Pharmacology Biochemistry & Behavior, Vol. 35, pp. 461–464. © Pergamon Press plc, 1990. Printed in the U.S.A. 0091-3057/90 $3.00 + .00

Effects of Perifornical Hypothalamic Microinjections of Phenylpropanolamine and Amphetamine on Latency to Feed and Mash Intake in Rats[1]

P. J. WELLMAN AND R. COCKROFT

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

Received 19 July 1989

WELLMAN, P. J. AND R. COCKROFT. *Effects of perifornical hypothalamic microinjections of phenylpropanolamine and amphetamine on latency to feed and mash intake in rats.* PHARMACOL BIOCHEM BEHAV **35**(2) 461–464, 1990.—To determine whether phenylpropanolamine (PPA) and amphetamine act on a common satiety mechanism, the present experiment compared the effect of unilateral microinjections (40, 80, and 160 nmol) of phenylpropanolamine hydrochloride (PPA: d,l-norephedrine) and of d-amphetamine sulfate within the perifornical hypothalamus (PFH) on consumption of a palatable sweetened-mash diet in adult male rats. Microinjection of d-amphetamine (40–160 nmol) within the PFH induced dose-dependent anorexia, whereas PPA microinjections were without effect on feeding. These results document that amphetamine and PPA do not act at a common CNS site, such as the PFH, to induce anorexia.

Phenylpropanolamine Amphetamine Anorexia Perifornical hypothalamus Food intake

PHENYLPROPANOLAMINE (PPA), the racemic mixture of d- and l-norephedrine, induces anorexia and weight loss (9, 14, 27) that is dose- and isomer-dependent (3, 8, 25, 27). Although PPA is structurally similar to amphetamine, the two drugs appear more dissimilar than similar (21,23). A number of studies have documented important differences between amphetamine and PPA with regard to anorexic magnitude, isomer potency and development of tolerance with chronic treatment (8, 21, 24, 25, 27).

The issue as to whether amphetamine and PPA induce anorexia via a common mechanism has not been resolved. The anorexic action of amphetamine has been linked to activation of dopaminergic and/or beta-adrenergic receptors within the perifornical hypothalamus [PFH: (12)]. Hoebel's group noted that application of PPA crystals within the lateral hypothalamus readily suppressed feeding induced by low-level electrical stimulation of the lateral hypothalamus, whereas application of PPA within the medial hypothalamus was without effect on elicited feeding (11). Their results suggested that PPA's anorexic activity is linked to catecholaminergic mechanisms within the lateral hypothalamus. Yet, no report to date has examined the action of intracranial application of various doses of PPA on deprivation-induced feeding behavior as has been accomplished for amphetamine (2, 16, 17).

The purpose of the present experiment was to compare the effects of microinjections of PPA (40, 80, and 160 nmol) into the perifornical hypothalamus on latency to feed (sec) as well as intake of a sweetened-mash diet during a 30-minute feeding trial. To provide a comparison, additional feeding trials were conducted using 40, 80 and 160 nmol d-amphetamine sulfate microinjected into the PFH.

METHOD

Animals

The animals were 19 male Sprague-Dawley albino rats (obtained from Harlan Industries; Houston, TX) weighing about 240 g at the beginning of the study. The rats were housed individually in standard plastic rodent cages in a colony room maintained at 23.0 ± 2°C under a 12-hr/12-hr illumination schedule (lights on at 0800 hr). The rats were provided continuous access to tap water and limited access to rodent pellets (Teklad) in the home cage throughout the experiment as well as to a test diet described below.

Diet

In the present experiment, the test diet was a modified version of the sweetened-mash diet described by Gilbert and Cooper (10). Briefly, the diet consisted of 140 g sweetened condensed milk

[1]The procedures of this study were reviewed and approved by the Texas A&M University Laboratory Animal Care Committee.

(Borden's Eagle Brand), 300 g ground rat chow (Teklad) and 400 ml tap water. The diet was prepared prior to each afternoon test and was stored in a chilled flask. The diet was presented to the rats in glass Petri dishes.

Drugs

In this experiment, a vehicle solution was prepared using sterile distilled water and 0.9% (w/v) sodium chloride. Amphetamine solutions (40, 80 and 160 nmol/0.5 μl) and phenylpropanolamine solutions (40, 80 and 160 nmol/0.5 μl) were prepared using d-amphetamine sulfate and phenylpropanolamine hydrochloride (Sigma Chemical Company) mixed into the vehicle solution. All drug solutions were calculated as the base of drug/μl of vehicle.

Procedure

The rats were deprived of food and water for 24 hr 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 (5-min interval) of ketamine (Ketaset: 60 mg/kg, IP) and sodium pentobarbital (20 mg/kg, IP). With the upper incisor bar of the stereotaxic instrument positioned 3.1 mm above the interaural line, the tip of a 28-gauge guide cannula (Plastic-One) was positioned 0.9 mm caudal to bregma, 1.3–1.4 mm lateral to the midline and 8.4 mm below the surface of the skull. The shaft of the guide cannula was affixed to the skull using 3 stainless steel screws and a pedestal of dental acrylic. The 32-gauge inner injector cannulae (Plastic-One) extended 0.4 mm beyond the tip of each guide cannula. Following surgery, each rat received 250,000 units sodium penicillin (IM). A 9-day recovery period was allowed following the surgical procedure during which the rats were weighed daily and given continuous access to rat pellets and water.

Baseline food intakes during a 30-minute test period were conducted on 8 consecutive tests. The rats were deprived of food, but not water, at 2200 hr prior to each intake test (16 hours food deprivation). Beginning at 1500 hr on the baseline tests 4–8, each rat received a sham microinjection of saline (0.5 μl over 10 seconds) with the injector cannula left in place for 30 sec after the end of the injection. Five minutes after the removal of the injector, a glass Petri dish containing 35–40 g of the sweet-mash diet was positioned on the wire grid floor of the test cage. Latency to initiate feeding (sec) was recorded for each rat following placement of the test diet in the cage (with a 60 sec maximum score). The mash diet, but not water, was available during the 30-minute feeding test. At the end of the test, food intake was recorded to the nearest 0.1 g after correction for the weight of spillage (collected on a paper pad positioned beneath the cage floor). The rats had continued access to pellets and tap water during the period after each intake test until 2200 hr each night.

The drug test sequence consisted of 11 trials conducted using the baseline procedures. The trials consisted of alternating drug (D) and vehicle (V) trials conducted on successive days in the order noted below:

D1-V-D2-V-D3-V-D4-V-D5-V-D6

For half of the rats, amphetamine microinjections were given on Trials D1–D3 and PPA microinjections on Trials D4–D6. The reverse drug order held for the remaining rats. Within each drug block, each rat was given each drug dose (40, 80 and 160 nmol) with dose order randomized for each rat. The vehicle test between successive drug trials served to allow rats to recover baseline levels of feeding after drug injection and to minimize carryover effects. The single vehicle test was sufficient because the rats also had access to food and water for about 6 hours after the end of each drug trial; an interval that allowed each rat to recover from a marked anorexic effect during the drug trial.

Histological Analyses

At the end of the experiment, each rat was sacrificed using a fatal overdose of sodium pentobarbital (80 mg/kg, IP) followed by exsanguination using 0.9% saline and fixation using intracardiac perfusion of 10% buffered formalin. After at least 72 hours fixation in 10% buffered formalin, each brain was sectioned in the plane used during surgery and the locus of each cannula tip was ascertained by examination of photographic enlargements ($\times 8$) of successive 80-micron frozen unstained sections.

Statistical Analyses

The present experiment employed a within-group factorial design with the factors of DRUG (amphetamine: AMP vs. phenylpropanolamine: PPA) and DRUG DOSE (40, 80 and 160 nmol). Following histological verification of cannula placement within the perifornical hypothalamus, separate analyses of variance (7) were computed for the latency data and for the 30-minute food intake data. These analyses used the difference between intake or latency after drug treatment and after vehicle treatment. Further comparisons between drug means and vehicle means were computed using a priori *t*-tests (13). Difference probabilities of less than 0.05 were deemed statistically significant.

RESULTS

In the present experiment, eight of the nineteen rats exhibited cannula placements that were within the region of the perifornical hypothalamus (i.e., within 0.3 mm lateral and dorsal to the fornix), whereas the remainder of the rats exhibited cannula placements that were either far lateral and/or dorsal/ventral to the fornix at the level of the lateral hypothalamus.

Figure 1 depicts the changes in food intake exhibited by rats with perifornical cannula placements after microinjections of vehicle, PPA and amphetamine. After vehicle treatment, the rats consumed an average of 24.1 grams of the mash diet. Microinjection of d-amphetamine (40, 80 and 160 nmol) into the PFH produced dose-dependent suppressions of mash intake of 21%, 28% and 48%, respectively. In contrast, PPA microinjections into the PFH were without effect on feeding. These changes in feeding behavior after drug treatment were confirmed by analyses of variance which revealed significant effects of DRUG, $F(1,7) = 21.8$, $p<0.0023$, of DOSE, $F(2,14) = 5.8$, $p<0.0148$, and a significant interaction between the factors of DRUG and DOSE, $F(2,14) = 3.6$, $p<0.05$. Further comparisons between the intakes after vehicle and the amphetamine doses revealed that the 40 nmol amphetamine dose significantly suppressed feeding relative to vehicle ($p<0.005$), that the 40 nmol and 80 nmol amphetamine doses induced comparable changes in anorexia ($p<0.12$) and that the 160 nmol amphetamine dose produced significantly greater anorexia than that produced by either 40 or 80 nmol amphetamine ($p<$ at least 0.005). Analyses of variance of the latency to feed data (not depicted) revealed no significant effects of either DRUG or DOSE nor a significant interaction between these factors.

DISCUSSION

In the present experiment, microinjection of amphetamine into the perifornical hypothalamus induced dose-dependent anorexia with significant anorexia observed to amphetamine at a dose of 40 nmol. In contrast, PPA even at a dose of 160 nmol, was without

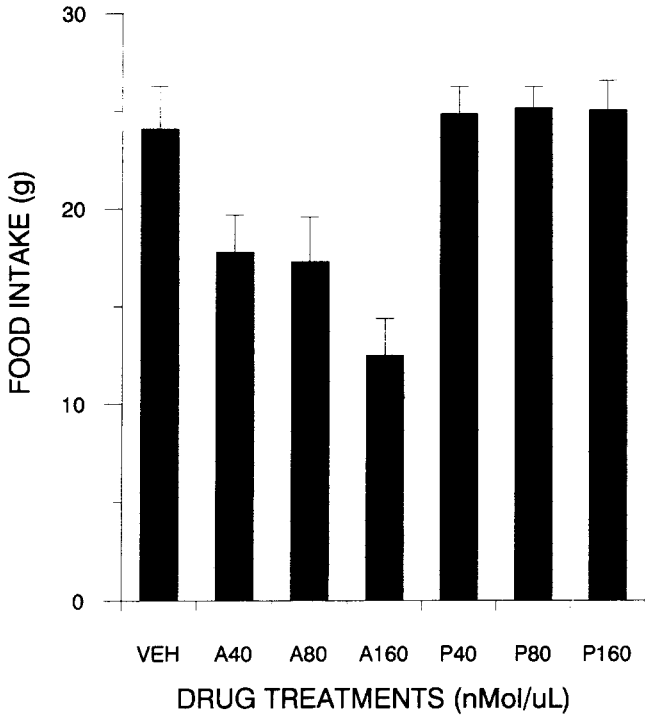

FIG. 1. Mean group intakes (g) of sweetened mash during a 30-minute period for rats after microinjections (40, 80 and 160 nmol/0.5 μl over 10 seconds) of amphetamine (A) and phenylpropanolamine (P) and vehicle (V). The line above each bar represents the mean plus one SEM.

effect on feeding. It might be argued that the doses of PPA selected for use in the present experiment were too low. Parametric dose-response comparisons of anorexia induced by systemic amphetamine and PPA, for example, revealed that the threshold for inducing significant anorexia for d-amphetamine was between 1 and 2 mg/kg, whereas that for PPA was 10 mg/kg, a 5–10-fold difference in drug potency (8). In the present study, a dose of 160 nmol PPA was without effect on feeding when injected into the PFH, whereas amphetamine induced significant anorexia at a dose as low as 40 nmol. Yet, it is our belief that the doses used in the present study were appropriate. Firstly, much of the difference between the potencies of amphetamine and PPA is due to their intrinsic differences in lipid solubilities; such that amphetamine more readily permeates the blood-brain barrier to reach a critical concentration within brain (22). Secondly, it is likely that a dose of amphetamine as low as 6.25 nmol within the PFH is at threshold for inducing anorexia (17). Finally, in a pilot study that compared the anorexic effect of 40, 80, 160 and 320 nmol PPA within the PFH, no change in feeding was observed even at 320 nmol PPA (Wellman and Cockroft, unpublished data), a dose that is 50 times greater than the amphetamine dose that is at threshold to suppress feeding. Rather, these data suggest that PPA, over a wide range of doses, has no intrinsic activity on neurons within the lateral hypothalamus that regulate feeding.

The present data support a growing body of literature that differentiate the mechanisms by which amphetamine and PPA suppress feeding. Dopaminergic and beta-adrenergic neurons that arise from the brainstem to terminate within the perifornical region of the lateral hypothalamus are critical for induction of anorexia by amphetamine (12). Direct injections of nanomole doses of amphetamine into the PFH suppress feeding (2, 5, 16, 17) and preinjections of dopamine and beta-adrenergic receptor antagonists into the PFH reliably antagonize anorexia induced by systemic amphetamine (17). Further, electrolytic lesions of the lateral hypothalamus as well as lesions that interrupt ascending catecholaminergic fibers that innervate the hypothalamus attenuate anorexia induced by amphetamine (1, 6, 19).

In contrast, catecholaminergic mechanisms within the PFH are unlikely to mediate PPA anorexia. Admittedly, PPA has weak indirect releasing effects on dopaminergic and adrenergic neurons (18,20), direct effects on adrenergic receptors (15) and binds to hypothalamic amphetamine-binding sites which are thought to be related to the induction of satiety (4); yet, these effects are not critical for the anorexic activity of PPA. Wellman and Peters (26), for example, noted that lesions of the dorsolateral aspects of the tegmentum, a site that likely interrupted ascending noradrenergic fibers, also attenuated amphetamine anorexia, but these lesions were without effect on anorexia induced by systemic injections of PPA (5, 10 or 20 mg/kg). Moreover, Wellman (23) noted that systemic pretreatment of rats with haloperidol (0.8 mg/kg) had no antagonistic effect on anorexia induced by systemic injection of l-NEP (l-norephedrine: one of the isomers that comprise PPA), whereas the same haloperidol treatment regimen significantly attenuated anorexia induced by d-amphetamine. Finally, although PPA does bind to low-affinity hypothalamic amphetamine-binding sites, the role played by these sites in the induction of satiety is questionable. Blosser, Barrantes and Parker (4) noted that although d-norephedrine and l-norephedrine exhibited comparable binding to the amphetamine-binding sites from hypothalamic fractions, these two isomers induce differential degrees of anorexia [l>d: (27)].

Prior reviews of anorexic activity of PPA have concluded that its site of action remains to be firmly established (15). The present experiment, in a similar vein, does not establish the mechanism by which PPA reduces feeding, but does serve to establish that PPA does not act at the level of the lateral hypothalamus, as does amphetamine, to reduce food intake. Future studies will be required to further evaluate the contribution of other hypothalamic and extrahypothalamic sites to the mediation of PPA anorexia.

ACKNOWLEDGEMENTS

The authors wish to thank Allyn Allard, Jason DeMott, Jamie Galpin, Sherry Keller, Marcel Brunel and Sabrina Crutcher for their assistance in this study and Thompson Medical Company for funds to carry out this study.

REFERENCES

1. Ahlskog, J. E.; Hoebel, B. G. Overeating and obesity from damage to a noradrenergic system in the brain. Science 182:166–169; 1973.
2. Angel, I.; Stivers, J. A.; Paul, S. M.; Crawley, J. A. Site of action of anorectic drugs: Glucoprivic- versus food deprivation-induced feeding. Pharmacol. Biochem. Behav. 27:291–297; 1987.
3. Arch, J. R. S.; Ainsworth, A. T.; Cawthorne, M. A. Thermogenic and anorectic effects of ephedrine and congeners in mice and rats. Life Sci. 30:1817–1826; 1982.
4. Blosser, J. C.; Barrantes, M.; Parker, R. B. Correlation between anorexic potency and affinity for hypothalamic (+)-amphetamine binding sites of phenethylamines. Eur. J. Pharmacol. 134:97–103; 1987.
5. Booth, D. A. Amphetamine anorexia by direct action on the adrenergic feeding system of rat hypothalamus. Nature 217:869–870; 1968.
6. Carlisle, H. J. Differential effects of amphetamine on food and water intake in rats with lateral hypothalamic lesions. J. Comp. Physiol. Psychol. 58:869–870; 1968.
7. Cody, R. P.; Smith, J. K. Applied statistics and the SAS program-

ming language. New York: North Holland; 1987.
8. Eisenberg, M. S.; Silverman, H. I.; Maher, T. J. A comparison of the effects of phenylpropanolamine, d-amphetamine and d-norpseudoephedrine on open-field locomotion and food intake in the rat. Appetite 9:31–37; 1987.
9. Epstein, A. N. Suppression of eating and drinking by amphetamine and other drugs in normal and hyperphagic rats. J. Comp. Physiol. Psychol. 52:37–45; 1959.
10. Gilbert, D. B.; Cooper, S. J. Analysis of dopamine D1 and D2 receptor involvement in d- and l-amphetamine-induced anorexia in rats. Pharmacol. Biochem. Behav. 15:385–389; 1985.
11. Hoebel, B. G.; Hernandez, L.; Thompson, R. D. Phenylpropanolamine inhibits feeding, but not drinking, induced by hypothalamic stimulation. J. Comp. Physiol. Psychol. 89:1046–1052; 1975.
12. 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 bodily disease. New York: Raven Press; 1981.
13. Kirk, R. J. Experimental design: Procedures for the behavioral sciences. Belmont, CA: Brooks/Cole; 1968.
14. 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.
15. Lasagna, L. Phenylpropanolamine: A review. New York: John Wiley and Sons; 1988.
16. Leibowitz, S. F. Amphetamine: Possible site and mode of action for producing anorexia in the rat. Brain Res. 98:160–167; 1975.
17. Leibowitz, S. F. Catecholaminergic mechanisms of the lateral hypothalamus: Their role in the mediation of amphetamine anorexia. Brain Res. 98:529–545; 1975.
18. Levitt, M.; Cumiskey, W. R.; Shargel, L. Studies on the physiologic disposition and activity of phenylpropanolamines in the mouse. Drug Metab. Dispos. 2:187–193; 1974.
19. McCabe, J. T.; Leibowitz, S. F. Determination of the course of brainstem catecholamine fibers mediating amphetamine anorexia. Brain Res. 311:221–224; 1984.
20. Maher, T. J. Dopamine-releasing potency of phenylpropanolamines and related isomers. Xth International Congress of Pharmacology; 1987:814.
21. Morgan, J. P. Phenylpropanolamine: A critical analysis of reported adverse drug reactions and overdosage. Fort Lee: Jack K. Burgess, Inc.; 1986.
22. Vree, T. B.; Muskens, A. T. J.; Van Rossum, J. M. Some physicochemical properties of amphetamine and related drugs. J. Pharm. Pharmacol. 21:774–775; 1969.
23. Wellman, P. J. Effects of haloperidol on anorexia induced by l-norephedrine and d-amphetamine in rats. Pharmacol. Biochem. Behav. 35:457–460; 1990.
24. Wellman, P. J.; Cockroft, R. Effects of amphetamine and phenylpropanolamine on latency to feed and liquid diet intake in rats. Pharmacol. Biochem. Behav. 32:147–150; 1989.
25. Wellman, P. J.; Miller, J. The effects of phenylpropanolamine on food intake and body weight in the genetically obese (ob/ob) mouse. Appetite 9:231–236; 1987.
26. Wellman, P. J.; Peters, R. H. Influence of amphetamine and phenylpropanolamine on food intake in rats with ventromedial hypothalamic and dorsolateral tegmental damage. Physiol. Behav. 25:819–827; 1980.
27. Wellman, P. J.; Sellers, T. Weight loss induced by chronic phenylpropanolamine: Anorexia and brown adipose tissue thermogenesis. Pharmacol. Biochem. Behav. 24:605–611; 1986.