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Effects of Haloperidol on Anorexia Induced by l-Norephedrine and d-Amphetamine in Adult Rats[1]

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WELLMAN, P. J. *Effects of haloperidol on anorexia induced by l-norephedrine and d-amphetamine in adult rats.* PHARMACOL BIOCHEM BEHAV **35**(2) 457–460, 1990.—Although amphetamine anorexia has been linked to activation of dopaminergic receptors within the lateral aspects of the hypothalamus, the receptor type by which phenylpropanolamine (PPA: the racemic mixture of d- and l-norephedrine) induces anorexia has not been identified. In the present experiment, separate groups of adult male rats were pretreated (IP) with either 0.9% saline or haloperidol (either 0.4 or 0.8 mg/kg) 45 minutes prior to treatment (IP) with either saline or 20 mg/kg l-NEP (the active enantiomer of PPA) and were then allowed 180 minutes access to food and water. Treatment with 20 mg/kg l-NEP induced comparable reductions in food intake of approximately 30% in rats pretreated with either dose of haloperidol or saline. In a sub-experiment, it was demonstrated that 1.0 mg/kg d-amphetamine sulfate reduced food intake by 25%, but this anorexic action was completely attenuated by 0.8 mg/kg haloperidol given 45 minutes prior to feeding. These results add to a growing body of literature that documents important differences between the mechanisms by which amphetamine and PPA produce their anorexic actions.

Phenylpropanolamine l-Norephedrine (l-NEP) Anorexia Haloperidol d-Amphetamine

PHENYLPROPANOLAMINE (PPA) is a racemic mixture of d- and l-norephedrine (16,21). Studies on the acute and chronic actions of this drug reveal that PPA reduces food intake as well as body weight in a variety of species (3, 8, 11, 16, 27). Moreover, the component isomers of PPA exhibit characteristic differences in potency with a two-fold greater anorexia and weight loss observed (26) in rats after chronic treatment with l-norephedrine (l-NEP) than after d-norephedrine (d-NEP) (27). Although PPA is often likened to amphetamine in both structure as well as functional pharmacology [(5), cf. (21)], the differences between these drugs are at least as salient as their similarities. Their isomer potency ratios are reversed, with d-amphetamine exerting 4–10-fold greater reductions in feeding behavior than l-amphetamine (4,27) and amphetamine increases eating rate, whereas PPA depresses rate (24). These differences suggest that these compounds may act on different mechanisms to reduce feeding behavior.

The literature is replete with studies that point to central noradrenergic (NE) and dopaminergic (DA) mechanisms as mediating the anorexic action of amphetamine (14, 18, 19). Microinjections of amphetamine into the lateral hypothalamus produces marked anorexia, whereas lateral hypothalamic lesions attenuate the anorexic activity of amphetamine (6,14). Systemic injections of dopaminergic receptor antagonists (haloperidol) reliably antagonize amphetamine anorexia (7, 9, 28). Moreover, injection of dopaminergic (haloperidol) and beta-adrenergic (propranolol) receptor antagonists within the lateral hypothalamus blocks the anorexia induced by peripheral injection of amphetamine in rats (19). In contrast, few studies have examined the role played by catecholaminergic systems in the anorexia induced by PPA. Although amphetamine anorexia is attenuated by lesions that interrupt fibers of the ventral noradrenergic bundle, PPA-induced anorexia is left intact (1, 2, 26).

The purpose of the present experiment was to assess the relative impact of dopaminergic receptor antagonism using systemic haloperidol injections (0.4, 0.8 mg/kg, IP) on the anorexia induced by systemic 20 mg/kg (IP) l-NEP in rats. PPA exerts a weak action, relative to amphetamine, on dopamine release in striatal slices (20) and increases extracellular dopamine concentration as measured by in vivo microdialysis probes implanted into the nucleus accumbens (12). Given the prominent role played by dopamine in amphetamine anorexia, it was deemed important to compare the effects of haloperidol on anorexia induced by l-NEP and by d-amphetamine. The dose of l-NEP chosen for the present study has been shown elsewhere to induce a moderate degree of anorexia (3,27) and was selected to provide an opportunity to detect either attenuation or potentiation of l-NEP anorexia induced by dopaminergic receptor antagonism.

METHOD

Sub-Experiment A

Animals. Male Sprague-Dawley albino rats (n = 31) weighing

[1]Portions of this data were reported at the satellite meeting of the 15th Annual Meeting of the Society for Neuroscience, 1985, San Antonio.

between 184–228 grams were obtained from Timco, Inc. (Houston, TX). The rats were maintained in individual plastic rodent cages under constant temperature control (23.0 ± 1.0 degrees C) and a reversed day/night illumination schedule (lights off at 0800 hr). The rats were allowed continuous access to chow pellets (Teklad) and tap water except as noted in the schedules below.

Drugs. A saline solution was prepared by dissolving sodium chloride (0.9% w/v) into sterile distilled water. Drug solutions were prepared prior to use by dissolving each compound into sterile distilled water and were calculated as the weight of base and salt per 1.0 ml. Drugs and sources were: haloperidol (MacNeil, Lot 7703890) and l-norephedrine hydrochloride (Roehr, Lot X-4013).

Procedures. The rats were allowed 180 minutes daily access to the chow pellet diet and tap water on 10 consecutive days. Each test period began at 0900 hr on each day. Food intake was measured to the nearest 0.1 gram and was corrected for spillage collected on paper towels placed beneath the wire floor of each cage. Water intake was measured to the nearest 1.0 ml using calibrated 100 ml drinking tubes (Wahmann). On Days 4–6, each rat was injected (IP) with 0.9% saline (1.0 ml/kg) at 0815 hr and then again at 0845 hr. The intake measures during these 3 days were used to form groups of comparable average food and water intake. The matched groups were then assigned to either saline (n = 15) or to either 0.4 or 0.8 mg/kg haloperidol (n = 8 each) pretreatment conditions. The pretreatment injections were given on Days 7 and 10 at 0815 hr. Each rat always received the same pretreatment injection and dose on Days 7 and 10 and received either saline or l-NEP treatments (on Days 7 and 10) with treatment order counterbalanced. On Days 8 and 9, each rat was injected (IP) with 1.0 ml/kg 0.9% saline at 0815 and 0845 hr; these tests served to minimize drug carry-over effects.

Data analyses. The design of this study represents a split-plot factorial with a between-group factor of DRUG PRETREATMENT (saline, 0.4 mg/kg haloperidol and 0.8 mg/kg haloperidol) and a within-group factor of DRUG TREATMENT (saline, 20 mg/kg l-NEP). Analyses of variance were computed for the food and water intake data and were followed by subsequent comparisons between groups using a posteriori Tukey *t*-tests (15). Difference probabilities less than 0.05 were deemed statistically significant.

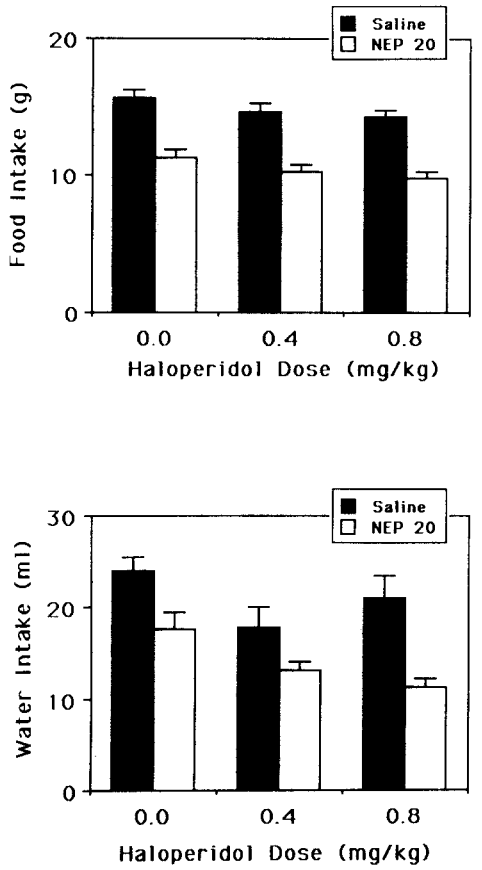

FIG. 1. Mean group food intake (upper panel) and water intake (lower panel) during a 180-minute test period. The rats were pretreated with either saline (0.0 mg/kg), 0.4 mg/kg haloperidol or 0.8 mg/kg haloperidol and then treated on separate tests with either saline or 20 mg/kg l-NEP (NEP 20). The line above each bar represents the standard error of the mean.

RESULTS

The impact of the pretreatment and treatment conditions on mean group food intake are depicted in the top panel of Fig. 1. Rats that received the saline pretreatment and saline treatment consumed an average of 15.9 grams of food during the 180-minute test period. The haloperidol pretreatments alone only slightly altered baseline food intakes [i.e., a comparison of rats pretreated with either saline, 0.4 mg/kg haloperidol or 0.8 mg/kg haloperidol and then treated with saline: $q(0.05,26) \leq 2.7$, $p > 0.05$]. The 20 mg/kg dose of l-NEP produced a significant decline in food intake of approximately 30% in each pretreatment group, $F(1,26) = 106.1$, $p < 0.0001$, and there were no significant differences in the magnitude of anorexia induced by l-NEP in each pretreatment group, $F(2,26) = 0.03$, $p < 0.97$. Thus, antagonism of dopaminergic synapses, achieved using systemic administration of haloperidol, neither attenuated nor potentiated the anorexic activity of l-NEP.

The bottom panel of Fig. 1 depicts the changes in water intake induced by the pretreatment and treatment conditions of this study. Rats pretreated and then treated with saline consumed an average of 24 ml of water. Rats pretreated with either 0.4 mg/kg haloperidol or 0.8 mg/kg haloperidol exhibited only slight, but nonsignificant, differences in water intake relative to that of the saline pretreatment group [food intakes collapsed across drug treatment: $q(0.05,26) \leq 3.5$, $p > 0.05$]. The pretreatment groups exhibited a significant reduction in water intake of between 17% and 32% (comparison of water intake after l-NEP relative to after saline treatment). Analyses of variance revealed a significant effect of the 20 mg/kg l-NEP treatment on water intake, $F(1,26) = 36.6$, $p < 0.0001$, but no significant interaction between the haloperidol pretreatment and 20 mg/kg l-NEP treatment conditions, $F(2,26) = 0.07$, $p < 0.093$. As was noted for the food intake data, neither dose of haloperidol alone had an effect on drinking, and neither haloperidol dose altered the magnitude of the hypodipsic effect of 20 mg/kg l-NEP.

Sub-Experiment B

The results of Sub-Experiment A document that the dopaminergic receptor antagonist haloperidol was without effect on l-NEP-induced anorexia and hypodipsia. Yet, these negative results are difficult to interpret in that the drug pretreatment regimen for haloperidol may have been less than optimal. Others have demonstrated a reversal of amphetamine anorexia using lower doses of haloperidol that were given at intervals ranging in length from 45 minutes (27) to 2.0–2.5 hours (9) before the start of an ingestive test. The possibility exists that a 45-minute pretreatment interval,

even with a high dose of haloperidol, is below or at the threshold for induction of adequate blockade of central dopaminergic receptors. To determine whether the haloperidol treatment regimen used in Sub-Experiment A effectively blocked central dopaminergic receptors, a positive control experiment was conducted in which the haloperidol pretreatment regimen (0.8 mg/kg, given IP 45 min prior to testing) was used to antagonize the anorexic activity of 1.0 mg/kg d-amphetamine sulfate.

METHOD

Animals

The animals were 16 male Sprague-Dawley albino rats obtained from Timco Farms, Inc. (Houston, TX) maintained under conditions identical to those of Sub-Experiment A.

Procedures

The procedures of Sub-Experiment B were identical to those described above except that the rats were pretreated with either saline or haloperidol prior to treatment with either saline or 1.0 mg/kg amphetamine. As before, the rats were allowed to feed and drink for 180 minutes on each day beginning at 0900 hours. At 0815 hours on Days 7 and 10, two groups of rats (n = 8 each) were treated (IP) with either 0.9% saline or 0.8 mg/kg haloperidol and were then treated (IP) with either saline or 1.0 mg/kg d-amphetamine sulfate (Sigma Chemical, St. Louis, MO) at 0845 hours. Each rat within each pretreatment condition received both saline and amphetamine treatments (with drug order counterbalanced across Days 7 and 10). On Days 8 and 9, the pretreatment and treatment injections were saline (1.0 ml/kg) for all rats.

RESULTS

Figure 2 depicts the changes in food intake and water intake induced by the pretreatments and treatments of this experiment. Food intake was significantly reduced by 1.0 mg/kg d-amphetamine over a three hour period in rats pretreated with saline, $t(7) = 3.4$, $p < 0.02$. The haloperidol pretreatment, at 0.8 mg/kg, had no effect on baseline food intakes, but completely reversed the anorexic action of amphetamine [comparison of haloperidol-saline mean with haloperidol-amphetamine mean, $t(7) = 0.7$, $p < 0.99$]. Amphetamine slightly reduced water intake in rats pretreated with saline ($p < 0.2$), but not in rats pretreated with haloperidol ($p < 0.9$). Moreover, haloperidol in this experiment significantly reduced baseline water intake, $t(14) = 3.7$, $p < 0.01$. Recall that in Sub-Experiment A, the same directional finding was obtained, but that difference was not significant. An effect of haloperidol on water intake, but not food intakes is not uncommon as evident in the research of Rowland and Engle (22). The important aspect of Sub-Experiment B is that haloperidol, given at 0.8 mg/kg only 45 minutes before a 3-hour ingestive test, clearly antagonized the anorexic activity of 1.0 mg/kg d-amphetamine sulfate.

DISCUSSION

In the present experiments, a moderate degree of anorexia and hypodipsia was induced by 20 mg/kg l-NEP as well as by 1.0 mg/kg d-amphetamine in rats. Pretreatment with the dopaminergic receptor antagonist haloperidol (0.4, 0.8 mg/kg) was without effect on either anorexia or hypodipsia induced by l-NEP. In contrast, the 0.8 mg/kg haloperidol pretreatment regimen produced a complete blockade of the anorexia induced by 1.0 mg/kg d-amphetamine sulfate. The failure of haloperidol to antagonize PPA anorexia stands in marked contrast to the attenuation of the anorexic activity of amphetamine induced by antagonism of

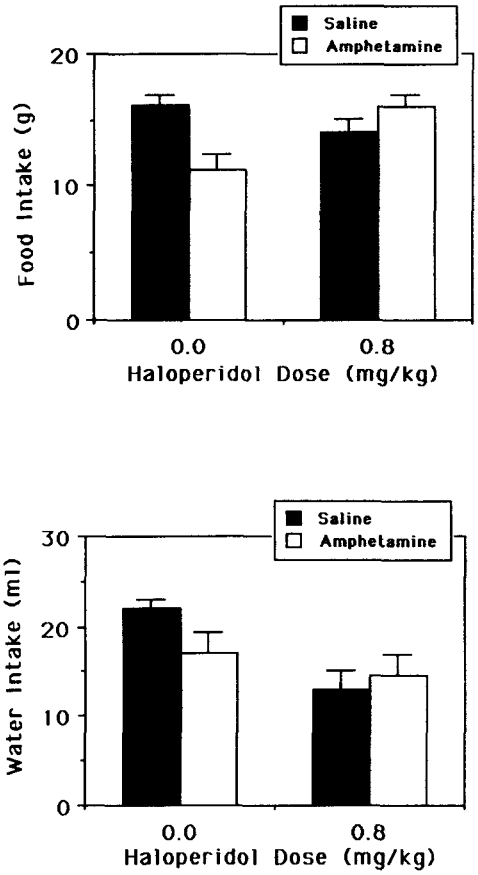

FIG. 2. Mean group food intake (upper panel) and water intake (lower panel) during a 180-minute test period for rats pretreated with either 0.9% saline or 0.8 mg/kg haloperidol and then treated with 0.9% saline and 1.0 mg/kg d-amphetamine sulfate.

dopaminergic receptors (4, 7, 9, 18, 28).

The anorexic activity of PPA is believed to be dependent on functional catecholaminergic terminals within the lateral hypothalamus (14). This link is based on a number of studies demonstrating that: a) application of crystalline PPA to the lateral hypothalamus suppresses electrically elicited feeding (13); b) the perifornical hypothalamus is rich in dopaminergic terminals (14); c) PPA has weak activity on dopamine release as assessed using either in vitro measurements of dopamine release in striatal slices (20) or as assessed in vivo microdialysis measurements in nucleus accumbens (12); and d) PPA produces a drug cue that generalizes to the amphetamine drug cue and this generalization is blocked by haloperidol (17).

The present results suggest that although PPA has weak actions on dopaminergic neurons, the anorexic activity of PPA is not mediated via a dopaminergic system. It is also unlikely that PPA acts within the perifornical hypothalamus to reduce feeding. In a recent study from this lab, microinjection of d-amphetamine (40, 80 and 160 nmol) into the perifornical hypothalamus suppressed feeding, but equimolar microinjections of PPA had no significant effect on feeding (25).

The present study adds to a growing body of literature in which distinct pharmacological profiles are observed between amphetamine and PPA. Amphetamine is more lipid soluble than is PPA and as a consequence easily permeates into the central nervous

system (21,23). Amphetamine, but not PPA, induces a variety of behavioral effects thought to be related to central DA activity, including increased locomotion, euphoria, self-administration, and finally, reduced feeding [(8, 10, 16), the present study]. The present study suggests that PPA is not dependent on dopaminergic mechanisms to suppress appetite and further strengthens the suggestion by Wellman and Peters (25) that PPA and amphetamine do not act via a common mechanism to suppress appetite.

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