Pharmacology Biochemistry & Behavior, Vol. 38, pp. 905–908. © Pergamon Press plc, 1991. Printed in the U.S.A. 0091-3057/91 $3.00 + .00

RAPID COMMUNICATION

Reversal of Phenylpropanolamine Anorexia in Rats by the Alpha-1 Receptor Antagonist Benoxathian

PAUL J. WELLMAN AND BECKY T. DAVIES

Department of Psychology, Texas A&M University, College Station, TX 77843

Received 14 November 1990

WELLMAN, P. J. AND B. T. DAVIES. *Reversal of phenylpropanolamine anorexia in rats by the alpha-1 receptor antagonist benoxathian.* PHARMACOL BIOCHEM BEHAV **38**(4) 905–908, 1991.—Phenylpropanolamine (PPA) is a phenethylamine anorectic drug that exerts direct agonist effects predominantly on alpha-1 adrenergic receptors, with some alpha-2 adrenergic activity. Direct injections of PPA as well as the alpha-1 agonist l-phenylephrine into rat paraventricular nucleus (PVN) suppress feeding. In the present study, we evaluate the hypothesis that systemic PPA acts within the PVN on an alpha-1 receptor population to suppress feeding. Accordingly, adult male rats were prepared with a unilateral guide cannula aimed at the PVN. Microinjection of the alpha-1 adrenergic receptor antagonist benoxathian (0, 2.5, 5.0 or 10.0 nmol) into the PVN was found to have no effect on baseline feeding behavior. Microinjection of 10.0 nmol benoxathian into the PVN completely reversed the anorexia induced by 2.5, 5.0 or 10.0 mg/kg PPA (IP), yet did not alter the hypodipsia produced by PPA. These data strongly suggest that PPA anorexia is mediated by an alpha-1 adrenergic satiety mechanism within the PVN.

Paraventricular nucleus Phenylpropanolamine Anorexia Alpha-1 adrenergic receptors Benoxathian

INJECTION of phenylpropanolamine (PPA) into the paraventricular hypothalamus (PVN), but not the perifornical hypothalamus (PFH), significantly reduces feeding behavior in rats (14,15). Studies of alpha receptor binding using vas deferens tissue reveal that PPA binds to alpha-1 receptors (7) and studies of the cardiac system reveal that increases in blood pressure induced by PPA are mediated predominantly by activation of alpha-1 receptors, with partial activation of alpha-2 receptors (8). The anorectic effect of PPA on feeding after administration into the PVN is mimicked by injection of the alpha-1 agonist l-phenylephrine (16). Yet, these studies do not permit a conclusion as to whether an alpha-1 receptor system (either systemic or central) mediates the anorexic action of PPA, or the issue of whether this system resides solely within the PVN. Systemic as well as central hypotheses have been advanced to account for the anorexic action of PPA but these have been difficult to test in a differential manner (1,13).

A variety of specific alpha-1 antagonists have been developed over the last ten years. One of these, benoxathian, readily binds to alpha-1 receptors in the periphery and is reported to exhibit greater binding to alpha-1 receptor than to alpha-2 receptors (2, 6, 10). In the present study, we evaluate the effect on feeding of direct injections of benoxathian alone (0, 2.5, 5.0 and 10.0 nmol/1 μl) into the PVN in rat. Moreover, we demonstrate that preinjection of 10.0 nmol benoxathian into the PVN reliably and completely reverses the anorexia induced by systemic injection of phenylpropanolamine (0, 2.5, 5.0 and 10.0 mg/kg, IP).

METHOD

Subjects

The subjects were 9 male Sprague-Dawley viral-free albino rats (obtained from Harlan Industries; Houston, TX) weighing approximately 385 g at the beginning of the study. The rats were housed individually in standard plastic rodent cages in a colony room maintained at 21.0 ± 1°C under a 12-h/12-h illumination schedule (lights on at 0700 h). The rats were provided continuous access to tap water and to rodent pellets (Teklad) in the home cage.

Drugs

A vehicle (Ringers) solution consisted of 1.2 mM $CaCl_2$, 4.0 mM KCl and 145.8 mM NaCl dissolved in sterile distilled water. All drug solutions were calculated as the weight of the base molecule mixed in 1.0 μl vehicle. Benoxathian solutions were prepared using the hydrochloride salt of benoxathian (RBI) dissolved in vehicle solution. The norepinephrine solution (25 nmol) was prepared using ±-norepinephrine hydrochloride (Sigma) dissolved in vehicle. The phenylpropanolamine solutions (2.5, 5.0 and 10.0 mg/ml) were prepared using PPA hydrochloride (Sigma: Lot 21F-0215) dissolved in 0.9% saline.

Procedure

The rats were maintained in the colony room for 90 days prior to the start of the experiment to acclimate them to daily handling and routine colony procedures. At the end of the adaptation period, the rats were deprived of food and water for approximately 15 h prior to the surgical procedure.

On the day of surgery, each rat was injected (IP) with 0.7 mg/kg atropine sulfate (to minimize bronchial secretions) and then anesthetized using separate injections (spaced 5 min apart) of ketamine (Ketaset: 60 mg/kg, IP) and sodium pentobarbital (20 mg/kg, IP). With the upper incisor bar of the stereotaxic instrument positioned 3.0 mm above the interaural line, the tip of each cannula was positioned 0.4 mm caudal to bregma, 0.4 mm lateral to the midline, and 8.0 mm below the surface of the skull. The shaft of each guide cannula was affixed to the skull using stainless steel screws and a pedestal of dental acrylic. Following surgery, each rat received a single injection (IM) of penicillin (300,000 units).

A 7-day recovery period followed the surgical procedure, during which the rats were weighed daily and given continuous access to food pellets and water.

Phase 1: Effects of benoxathian injections on baseline food intake. Following recovery from surgery, each rat underwent a series of 8 baseline feeding trials. The rats were not deprived of food or water prior to each intake trial. Beginning at 1600 h on baseline Days 1–8, a clean cage (with DACB pad placed beneath a grid floor) was provided for each rat. The feeder was not returned to the home cage, and each rat was handled prior to the start of its feeding trial. Approximately 12 g of the pellet diet was positioned on the grid floor of each home cage beginning six minutes after the start of the trial. Each rat was allowed 60 minutes access to the pellet diet and to water. At the end of each feeding trial, the remaining food and spillage was removed from the cage and was weighed to determine individual food intake (to the nearest 0.1 g). Water intakes were not measured in the first phase of the present study. The rats were allowed free access to food and water between feeding measurement trials.

On drug test 1, the rats were injected with 25 nmol norepinephrine (NE) to determine the probable locus of the injection cannula within the PVN. Prior studies have noted that this dose of NE reliably increases feeding behavior after injection into the PVN (4,11). After insertion of the microinjector into the guide shaft (positioned so as to extend 1.0 mm beyond the tip of the guide shaft), 1.0 μl of NE solution was injected over a 10-s period, with the injector left in place for an additional minute. Rats that did not respond to NE injection with a food intake of at least 3.0 grams were discarded from the experiment.

On drug tests 2–5, the rats were injected with either vehicle, 2.5, 5.0 or 10.0 nmol benoxathian, in random order, using the procedures outlined above. Two no-injection food intake trials were interposed between each drug trial.

Phase 2: Effects of PVN benoxathian on anorexia induced by systemic PPA. In this phase, the rats were shifted to a 30-min testing procedure in which both food intake (to the nearest 0.1 g) and water intake (to the nearest ml) were recorded for each rat after a PVN injection of either vehicle or 10 nmol benoxathian and a subsequent systemic injection of either vehicle or 2.5, 5.0 or 10.0 mg/kg PPA. The duration of the ingestive test was shortened during this phase because of the relatively short duration of action of benoxathian (6). The interval between PVN and systemic injection was about 1 min, and each rat was offered about 12 g of food pellets and access to a drinking tube 6 minutes after the injection procedure. Each rat received each combination of PVN injection and systemic PPA yielding 8 drug trials. For the first 4 trials, half of the rats received the benoxathian injections (with systemic PPA injections administered in random order), whereas the remaining rats received vehicle PVN injections (with randomized systemic PPA injections). In the next 4 trials, the pretreatment injection conditions (benoxathian or vehicle) were reversed and the rats again received PPA treatments in random order. In this series, only a single no-injection feeding trial was interposed between successive drug trials as there were no apparent carry-over effects noted in Phase 1.

Histological Analyses

At the conclusion of the experiment, the rats were overdosed with sodium pentobarbital (60 mg/kg, IP) and perfused through the heart with 0.9% saline followed by 10% formalin. Further fixation in 10% formalin proceeded for at least 72 hours prior to sectioning each brain. Alternate 80-micron frozen sections were photographically enlarged and compared to the atlas plates from Paxinos and Watson (9) to verify cannula placements.

RESULTS

Two rats failed to consume food during the baseline phase and testing phases and one rat became ill and lost approximately 50 grams body weight during the baseline phase. Data analyses were calculated for 6 rats in the present study. Histological analysis revealed that these 6 rats exhibited cannula placements that were positioned in the medial and posterior parvocellular aspects of the paraventricular nucleus. The cannula placements within the PVN were bounded ventrally by the dorsal aspects of the medial preoptic nucleus and dorsally by the ventral thalamic reuniens nucleus and were positioned between the lateral posterior and medial parvocellular aspects of the parventricular nucleus.

Phase 1. In the present experiment, feeding was recorded during a 60-min period in the late afternoon for rats maintained on an ad lib feeding and drinking schedule. Under these conditions, the rats ate an average of 2.0 grams (SEM = 0.25 g) during the last 3 days of the baseline period. Food intakes after microinjection of NE into the PVN on trial 1 produced a 94% increase in food intake to 3.9 g (SEM = 0.6 g). On drug trials 2–5, PVN injection of the vehicle, 2.5, 5.0 and 10.0 nmol benoxathian resulted in intakes of 1.9, 2.6, 2.2 and 2.5 g, respectively. Although benoxathian slightly increased food intake relative to the vehicle value, these changes were not statistically significant, $F(5,15) = 0.48$, $p<0.99$.

Phase 2. In phase 2, rats were pretreated with either vehicle or 10.0 nmol benoxathian into the PVN and then injected with either vehicle or one of 3 systemic doses of PPA. Figure 1 depicts the changes in feeding behavior produced by these treatments. Analysis of variance revealed a significant interaction between Pretreatment (benoxathian vs. vehicle) and Treatment (0, 2.5, 5.0, 10.0 mg/kg PPA) factors, $F(3,15) = 5.6$, $p<0.009$. Subsequent within-pretreatment-comparisons (of 10 mg/kg PPA relative to 0.0 mg/kg PPA) revealed that PPA significantly suppressed feeding in rats given vehicle injection into the PVN, $t(40) = 2.8$, $p<0.02$, but not in rats given 10 nmol benoxathian ($p>0.05$). This effect of benoxathian on PPA anorexia was not due to an algebraic summation, as there was no difference in food intake between the vehicle-0.0 mg/kg PPA condition and the benoxathian-0.0 mg/kg PPA condition.

Figure 2 depicts the changes in drinking produced by the benoxathian pretreatment and PPA treatments in the present study. Again, PPA produced a significant suppression of drinking in rats given vehicle injection into the PVN. In contrast to the results obtained with feeding, however, benoxathian injection into the PVN did not reverse the hypodipsic action of PPA. These results were confirmed by analysis of variance which revealed no signif-

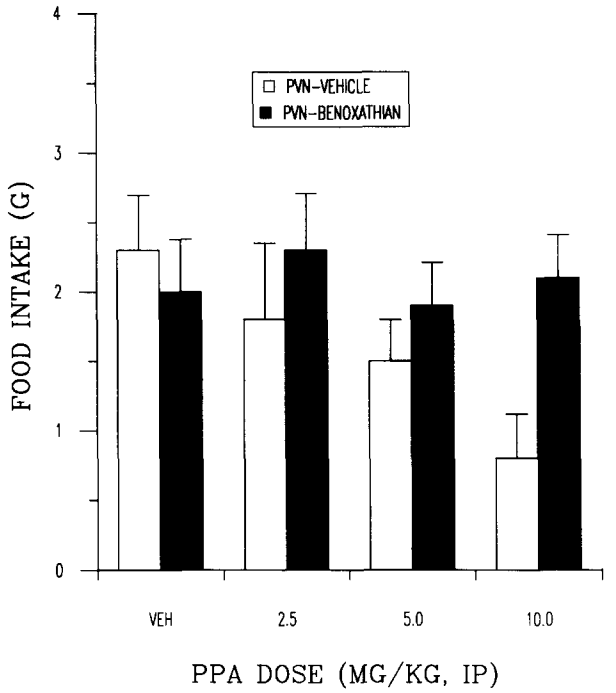

FIG. 1. Mean group pellet intake (g) during a 30-minute period for 6 rats receiving a PVN microinjection of vehicle (PVN-VEHICLE) or 10.0 nmol benoxathian (PVN-BENOXATHIAN) 1 min prior to systemic (IP) injection of 0.0, 2.5, 5.0 or 10.0 mg/kg PPA. The lines above each bar represent the standard error of the mean.

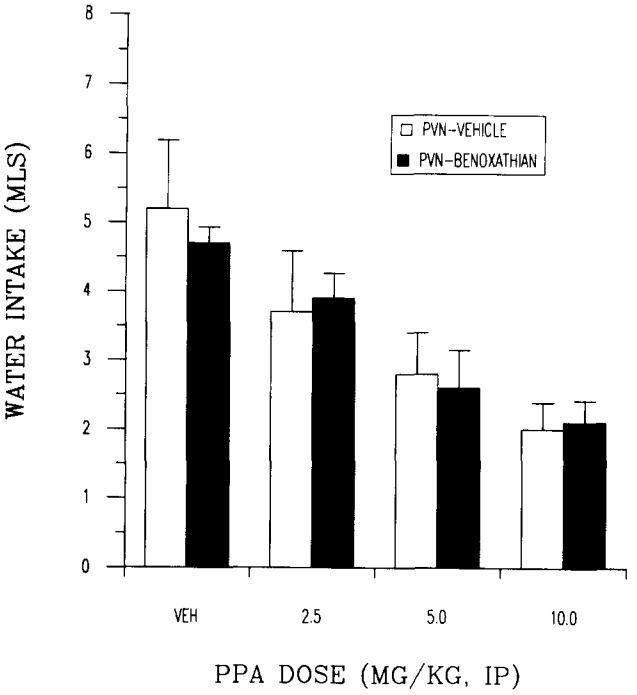

FIG. 2. Mean group water intake (ml) during a 30-minute period for 6 rats receiving a PVN microinjection of vehicle (PVN-VEHICLE) or 10.0 nmol benoxathian (PVN-BENOXATHIAN) 1 min prior to systemic (IP) injection of 0.0, 2.5, 5.0 or 10.0 mg/kg PPA. The lines above each bar represent the standard error of the mean.

icant interaction between the factors of Pretreatment and Treatment, $F(3,15)=0.1$, $p<0.9768$.

Finally, at the end of the experiment, the rats continued to respond to an intra-PVN injection of 25 nmol NE with an average group food intake of 4.0 g. Thus the cannulae remained patent throughout the experiment.

DISCUSSION

As recently as 1989, the mechanism by which phenylpropanolamine suppresses feeding was unknown (12). Wellman and Cockroft (14) noted that microinjection of PPA into the rat paraventricular hypothalamus resulted in a significant suppression of feeding, a finding that was confirmed by Wellman and Davies (15). These reports linked the anorexic action of PPA to the PVN, but did not establish that systemic PPA acted solely on cells within the PVN, nor did these reports suggest a neuropharmacological explanation for the anorexic action of PPA.

PPA is considered to be a primarily direct-acting agonist at the alpha-1 receptor in the rat cardiac system as well as in rat vas deferens (7,8). That PPA might act via an alpha-1 mechanism within the PVN was strengthened by two lines of evidence: 1) binding studies established the existence of alpha-1 receptors within the PVN (5); and 2) microinjection of the alpha-1 receptor agonist l-phenylephrine into the PVN suppressed feeding at doses comparable to that at which PVN microinjections of PPA suppress feeding (16).

In the present study, injection of the selective alpha-1 antagonist benoxathian into the PVN completely reversed the anorexic action of systemic injections of PPA. Although PPA has demonstrable effects within the autonomic nervous system [cf. (3,12)], the present results demonstrate that the systemic effects of PPA are not necessary for the expression of the anorexic action of PPA. Moreover, the demonstration of a reversal of PPA anorexia at a dose of benoxathian (10 nmol), that alone did not alter baseline food intake or reverse the hypodipsic action of PPA, provides compelling evidence for a selective effect on feeding of alpha-1 receptors within the PVN.

ACKNOWLEDGEMENTS

The authors wish to thank Vanessa Blasingame, James Fojt, Kathleen Isaac and Alisa Rothermel for their assistance in this study and Thompson Medical Company for funds to carry out this study.

REFERENCES

1. 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.
2. Hoyer, D.; Jones, C. R.; Ford, W.; Palacios, J. M. Subtypes of alpha1-adrenoreceptors in hippocampus of pigs, guinea-pigs, calves and humans: regional differences. Eur. J. Pharmacol. 188:9–16; 1990.
3. Lasagna, L. Phenylpropanolamine: A review. New York: John Wiley and Sons; 1988.
4. 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.
5. Leibowitz, S. F.; Jhanwar-Uniyal, M.; Dvorkin, B.; Makman, M. H. Distribution of alpha-adrenergic, beta-adrenergic and dopaminergic and dopaminergic receptors in discrete hypothalamic areas of rat.

Brain Res. 233:97–114; 1982.
6. Massi, M.; Venturi, F.; Brasili, L.; Melchiorre, C. Hypotensive effect in dogs and rats of intravenous injections of the alpha1-adrenoceptor antagonist benoxathian. Pharmacol. Res. Commun. 18:813–829; 1986.
7. Minneman, K. P.; Fox, A. W.; Abel, P. W. Occupancy of alpha-1 adrenergic receptors and contraction of rat vas deferens. Mol. Pharmacol. 23:359–36; 1983.
8. Moya-Huff, F.; Maher, T. J. Adrenergic receptor subtype activation by (+)-, (−)- and (±)-norephedrine in the pithed rat. J. Pharm. Pharmacol. 39:108–112; 1987.
9. Paxinos, G.; Watson, C. The rat brain in stereotaxic coordinates, 2nd ed. New York: Academic Press; 1986.
10. Pigini, M.; Brasili, L.; Giannella, M.; Giardina, D.; Gulini, U.; Quaglia, W.; Melchiorre, C. Structure-activity relationships in 1,4-benzodioxan-related compounds. Investigation on the role of the dehydrodioxane ring on alpha1-adrenoceptor blocking activity. J. Med. Chem. 31:2300–2304; 1988.
11. Roland, C. R.; Oppenheimer, R. L.; Chang, K.; Leibowitz, S. F. Hypophysectomy disturbs the noradrenergic feeding system of the paraventricular nucleus. Psychoneuroendocrinology 10:109–120; 1985.
12. 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.
13. Wellman, P. J.; Arasteh, K.; Ruddle, J.; Strickland, M. D. Effects of phenylpropanolamine on gastric retention in the adult rat. Brain Res. Bull. 17:127–128; 1986.
14. 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: 461–465; 1990.
15. Wellman, P. J.; Davies, B. T. Effects of paraventricular hypothalamic microinjections of phenylpropanolamine and d-ampetamine on mash intake in rats. Brain Res. Bull. 25:335–338; 1990.
16. Wellman, P. J.; Davies, B. T. Suppression of feeding induced by phenylephrine microinjections within the paraventricular hypothalamus in rats. Appetite, in press; 1991.