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Reversal of Cirazoline- and Phenylpropanolamine-Induced Anorexia by the α_1-Receptor Antagonist Prazosin

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WELLMAN, P. J. AND B. T. DAVIES. *Reversal of cirazoline- and phenylpropanolamine-induced anorexia by the* α_1-receptor antagonist prazosin. PHARMACOL BIOCHEM BEHAV **42**(1) 97–100, 1992.—Phenylpropanolamine (PPA) is a phenethylamine anorectic drug that exerts direct agonist effects predominantly on α_1-adrenoceptors, with some α_2-adrenergic activity. Microinjections of PPA, as well as the α_1-adrenergic receptor agonists cirazoline, methoxamine, and 1-phenylephrine, into rat paraventricular nucleus (PVN) suppress feeding. The present study further evaluates the α_1-adrenergic basis of PPA-induced anorexia by examining the effects of systemic injections of the α_1-adrenergic antagonist prazosin (PRAZ, 2 and 5 mg/kg, IP) on the anorexia induced by systemic injections of PPA (5, 10, and 20 mg/kg, IP), as well as cirazoline (0.05, 0.1, and 0.2 mg/kg, IP). Although neither PRAZ dose alone altered food intake in the present study, 2 mg/kg PRAZ effectively reversed the feeding-suppressive effects of both PPA and cirazoline. These results strongly support the hypothesis that α_1-adrenoceptor stimulation mediates the anorexia induced by drugs such as PPA and cirazoline.

Phenylpropanolamine Anorexia Cirazoline α_1-Adrenergic receptors Prazosin

PHENYLPROPANOLAMINE (PPA), a variant of the phenethylamine molecule, suppresses food intake and reduces body fat content (8,10,19,24). The anorexic action of PPA is thought to result from its efficacy as an α_1-adrenergic receptor agonist (22). Pharmacological studies have demonstrated that PPA exerts predominantly direct α_1-adrenergic receptor stimulation (6,14,15). Recent studies have shown that microinjections of PPA into the hypothalamic paraventricular nucleus (PVN) reduce food intake in rats (20,22). Moreover, intra-PVN injection of other α_1-adrenergic agonists including cirazoline (3a), methoxamine (3b), and phenylephrine (23) also suppress feeding.

Prior work has established that stimulation of PVN α_2-adrenergic receptors enhances food intake (5,11,12), presumably by inhibiting PVN neurons that in turn suppress food intake (7). The demonstration that injection of α_1-adrenergic agonists suppress food intake suggests these adrenergic receptor populations (13,25) might exert reciprocal inhibition to modulate feeding behavior. Such interactions between α_1- and α_2-adrenergic receptors have been previously noted in the PVN (9), ventromedial hypothalamus (17), and nucleus tractus solitarius (4).

The hypothesis that PPA's anorectic effect results from stimulation of PVN α_1-adrenergic receptors is further supported by a study by Wellman and Davies (22) in which it was demonstrated that the anorexic action of systemically administered PPA is reversed by intra-PVN administration of the α_1-adrenergic receptor antagonist benoxathina. However, although the aforementioned studies provide a strong indication that PPA acts via stimulation of PVN α_1-adrenergic receptors, whether other adrenergic agonists also suppress feeding via α_1-adrenoceptor stimulation is unknown. Cirazoline, for example, readily crosses the blood-brain barrier and is more potent than PPA in reducing food intake (3a,21). However, this agonist may bind not only to the α_1-adrenoceptor but to the newly proposed "idazoxan receptor" (1,16,18). It therefore remains to be determined whether the anorexia induced by systemic cirazoline, like that of systemic PPA, is reversible by administration of an α_1-adrenergic receptor antagonist. Moreover, the demonstration that systemic administration of an α_1-adrenergic antagonist also reverses the anorexic activity of systemically administered PPA would further support the notion of PPA's action at the α_1-adrenoceptor. Thus, the present experiment examines the effects of systemic pretreatment with the α_1-antagonist prazosin (PRAZ; 2 and 5 mg/kg, IP) on the anorexia induced by systemic injection of PPA (5, 10, and 20 mg/kg, IP), as well as cirazoline (0.05, 0.01, and 0.2 mg/kg, IP).

METHOD

Subjects

Subjects were 21 male Sprague-Dawley viral-free albino rats (obtained from Harlan Industries, Houston, TX) weighing approximately 400–500 g at the beginning of the study.

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Rats were housed individually in standard plastic rodent cages in a colony room maintained at 21.0 ± 1°C under a 12 L : 12 D illumination schedule (lights on at 0700 h). Rats were provided continuous access to tapwater and rodent pellets (Teklad) in the home cage.

Drugs

A vehicle solution was prepared using 0.9% sodium chloride dissolved in sterile distilled water. The cirazoline solutions (0.05, 0.1, and 0.2 mg/ml) were prepared by dissolving cirazoline hydrochloride (a gift from Dr. David Sanger, Synthelabo Labs, Paris, France) into the vehicle solution. The PPA solutions (2.5, 5, and 10 mg/ml) were prepared using PPA hydrochloride (Sigma Chemical, St. Louis, MO) dissolved in 0.9% saline. Solutions of PRAZ (2 and 5 mg/ml) were similarly prepared.

Procedure

Rats were maintained in the colony room for a minimum of 1 week prior to the start of the experiment to acclimate them to daily handling and routine colony procedures. Each rat underwent a series of six baseline feeding trials during which they had access to food and water prior to and following each intake trial (thus, they were tested in a nondeprived condition). Beginning at 1300 h each day, clean cages were provided with cardboard pads beneath the grid floors. The feeders and water bottles were removed and each rat was handled prior to the start of its feeding trial. Approximately 12 g of the pellet diet was placed on the grid floor of each home cage and the weighed water bottles were returned. Each rat was allowed 30-min access to the pellet diet and water. Following each feeding trial, the remaining food and spillage was removed from the cage and weighed to determine individual food intake (to the nearest 0.1 g). Water intakes were measured to the nearest 0.1 ml.

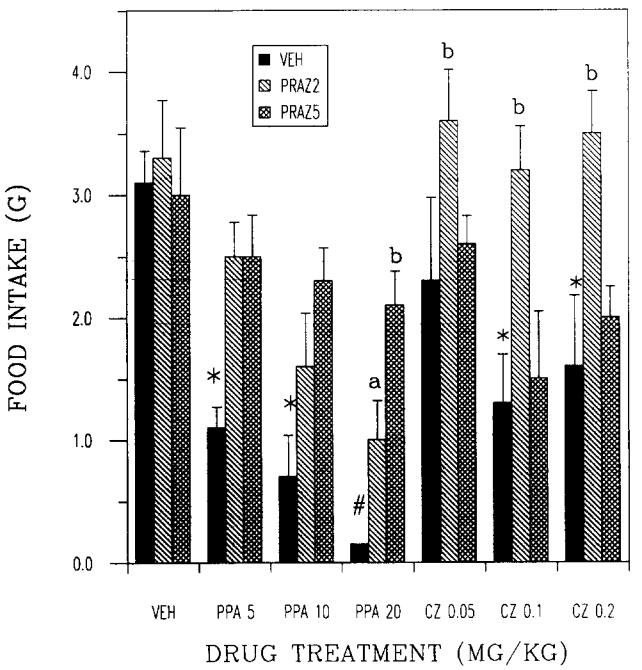

FIG. 1. Reversal of phenylpropanolamine and cirazoline anorexia in rats by systemic administration of prazosin. Mean group pellet intake (g) during a 30-min period for rats pretreated (−40 min) with either VEH, 2 mg/kg PRAZ (PRAZ2), or 5 mg/kg PRAZ (PRAZ5) and treated (−20 min) with VEH, 5, 10, and 20 mg/kg PPA, and 0.05, 0.1, and 0.2 mg/kg cirazoline. The lines above each bar represent the SEM. For comparisons of VEH vs. drug conditions for VEH-pretreated rats, levels of significance are denoted by: (*) $p < 0.05$, (#) $p < 0.001$. For comparisons of drug vs. vehicle pretreatment conditions within a drug treatment condition, the levels of significance are denoted by: (a) $p < 0.05$, (b) $p < 0.01$.

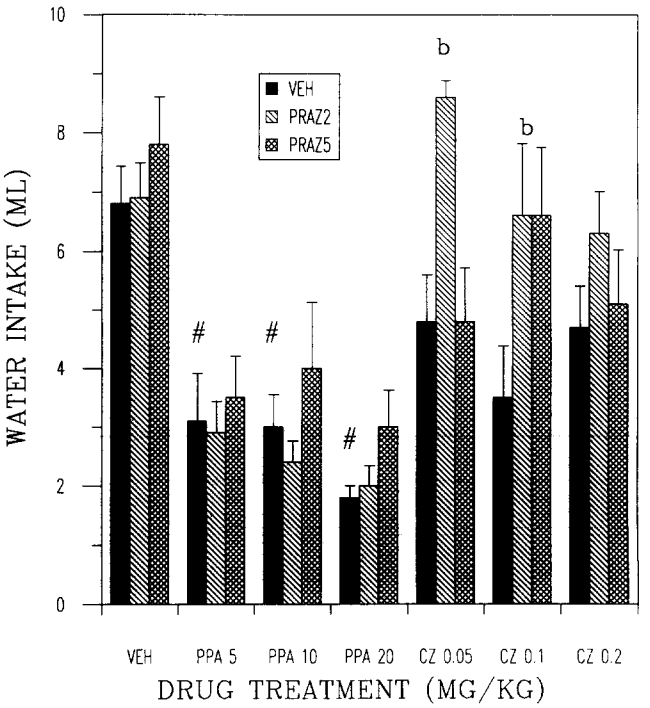

FIG. 2. Effects of systemic prazosin on the hypodipsia induced by systemic cirazoline or phenylpropanolamine. Mean group water intake (ml) during a 30-min period for rats pretreated (−40 min) with either VEH, 2 mg/kg PRAZ (PRAZ2), or 5 mg/kg PRAZ (PRAZ5) and treated (−20 min) with VEH, 5, 10, and 20 mg/kg PPA, and 0.05, 0.1, and 0.2 mg/kg cirazoline. The lines above each bar represent the SEM. Symbols for statistical significance are as for Fig. 1.

Following baseline intake trials, rats were randomly assigned to one of three groups of comparable mean baseline food intake. The three groups formed were randomly assigned a drug pretreatment (VEH, 2 mg/kg PRAZ, or 5 mg/kg PRAZ) to be administered for the duration of the study. During Trials 1-3, rats received their respective pretreatment injections of either VEH, 2 mg/kg PRAZ, or 5 mg/kg PRAZ 40 min prior to the feeding test and a randomly assigned dose of either PPA or cirazoline 20 min prior to feeding. For Trial 4, all rats received their respective pretreatment injections followed by a treatment injection of VEH. In Trials 5-7, drug treatments were shifted so that those rats that formerly received PPA received the cirazoline doses and those rats that had received cirazoline were treated with PPA. Two no-injection trials separated each drug test to allow rats to recover their baseline food intakes.

Data Analyses

The design of the experiment was a split-plot factorial with a between-group factor of drug pretreatment (0, 2, or 5 mg/

kg PRAZ) and a within-group factor of drug dose (0, low, medium, high). Separate analyses of variance (ANOVAs) (2) were computed for PPA and cirazoline tests for the dependent variables of food intake and water intake. Additional contrasts were made using Tukey's procedure. Difference probabilities less than 0.05 were deemed statistically significant.

RESULTS

Figure 1 depicts the changes in food intake produced by the PRAZ pretreatments and drug treatments in the present experiment. PRAZ pretreatment, at doses of 2 and 5 mg/kg, did not alter food intake in rats treated with vehicle ($p > 0.05$). ANOVAs computed for the PPA injection series revealed a significant effect of PPA dose, $F(3, 54) = 32.9$, $p < 0.0001$, a significant effect of PRAZ pretreatment, $F(2, 18) = 6.7$, $p < 0.007$, but also a significant interaction between the factors of PRAZ pretreatment and PPA dose, $F(6, 54) = 3.5$, $p < 0.006$. PPA produced a dose-dependent suppression of food intake in the group of rats pretreated with VEH. A priori contrasts of food intake in VEH-pretreated rats revealed that food intake after 5 mg/kg PPA was significantly less than after vehicle ($p < 0.05$); that food intake after 10 mg/kg PPA was not different from either 5 mg/kg PPA or 20 mg/kg PPA; and that food intake after 20 mg/kg PPA was significantly less than after vehicle ($p < 0.001$) or 5 mg/kg PPA ($p < 0.01$). The PRAZ pretreatments reversed the anorexic action of all PPA doses administered. Moreover, contrasts computed using Tukey's test at the 20-mg/kg PPA dose revealed that food intake after pretreatment with 5 mg/kg PRAZ was significantly greater than that after 2 mg/kg PRAZ, which was significantly greater than that recorded after vehicle pretreatment (HSD > 0.87, $p < 0.05$).

Figure 1 also depicts the effects of PRAZ pretreatment on the anorexia induced by cirazoline. ANOVAs revealed a significant effect of cirazoline dose, $F(3, 54) = 5.5$, $p < 0.0024$, and a significant effect of PRAZ pretreatment, $F(2, 18) = 5.1$, $p < 0.0187$, but no significant interaction between these factors, $F(6, 54) = 1.5$, $p < 0.1846$. Subsequent contrasts revealed that 0.1 and 0.2 mg/kg cirazoline treatments significantly suppressed food intake relative to vehicle treatment in rats pretreated with vehicle ($p < 0.05$), but that food intake after 0.2 mg/kg cirazoline was not different from that after 0.1 mg/kg cirazoline. Additional contrasts revealed that PRAZ pretreatment at 2 mg/kg significantly increased food intake (collapsed across cirazoline doses) (HSD > 0.81, $p < 0.01$). Surprisingly, food intake following pretreatment with the higher dose of PRAZ (5 mg/kg) was not different from food intake after VEH pretreatment.

Figure 2 depicts the changes in water intake produced by the PRAZ pretreatments and drug treatments used in the present study. PRAZ alone did not alter water intake in that water intakes after vehicle treatment were not different between rats pretreated with either VEH, 2 mg/kg PRAZ, or 5 mg/kg PRAZ ($p > 0.05$). ANOVAs revealed a significant suppressive effect of PPA dose, $F(3, 54) = 50.7$, $p < 0.0001$, on water intake, but no significant effect of PRAZ pretreatment, $F(2, 18) = 0.97$, $p < 0.3968$, nor any interaction between PPA dose and PRAZ pretreatment, $F(6, 54) = 0.3$, $p < 0.9325$. In contrast to the effects of PPA, ANOVAs revealed no significant effect of cirazoline dose, $F(3, 54) = 2.5$, $p < 0.0715$, on water intake. However, there was a significant effect of PRAZ pretreatment, $F(2, 18) = 9.22$, $p < 0.0018$, as well as a significant interaction between cirazoline dose and PRAZ pretreatment, $F(6, 54) = 2.46$, $p < 0.0375$. Although there was no difference in water intake between the vehicle and 5-mg/kg PRAZ pretreatment conditions (collapsed across cirazoline doses), water intake of the 2-mg/kg PRAZ group was significantly greater than that of the vehicle pretreatment condition.

DISCUSSION

A converging series of studies employing intra-PVN injections of PPA, phenylephrine, methoxamine, and cirazoline, as well as systemic injection of α_1-adrenergic agonists PPA and cirazoline, reveal that stimulation of α_1-adrenergic receptors reduces food intake (3,21–24). However, such reductions in food intake following injections of α_1-agonists do not firmly establish that this effect reflects an action on a receptor population that modulates feeding behavior. Such a demonstration requires reversal of the behavioral effect using pharmacological receptor antagonists. Wellman and Davies (22) documented that the anorexia induced by systemic PPA, a route capable of delivering drug both peripherally and to brain, is reversed by prior central injection of the α_1-adrenergic antagonist benoxathian into the PVN. Moreover, the reversal induced by intra-PVN benoxathian was evident only for the inhibitory effect of PPA on food intake in that PPA-induced hypodypsia was not altered by benoxathian pretreatment.

The intent of the present study was to further extend the antagonist method using a different route of exposure (systemic), a different α_1-adrenergic antagonist (PRAZ), and comparison of the effects of these variables on the anorexia induced by systemic injections of PPA, as well as the novel α_1-agonist cirazoline. Pretreatment with PRAZ alone did not alter baseline food or water intake. It did, however, reverse the anorexic action of PPA, with the 5-mg/kg dose of PRAZ exerting a greater effect than the 2-mg/kg dose. The effect of PRAZ in the PPA injection series was specific to food intake in that the hypodipsic action of PPA was not altered by either dose of PRAZ. In contrast, the effect of PRAZ on the anorexia induced by the novel α_1-adrenergic anorexic agent cirazoline was evident at 2 mg/kg PRAZ, but the 5-mg/kg PRAZ dose did not alter the effects of cirazoline on ingestive behavior. The precise reason for the lack of reversal by 5 mg/kg PRAZ on cirazoline anorexia is unclear but may be related to a sedative action of the combination of these two compounds. Observation of animals during the study suggests that 5 mg/kg PRAZ did produce sedation of rats during the cirazoline series, and this effect could be responsible for the reduced food intake.

The present study documents that both PPA and cirazoline induce anorexia in rats that is reversible by the α_1-adrenergic antagonist PRAZ. The two drugs, however, exhibited some differences in the pattern of reversal. PRAZ reversed the anorexia, but not the hypodypsia, induced by all doses of PPA, whereas it (2 mg/kg) reversed both anorexia and hypodypsia induced by cirazoline. These results suggest that hypodypsia induced by these anorexic drugs may have two bases: one related to stimulation of α_1-adrenoceptors (as in the case of cirazoline) and one nonadrenergic (as in the case of PPA).

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REFERENCES

1. Cavero, I.; Lefevre-Borg, F.; Roach, A. G.; Gomeni, R.; Scatton, B. Functional and biochemical evidence for lack of cardiac presynaptic α_2-adrenoceptor stimulant properties of cirazoline (LD 3098), a potent α_1-adrenoceptor agonist in dogs and rats. J. Pharmacol. Exp. Ther. 223:241–250; 1982.
2. Cody, R. P.; Smith, J. K. Applied statistics and the SAS programming language. New York: North Holland; 1987.

3a. Davies, B. T.; Wellman, P. J. Effects on ingestive behavior of the α_1-adrenoceptor agonist cirazoline. Eur. J. Pharmacol. 210: 11–16; 1992.

3b. Davies, B. T.; Wellman, R. J.; DiCarlo, B. Microinjection of the α_1-agonist methoxamine into the paraventricular hypothalamus induces anorexia in rats. Brain Res. Bull. (in press).

4. Feldman, P. D.; Felder, R. B. Alpha-adrenergic influences on neuronal responses to visceral afferent input in the nucleus tractus solitarius. Neuropharmacology 28:1081–1087; 1989.
5. Goldman, C. K.; Marino, L.; Leibowitz, S. F. Postsynaptic α_2-noradrenergic receptors mediate feeding induced by paraventricular nucleus injection of norepinephrine and clonidine. Eur. J. Pharmacol. 115:11–19; 1985.
6. Hettiarachchi, M.; Colquhoun, E. Q.; Ye, J. M.; Rattigam, S.; Clark, M. G. Norephedrine (phenylpropanolamine) stimulates oxygen consumption and lactate production in the perfused rat hindlimb. Int. J. Obes. 15:37–43; 1991.
7. Hoebel, B. G.; Leibowitz, S. F. Brain monoamines in the modulation of self-stimulation, feeding and body weight. In: Weiner, H.; Hofer, M. A.; Stunkard, A. J., eds. Brain, behavior and disease. New York: Raven Press; 1981:103–142.
8. Kornblith, C. L.; Hoebel, B. G. A dose–response study of anorectic drug effects on food intake, self-stimulation, and stimulation-escape. Pharmacol. Biochem. Behav. 5:215–218; 1976.
9. Kow, L.-M.; Pfaff, D. W. Responses of hypothalamic paraventricular neurons in vitro to norepinephrine and other feeding-relevant agents. Physiol. Behav. 46:265–271; 1989.
10. Lasagna, L. Phenylpropanolamine: A review. New York: John Wiley and Sons; 1988.
11. Leibowitz, S. F. Paraventricular nucleus: A primary site mediating adrenergic stimulation of feeding and drinking. Pharmacol. Biochem. Behav. 8:163–175; 1978.
12. Leibowitz, S. F. Hypothalamic paraventricular nucleus: Interaction between α_2-noradrenergic system and circulating hormones and nutrients in relation to energy balance. Neurosci. Biobehav. Rev. 12:101–109; 1988.
13. Leibowitz, S. F.; Jhanwar-Uniyal, M.; Dvorkin, B.; Makman, M. H. Distribution of alpha-adrenergic, beta-adrenergic and dopaminergic receptors in discrete hypothalamic areas of rat. Brain Res. 233:97–114; 1982.
14. Minneman, K. P.; Fox, A. W.; Abel, P. W. Occupancy of alpha-1 adrenergic receptors and contraction of rat vas deferens. Mol. Pharmacol. 23:359–368; 1983.
15. Moya-Huff, F.; Maher, T. J. Adrenergic receptor subtype activation by (+)-, (−)- and (+)-norephedrine in the pithed rat. J. Pharm. Pharmacol. 39:108–112; 1987.
16. Ruffulo, R. R.; Waddell, J. E. Receptor interactions of imidazolines. IX. Cirazoline is an α_1-adrenergic receptor agonist and an α_2-adrenergic antagonist. J. Pharmacol. Exp. Ther. 222:29–36; 1982.
17. Saad, W. A.; Camargo, L. A. A.; Saad, W. A. Effects of the application of α_1- and α_2-adrenoceptor agonists and antagonists into the ventromedial hypothalamus on the sodium and potassium excretion. Pharmacology 28:228–238; 1984.
18. Sanger, D. Discriminative stimulus effects of the α_2-adrenoceptor antagonist idazoxan. Psychopharmacology (Berl.) 99:117–121; 1989.
19. Wellman, P. J. A review of the physiological bases of the anorexic action of phenylpropanolamine (*d,l*-norephedrine). Neurosci. Biobehav. Rev. 14:339–355; 1990.
20. Wellman, P. J.; Cockroft, R. Effects of perifornical hypothalamic microinjections of phenylpropanolamine and amphetamine on latency to feed and mash intake in rats. Pharmacol. Biochem. Behav. 35:461–464; 1990.
21. Wellman, P. J.; Davies, B. T. Effects of paraventricular hypothalamic microinjections of phenylpropanolamine and *d*-amphetamine on mash intake in rats. Brain Res. Bull. 25:335–338; 1990.
22. Wellman, P. J.; Davies, B. T. Reversal of phenylpropanolamine anorexia in rats by the alpha-1 receptor antagonist benoxathian. Pharmacol. Biochem. Behav. 38:905–908; 1991.
23. Wellman, P. J.; Davies, B. T. Suppression of feeding induced by phenylephrine microinjections within the paraventricular hypothalamus in rats. Appetite 7:121–128; 1991.
24. Wellman, P. J.; Sellers, T. L. Weight loss induced by phenylpropanolamine: Anorexia and brown adipose tissue thermogenesis. Pharmacol. Biochem. Behav. 24:605–611; 1986.
25. Wilcox, B. J.; Raskind, M. A.; Ko, G. N.; Pascualy, M.; Dorsa, D. M. Localization of ^{3}H-prazosin binding sites in the supraoptic and paraventricular nuclei of the human hypothalamus. Neuroendocrinology 51:315–319; 1990.