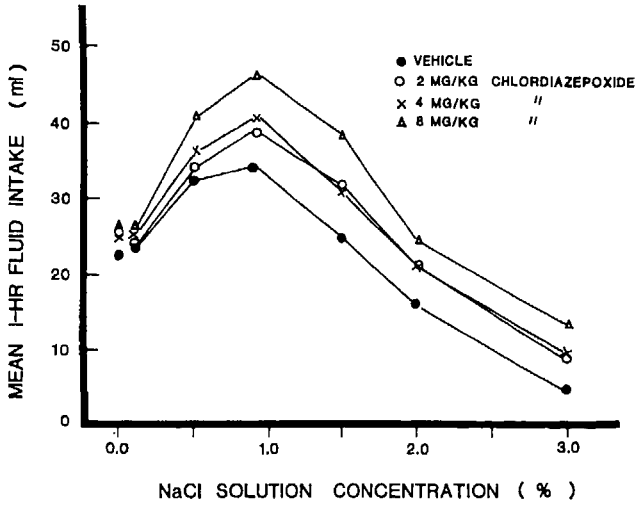

FIG. 1. Mean fluid intakes (ml) during daily rehydration periods for treatment groups of rats (N=5 each group) given acute injections of chlordiazepoxide (vehicle, 2, 4 or 8 mg/kg). Water or an NaCl solution was available for 1 hr starting 15 min after injection.

animals injected with either distilled water (vehicle) or various dose levels of chlordiazepoxide. (Means of the two injections given each animal at each NaCl solution concentration were calculated and these were then averaged for each group.) The NaCl solution acceptance-rejection function of the vehicle group resembles those functions usually found when rehydrating animals are given NaCl single-bottle acceptance tests [12, 17, 22]. An overall analysis of variance performed on the fluid intakes for all groups indicates an overall group effect, $F(3,16)=24.9$, $p<0.01$. The group given the largest dose (8 mg/kg) consumed significantly more NaCl solution than the vehicle group, $F(1,8)=30.9$, $p<0.001$, with the two lower dose levels lying in between. Further, the figure shows that chlordiazepoxide increased the height of the entire NaCl solution acceptance-rejection function at and beyond the 0.5% NaCl concentration without shifting peak acceptance.

The effect of chlordiazepoxide on distilled water intake is shown in the points plotted to the left of the NaCl solution curves (Fig. 1). For each of the four dose-level groups, the drug or vehicle was injected twice (separated by 3–4 days) and the mean difference in ml of water drunk from the mean of the two preceding baseline interdays for the replicated drug treatment was calculated. As shown in the figure, all chlordiazepoxide dose levels yielded about the same effect on water intake and the group effect was significant: $F(3,16)=3.64$, $p<0.05$.

DISCUSSION

In a dose-related fashion chlordiazepoxide increased NaCl solution intake in the 0.5–3.0% NaCl range. At the 0.1% NaCl concentration intakes did not differ from the vehicle-injected group. The difference for water intake, while small, was statistically significant when tested in terms of intake differences between baseline interdays and drug-treatment days. These findings confirm previous studies which showed increases in NaCl solution [5, 15, 18, 19] and water [1, 2, 8, 11, 13, 16] intakes resulting from the injection of various punishment-attenuating agents such as the benzodiazepines and barbiturates.

In the light of these findings it is interesting to speculate on the previous suggestion that the increased intake of hypertonic NaCl solution produced by chlordiazepoxide follows from its punishment-attenuating property [5]. Throughout most of the NaCl solution concentration range chlordiazepoxide increased solution intake. Furthermore, the increase was greater than when water was the available drinking fluid. But since the drug-produced NaCl solution intake increase was not limited to the relatively rejected NaCl concentration range, i.e., the descending limb of the acceptance-rejection function, it is difficult to maintain that the observed increase is mediated solely by a punishment-attenuation mechanism.

The increment in NaCl solution drinking produced by chlordiazepoxide injection was approximately of the same magnitude at 0.5% NaCl and all greater concentrations. Hence, the point of peak NaCl concentration acceptance does not appear to change as a function of chlordiazepoxide treatment. Similar results were obtained by Schmidt [15] using phenobarbital. The absence of a shift in this peak can be contrasted with conditions which do alter the peak location of the acceptance-rejection function: diabetes insipidus, schedule-induced polydipsia and hypertonic NaCl solution loading shift the peak to the left [3, 4, 17, 20].

Within what interpretive context can the markedly increased ingestion of NaCl solutions produced by punishment-attenuating drugs be placed? It could conceivably be related to a drug-induced specific sodium appetite, but the increased intake of water and other non-sodium-containing fluids [9,21] makes this alternative unlikely. Neither is it a function of renal factors [18]. Perhaps it is related to the increases in both water and food [14] intake produced by such agents. In the present experiment food was not available during the drinking period making a prandial-drinking explanation untenable. It is possible that the rather uniform increase in drinking produced over the range of NaCl solutions by each dose level of chlordiazepoxide occurs because the drug somehow has effects similar to an increase in water deprivation. However, increasing water deprivation from 21 to 69 hours elevates the acceptance-rejection NaCl function only in the ascending limb [3]. At this point it would appear that the simple addition of a tastant to the drinking fluid exaggerates the intake-enhancing effect of punishment-attenuating drugs for reasons that are at present unknown. Nevertheless, the enhancement of drug effects by the addition of NaCl to the drinking fluid permits a more unequivocal evaluation of pharmacological action.

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