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Conditioned Taste Aversion in Rats Induced by the α_1-Adrenoceptor Agonist Cirazoline

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McMAHON, L. R., A. MORIEN, B. T. DAVIES AND P. J. WELLMAN. *Conditioned taste aversion in rats induced by the α_1-adrenoceptor agonist cirazoline.* PHARMACOL BIOCHEM BEHAV **48**(3) 601–604, 1994. – Recent studies have indicated that α_1-adrenoceptor agonists such as phenylpropanolamine (PPA), cirazoline, amidephrine, and SK&F-89748 suppress food intake in rats. These compounds activate α_1-adrenoceptors within the paraventricular hypothalamic nucleus (PVN) and may excite efferent fibers that inhibit feeding. Studies of the effects of α_1-agonists suggest a specificity for feeding behavior, but no study to date has evaluated whether these agonists may suppress feeding behavior by the induction of malaise. Accordingly, the present experiment examined the ability of systemically administered cirazoline (0.1, 0.2, and 0.4 mg/kg, IP) to induce conditioned taste aversion (CTA) to a saccharin solution. Significant CTA was noted for 0.2 and 0.4 mg/kg cirazoline but not for 0.1 mg/kg cirazoline, compared to a vehicle treatment. The ED_{50} for cirazoline-induced aversion was computed to be 0.3 mg/kg, which contrasts with an ED_{50} value of 0.09 mg/kg for the effect of cirazoline on food intake (computed in other studies). More importantly, a 0.1 mg/kg dose of cirazoline, which is slightly greater than that of the ED_{50} value for suppression of feeding, did not induce significant CTA in the present study. These results suggest that malaise is not a prominent factor in the suppressive activity of cirazoline on food intake and advocate the use of cirazoline as an effective appetite suppressant.

Cirazoline Conditioned taste aversion Malaise Anorexia α_1-Adrenoceptor agonists/antagonists

THERE is a developing literature that links activation of α_1-adrenergic receptors to the suppression of food intake (18,19). Studies involving systemic injections of α_1-agonists such as cirazoline (3,17,18), phenylpropanolamine (PPA) (2,14,18), amidephrine (10), and SK&F-89748 (10), reveal that these compounds suppress food intake. In addition, anorexia has been noted after intracranial injections within the paraventricular hypothalamus (PVN) of methoxamine (4), PPA (5,13), cirazoline (3), and phenylephrine (15). Moreover, a reversal of the anorexia induced by PPA and cirazoline has been demonstrated using pretreatment with the α_1-antagonist prazosin (17). Clearly, activation of α_1-receptors both in the periphery and in the PVN suppresses food intake.

One explanation of the feeding-inhibitory properties of α_1-agonists involves these compounds interacting with the hypothalamic neural pathways (satiety mechanism) responsible for the suppression of feeding behavior (7,19). Another interpretation involves the notion that these agonists suppress feeding by the induction of malaise (9,12). Such an internal state might disrupt a variety of behavioral sequences including feeding, drinking, and locomotion. Moreover, an internal state such as malaise might support conditioned taste aversion (CTA), a paradigm that pairs a novel taste stimulus with a compound that is known to produce malaise. If avoidance of the taste occurs in subsequent test presentations in a two-bottle preference test, such avoidance can be used as an index of the putative aversive properties of the administered compound. The present study examines the induction of CTA for a range of doses of cirazoline (0.1, 0.2, and 0.4 mg/kg, IP) which are known to suppress food intake (3). Systemically administered (IP) cirazoline suppresses food intake with an ED_{50} value of 0.09 mg/kg and a near maximal suppression of feeding evident at 0.4 mg/kg (3). As a positive control, the present study also included a treatment condition using 32 mg/kg (IP) lithium chloride, a dose known to effectively produce CTA (8).

METHOD

Subjects

Thirty-one male Sprague-Dawley albino rats (Harlan Industries, Houston, TX), weighing between 300–350 g at the beginning of the study, served as subjects. The animals were housed individually in standard plastic rodent cages and were allowed a 1-week adaptation period prior to onset of behavioral testing to acclimate them to daily handling and mainte-

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nance. The rats received continuous access to rodent pellets (Teklad) throughout the experiment, except during the testing periods described in the experimental protocol below. The animal holding room was maintained at 23 ± 1.0°C, with a 12 L : 12 D schedule (lights on at 0600 h).

Drugs

A vehicle solution was prepared using 0.9% (w/v) sodium chloride dissolved in sterile distilled water. Cirazoline solutions (0.1, 0.2, 0.4 mg/ml) were prepared by dissolving cirazoline hydrochloride (a gift from Dr. David Sanger of Synthelabo Laboratories, Paris, France) into the vehicle solution. A solution of lithium chloride (32 mg/ml: Fisher Scientific) was similarly prepared. All drug solutions were calculated as the weight of chemical (base and salt) per volume and were prepared immediately prior to injection. A 0.1% saccharin solution (1.0 g saccharin/1.0 liter tap water: Sigma Chemical) was prepared for use on the training day and subsequent extinction trials.

Procedure

The rats were trained to consume tap water from metal sipper tubes attached to Wahmann 100 ml graduated drinking bottles during a 30-min test session on 7 consecutive baseline days. The sipper tubes were inserted through holes in the wire mesh above each cage. Fluid intakes were directly measured from the bottles to the nearest 1.0 ml and recorded for each subject. All fluid intake trials were conducted in the home cages between 1530 and 1730 h with food available at all times. Our intention was to conduct these CTA trials under conditions similar to those which previous feeding tests were conducted. The rats were weighed daily following each trial. Following this baseline period, the rats were rank ordered according to mean water intakes for baseline trials 5–7 and randomly assigned to five groups based on quantitatively similar water intakes (average water consumption/group = 5.9 ml, SEM = 0.97) and similar mean weights (average weight/group = 335 g, SEM = 5.15). Each group was then randomly assigned to one of five drug treatment groups: vehicle (VEH: n = 6), lithium chloride (LiCl: n = 5), 0.1 (n = 5), 0.2 (n = 8), or 0.4 (n = 7) mg/kg cirazoline.

On day 8, the training day, all rats were presented with a 0.1% saccharin solution instead of tap water during the 30-min test session. Immediately following the 30-min access to saccharin, the bottles were removed and each rat was injected (IP) with either VEH, 32 mg/kg LiCl, or 0.1, 0.2, or 0.4 mg/kg cirazoline. Continuous access was allowed to food and to tap water except as specified during the test sessions. Days 9–15 constituted the extinction phase of the experiment. A two-bottle preference procedure (6) during each 30 min trial was used in which the subjects were presented with two Wahmann drinking bottles containing either tap water or the 0.1% saccharin solution. Bottle position was alternated daily according to a left or right position above the cage to control for position preference. Both bottles were removed from each cage immediately following the 30-min trial. Access to tap water was again allowed between the test sessions.

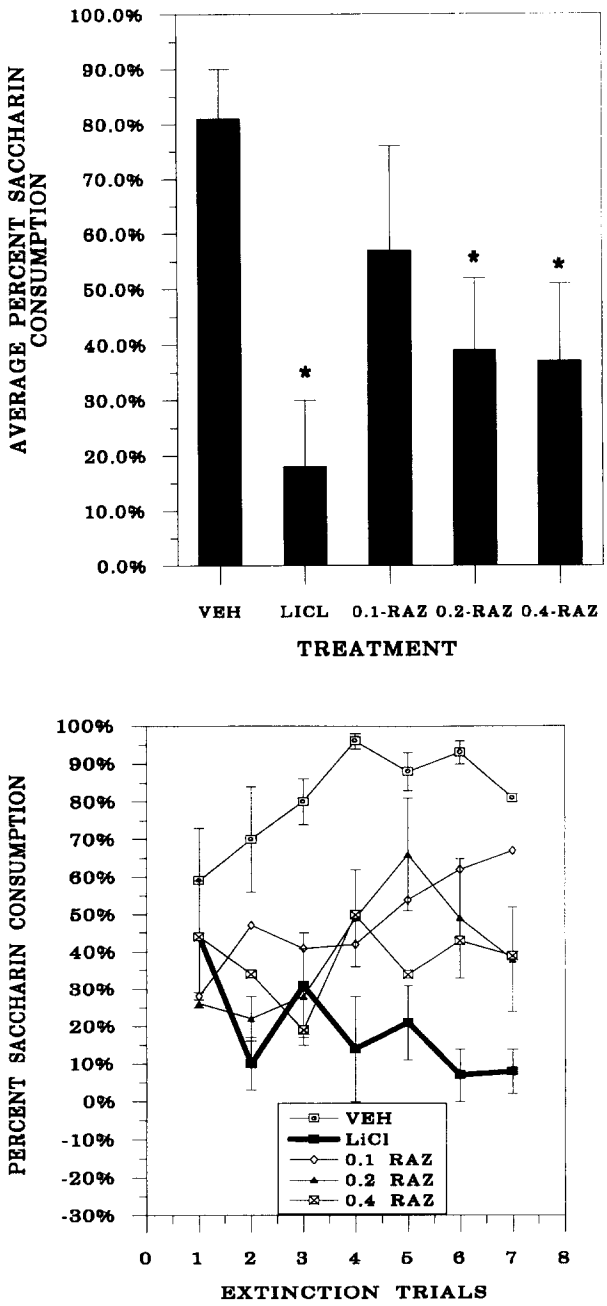

FIG. 1. Top panel: saccharin consumption ratios for each group of rats on extinction days 1–7 following treatment on training day with either vehicle (VEH), 32 mg/kg LiCl (LiCl), or 0.1, 0.2, or 0.4 mg/kg cirazoline (RAZ). Bottom panel: percent saccharin consumption ratios, collapsed across extinction trials 1–7, for the data of the top panel. Standard error of the mean values for VEH and drug-treated groups are represented by bars extending above the symbols. *Indicates significant difference from the control group, $p < 0.05$.

RESULTS

A one-way analysis of variance procedure (1), using the between-group factor of GROUP (VEH, LiCl, 0.1, 0.2 and 0.4 mg/kg cirazoline), was conducted comparing average group water intakes for baseline days 5–7 and indicated no significant group differences, $F(4, 10) = 1.01$, $p < 0.45$. A similar ANOVA failed to reveal significant group differences in saccharin consumption on the training day, $F(4, 26) = 0.26$, $p < 0.90$. Finally, the saccharin and water intake consumption measures collected during extinction days 1–7

were converted into standard suppression ratios [saccharin volume/(saccharin volume + water volume)] and subjected to ANOVA. The design of this ANOVA was a split-plot factorial with a between-group factor of drug treatment (VEH, 32 mg/kg LiCl, and 0.1, 0.2, or 0.4 mg/kg cirazoline) and a within-group factor of trials (1–7). This ANOVA revealed a significant effect of drug treatment, $F(4, 26) = 8.79$, $p < 0.0001$. There was, however, no significant effect of trials, $F(6, 156) = 1.98$, $p < 0.07$, and no significant drug treatment × trials interaction, $F(24, 156) = 1.43$, $p < 0.10$.

Because this analysis revealed no significant effect of trials, an additional ANOVA was computed using suppression ratios, collapsed across the seven trials, which were followed up by Duncan's multiple range procedure to discern between-group differences. Again, a significant effect of group was obtained (identical to that above). Duncan's procedure revealed no significant difference between the VEH group and the 0.1 mg/kg cirazoline group. In contrast, significant group differences were noted between the 0.2 mg/kg cirazoline, 0.4 mg/kg cirazoline, and LiCl groups vs. the VEH group ($\alpha(26) = 0.05$, MSE = 0.036), but these groups were not different from one another. An analysis was performed to confirm the data were normally distributed and the variances were homogenous.

A regression analysis was computed for the effects of cirazoline on conditioned taste aversion, again using the average saccharin consumption ratios collapsed across trials 1–7. Cirazoline doses of 0.0–0.4 mg/kg served as the X factor while suppression ratios served as the Y factor. A saccharin consumption ratio of 0.81 (i.e., that of vehicle-treated rats) was designated as 0% suppression while a suppression ratio of 0.0 was designated as 100% suppression. This analysis revealed that the ED_{50} for CTA was 0.3 mg/kg ± 0.17 for a confidence interval of 95%. A separate regression analysis was computed for food intake using the food intake values from Davies and Wellman (3). This analysis revealed that the ED_{50} for food intake was 0.09 ± 0.09 for a confidence interval of 95%. These ED_{50} values were significantly different, $t(13) = 21$, $p < 0.001$. The slopes of the dose-response curves for cirazoline's effect on CTA and food intake were −0.7 ± 0.17 and −0.15 ± 0.09, respectively (95% confidence interval). An analysis comparing the slopes (11) revealed that they were parallel, $t(13) = 0.082$, $p > 0.05$.

DISCUSSION

The present results document that systemic administration of the α_1-adrenoceptor agonist cirazoline at doses of 0.2 and 0.4 mg/kg induced significant aversion to saccharin relative to a vehicle treatment. A potential interpretation of these results would be that the demonstration of CTA to higher doses of cirazoline supports the view that this drug suppresses food intake by inducing malaise. This view, however, does not take into account appropriate contrasts of the dose-response curves for the effects of systemic cirazoline on conditioned taste aversion and on food intake. Gibbs and Smith (7) cogently argued that the observation of CTA after administration of a compound known to suppress food intake does not necessarily imply an influence of malaise. Rather, higher doses of a test compound may both induce taste aversion and suppress food intake, whereas lower doses may suppress food intake without inducing taste aversion. With regard to cirazoline, significant anorexia is observed at 0.05 mg/kg with a maximal suppression of feeding evident at 0.4 mg/kg (3). The ED_{50} value for the effect of cirazoline on feeding was computed to be 0.09 mg/kg (3). In the present study, cirazoline induced significant taste aversion at doses of 0.2 mg/kg or greater, but this aversion was not dose dependent. It should be noted that the present study explicitly conducted taste aversion testing under conditions similar to those used to determine the anorexic potency of cirazoline (3). That cirazoline has dose-dependent effects on the reduction of food intake but not for CTA, and that the ED_{50} value for the anorexic activity of cirazoline lies well below the ED_{50} value for the induction of taste aversion by cirazoline suggests that malaise is not a prominent factor in the suppression of food intake by this α_1-adrenoceptor agonist. Furthermore, cirazoline has little effect on water intake (3), an outcome inconsistent with a malaise position which would predict equivalent effects of cirazoline-induced malaise on feeding and drinking. A similar profile has been observed for the α_1-adrenoceptor agonist PPA. Wellman, Malpas, and Wikler (12) noted that doses of PPA that lie well below the threshold for CTA have been found to effectively suppress food intake (18).

These results further strengthen the notion that α_1-adrenergic agonists may represent a new generation of drugs that effectively suppress appetite by activating α_1-adrenoceptors within the paraventricular hypothalamus (5,18,19). Injections of a variety of α_1-adrenoceptor agonists into the PVN suppress appetite with minimal action on water intake, and these anorexic effects can be reversed by pretreatment with α_1-adrenoceptor antagonists (4,14,16,18). Moreover, a series of experiments suggest that systemic administration of a variety of α_1-adrenoceptor agonists, which cross the blood–brain-barrier, including PPA, cirazoline, amidephrine, and SK&F 89748, suppress food intake (2,3,10,16,18). Of these, cirazoline exerts marked potency, has minimal effect on water intake, and is effective with both systemic and intra-PVN routes of administration (3). With regard to side effects that would limit the utility of cirazoline as an appetite suppressant, systemic administration of cirazoline at the doses tested in this study has minimal effect on blood pressure (Dr. Timothy Maher, personal communication), does not have stimulant properties (16), and does not evoke the release of norepinephrine in the peripheral nervous system (16) or within the paraventricular hypothalamus (5). In the present experiment, cirazoline had no effect on CTA at a dose that in other studies reliably suppressed feeding behavior. These findings, when considered as a whole, are indicative of an anorexic compound with an identifiable mode of action that may function as an effective appetite suppressant.

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