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Phenylpropanolamine Decreases Food Intake in Rats Made Hyperphagic by Various Stimuli

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MOYA-HUFF, F. A. AND T. J. MAHER. *Phenylpropanolamine decreases food intake in rats made hyperphagic by various stimuli.* PHARMACOL BIOCHEM BEHAV **28**(1) 71–74, 1987.—Phenylpropanolamine (PPA, d,l-norephedrine), found in many over-the-counter appetite suppressants and nasal decongestants, induces anorexia by a yet unidentified mechanism. The present study evaluates the effects of PPA on different types of non-drug- and drug-induced hyperphagias (i.e., food deprivation, 2-deoxy glucose, ketocyclazocine and insulin). Phenylpropanolamine (15, 25 and 35 mg/kg IP) significantly reduced food intake in a dose-related fashion at the 1 hr and 3 hr time intervals in the food deprivation-, insulin- and 2-deoxy glucose-induced hyperphagic models. Phenylpropanolamine produced a non-dose-related 99% reduction of food intake in the ketocyclazocine-induced model at the 1 and 3 hr measurement, which was most likely due to a combination of the appetite suppressant activity of PPA and the sedation produced by ketocyclazocine in combination with PPA. We conclude that PPA is capable of suppressing appetite in rats made hyperphagic by various stimuli.

d,l-Norephedrine Phenylpropanolamine Insulin Hyperphagia Ketocyclazocine Food deprivation 2-Deoxy glucose

PHENYLPROPANOLAMINE (PPA, d,l-norephedrine) is commonly found in many over-the-counter appetite suppressants and nasal decongestants. While the efficacy of PPA as an appetite suppressant for use in weight loss programs in obese humans is well documented [18–20], the mechanism(s) by which PPA suppresses appetite is poorly understood largely as a result of the multitude of complex central and peripheral biological processes thought to be involved in the regulation of food intake. Although decreased food intake has been demonstrated with PPA in many experimental animal models, the vast majority of the appetite suppressant literature involves studies in food deprivation- (FD) induced hyperphagias [1, 4, 6, 21]. Phenylpropanolamine (1–10 mg/kg, IP) decreased food intake in rats maintained on a 20 hr fasting/4 hr feeding schedule during the first hr, but not at the fourth hr [9]. Higher doses of PPA (5–20 mg/kg, IP) produced a dose-dependent 10 to 50% decrease in food intake in rats placed on a 23 hr fasting/1 hr feeding schedule, when maintained at 80% of normal body weight [21]. In this study PPA was also effective in rats subjected to lesions of the ventromedial hypothalamus or dorsal lateral tegmentum (DLT), while amphetamine (a chemical analog of PPA)-induced anorexia was attenuated by lesions of the DLT.

Although structurally related to other stimulant-like beta-phenethylamines, PPA possesses a distinct pharmacological profile. In studies with food deprived rats, PPA was found to be equipotent to its stereoisomer, d-norpseudoephedrine, and approximately one-tenth as potent as d-amphetamine [6]. In contrast to their similar potency as anorectics, PPA and d-norpseudoephedrine differed significantly in their ability to stimulate locomotion. While d-norpseudoephedrine and d-amphetamine increased locomotion, all doses of PPA failed to stimulate locomotion [6]. These findings are consistent with studies in other rodents and primates.

To explore the appetite suppressant effiacy of PPA in other types of hyperphagias, we determined the effectiveness of PPA in hyperphagias induced by various stimuli, i.e., 24 hr of FD; 2-deoxy glucose (2-DG, an antimetabolite of glucose); insulin and the exogenous opioid kappa agonist, ketocyclazocine. During the course of the studies we observed a potent anorectic effect of PPA in the ketocyclazocine-induced hyperphagic model which appeared to be associated with decreased locomotor activity in these animals. Thus, in an attempt to determine if PPA's inhibion of food intake was due to a sedative effect in the ketocyclazocine-induced hyperphagia, we also tested the locomotor activities of PPA and other structurally related

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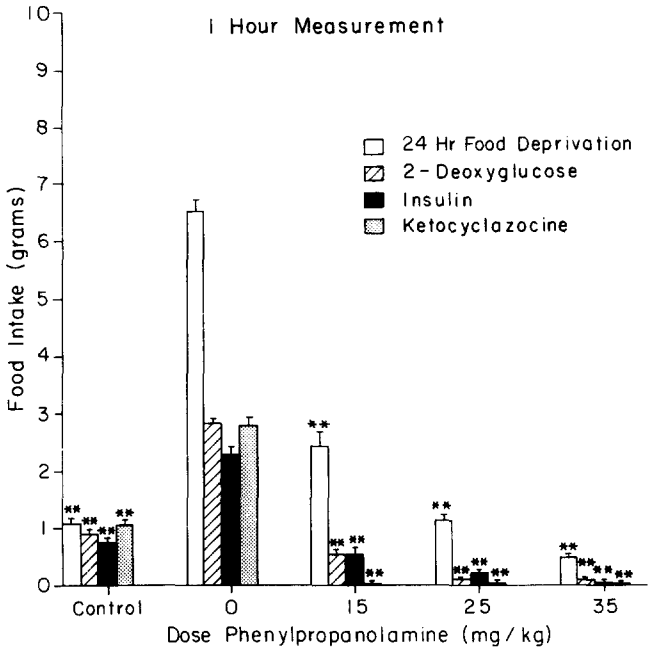

FIG. 1. One hour determination of the effect of phenylpropanolamine on 24 hour food deprivation-, 2-deoxy glucose-, insulin- and ketocyclazocine-induced eating. Rats were administered phenylpropanolamine (0–35 mg/kg) 30 min prior to the above hyperphagic stimuli. Control animals received the appropriate vehicle only. Food intake was then determined 1 hr later. Results are expressed as grams of food intake ±SEM. $^{**}p<0.01$ Significantly different than the respective 0 mg/kg phenylpropanolamine group by a one way analysis of variance and the Dunnett's Test.

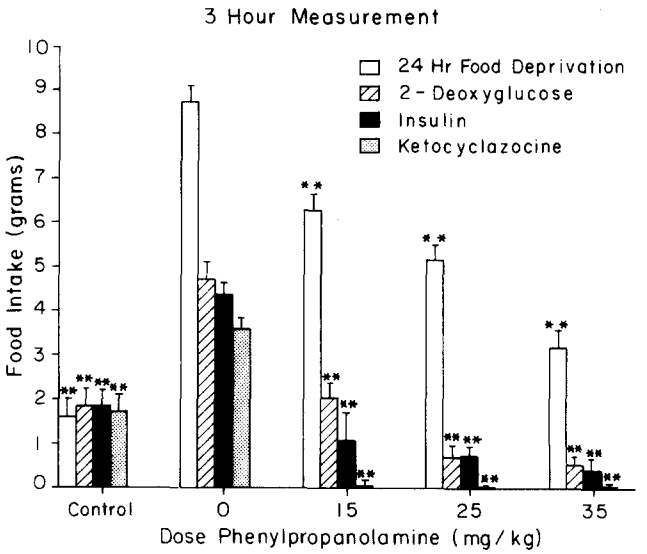

FIG. 2. Three hour determination of the effect of phenylpropanolamine on 24 hour food deprivation-, 2-deoxy glucose-, insulin- and ketocyclazocine-induced eating. Rats were administered phenylpropanolamine (0–35 mg/kg) 30 min prior to the above hyperphagic stimuli. Control animals received the appropriate vehicle only. Food intake was then determined 3 hr later. Results are expressed as grams of food intake ±SEM. Control animals received only the corresponding vehicle. $^{**}p<0.01$ Significantly different than the respective 0 mg/kg phenylpropanolamine group by a one way analysis of variance and the Dunnett's Test.

sympathomimetics with known anorectic activity (d-amphetamine and d-norpseudoephedrine) in combination with ketocyclazocine.

METHOD

Male Sprague-Dawley rats (N=224) weighing 220–375 grams (Charles River Breeding Laboratories) were housed individually in metal cages for at least one week prior to testing. The animals had free access to water and food (Wayne Lab Blox placed in a cup on the cage floor). Illumination was on a 12/12 hr schedule with lights on from 0700–1900 hr. The room temperature was maintained at 23–25°C. Animals were handled and given saline injections in three preliminary sessions to habituate them to the experimental procedure.

Daytime food intake was stimulated 3 hr after light onset by the subcutaneous (SC) injection of 2-DG (400 mg/kg), insulin (10 U/kg) and ketocyclazocine (3 mg/kg) or 24 hr of FD. Immediately after injection each rat was returned to its home cage and food intake (at 1 and 3 hr) measured to the nearest 0.1 grams by subtracting spillage on paper towels and uneaten food from a premeasured supply. Water was available ad lib. Experimental groups consisted of at least ten randomly assigned rats. Phenylpropanolamine in doses of 15, 25 and 35 mg/kg, was injected intraperitonally (IP) 30 min prior to the hyperphagic stimuli (e.g., 2-DG injection) and subsequent presentation of the food. All injections were given in a volume of 1 ml/kg and each group was compared to the 0 mg/kg PPA group which received the appropriate vehicle. Control animals were classified as those animals that were not fasted and/or had received the appropriate vehicle. Results were calculated and expressed as food intake (grams) ±S.E.M.

The open-field locomotion experiment followed the above described time schedule. Experimental groups consisted of at least six randomly assigned rats. All rats were injected IP with either PPA (20 mg/kg), d-amphetamine (2 mg/kg), d-norpseudoephedrine (15 mg/kg) or saline (1 ml/kg); and 30 min later with ketocyclazocine (3 mg/kg, SC). One hour after the ketocyclazocine injection the locomotor activity was measured for 10 min. Control and experimental groups were staggered throughout the test period to ensure that there were no diurnal variations in locomotion. The open field apparatus consisted of an opaque Plexiglas floor area of 70×70 cm, divided into 16 equal square fields and surrounded by walls 40 cm high. Locomotion (entering another field by at least 3/4 of the body) was monitored and recorded with a video system and the locomotor scores (number of fields entered per ten min test period) were later determined [10,16]. The appetite suppressant and open field locomotion data were analyzed by a one way analysis of variance and the Dunnett's Test [5]. All groups were compared to the vehicle treated groups.

All drugs were prepared daily in 0.9% saline, with the exception of ketocyclazocine which was dissolved in distilled water with an equimolar amount of HCl and back titrated with NaOH to pH 4.5 just prior to injection. The keto-

cyclazocine control animals received a pH 4.5 solution that matched the experimental pH 4.5 ketocyclazocine solution. 2-DG was purchased from Biochemical Co. (Cleveland, OH) and R-insulin (Iletin U-100, crystalline zinc) from Eli Lilly and Co. (Indianapolis, IN). Ketocyclazocine was a gift from Sterling-Winthrop Research Institute (Rochester, NY), PPA HCl and d-norpseudoephedrine from Rhoer Chemicals, Inc. (Long Island, NY) and d-amphetamine from Amend (Irvington, NJ).

RESULTS

Twenty-four hr of FD, the SC injection of 2-DG, insulin and ketocyclazocine significantly ($p<0.01$) increased food intake 1 and 3 hr after food presentation and injection of the hyperphagic stimulus. There was a significant ($p<0.01$) 504%, 212%, 210% and 165% increase, respectively, in food intake by the respective hyperphagic stimuli (24 hr FD, 2-DG, insulin and ketocyclazocine) when compared to control animals at the 1 hr food intake measurement (Fig. 1). At the 3 hr food intake measurement there was also a significant ($p<0.01$) increase in food intake (449%, 157%, 138% and 110%, respectively), when compared to control animals (Fig. 2).

The 15, 25 and 35 mg/kg doses of PPA significantly ($p<0.01$) inhibited feeding, induced by 24 hr of FD, by 62%, 83% and 92%, respectively, when compared to the 0 mg/kg PPA group, at the 1 hr food intake measurement (Fig. 1). At the 3 hr food intake measurement the 15, 25 and 35 mg/kg doses of PPA significantly ($p<0.01$) inhibited feeding by 28%, 41% and 63%, respectively, when compared to the 0 mg/kg PPA group (Fig. 2). These results demonstrate that PPA caused a significant ($p<0.01$) dose-related reduction of food intake 1 and 3 hr after food presentation.

The 15, 25 and 35 mg/kg doses of PPA significantly ($p<0.01$) inhibited feeding, induced by 2-DG, by 81%, 97% and 99%, respectively, when compared to the 0 mg/kg PPA group at the 1 hr food intake measurement (Fig. 1). At the 3 hr food intake measurement the 15, 25 and 35 mg/kg doses of PPA significantly ($p<0.01$) inhibited feeding by 56%, 85% and 89%, respectively, when compared to the 0 mg/kg PPA group (Fig. 2). These results demonstrate that PPA caused a significant ($p<0.01$) dose-related reduction of food intake 1 and 3 hr after the injection of 2-DG and food presentation.

The 15, 25 and 35 mg/kg doses of PPA significantly ($p<0.01$) inhibited feeding, induced by insulin, by 77%, 91% and 99%, respectively, when compared to the 0 mg/kg PPA group at the 1 hr food intake measurement (Fig. 1). At the 3 hr food intake measurement the 15, 25 and 35 mg/kg doses of PPA significantly ($p<0.01$) inhibited feeding by 76%, 84% and 91%, respectively, when compared to the 0 mg/kg PPA group (Fig. 2). These results demonstrate that PPA caused a significant ($p<0.01$) dose-related reduction of food intake 1 and 3 hr after the injection of insulin and food presentation.

The 15, 25 and 35 mg/kg doses of PPA significantly ($p<0.01$) inhibited feeding, induced by ketocyclazocine, by 99% (at all doses) when compared to the 0 mg/kg PPA group at the 1 hr and 3 hr food intake measurements (Figs. 1 and 2, respectively). At both the 1 and 3 hr food intake measurements, animals receiving both ketocyclazocine and PPA appeared significantly sedated, i.e., depressed locomotion was observed, which might have contributed to the observed decrease in food intake. Therefore, we tested the effects of the co-administration of ketocyclazocine and several structurally related anorectic sympathomimetic compounds (i.e., PPA, d-amphetamine and d-norpseudoephedrine) in an open field locomotor activity experiment. The results demonstrate that the ketocyclazocine-PPA group locomotor scores (94 ± 12) were significantly ($p<0.05$) lower than the ketocyclazocine-saline group locomotor scores (142 ± 20). Moreover, the ketocyclazocine-d-norpseudoephedrine and ketocyclazocine-d-amphetamine group locomotor scores (187 ± 16 and 197 ± 16, respectively) were significantly ($p<0.05$) higher than the ketocyclazocine-saline group locomotor scores (142 ± 20).

DISCUSSION

This study demonstrates that PPA (15, 25 and 35 mg/kg) is capable of effectively reducing daytime food intake in a dose-related fashion 1 and 3 hr after the SC injection of 2-DG and insulin, or by 24 hr of FD. PPA also has a non-dose-related inhibition of food intake following 1 and 3 hr of ketocyclazocine administration. This latter effect was most likely due to the increased sedation observed in the animals that received the ketocyclazocine and PPA combination. Moreover, this sedation was confirmed by a decrease in locomotion following the ketocyclazocine and PPA co-administration. Co-administration of ketocyclazocine with d-amphetamine or d-norpseudoephedrine in animals produced higher locomotor scores than in ketocyclazocine-saline animals, suggesting that the sedation observed by the combination of PPA and ketocyclazocine could be classified as "unique" when compared to the responses observed with other structurally related compounds. Therefore, we conclude that both, the appetite suppressant activity of PPA and the observed sedation, most likely contributed to the non dose-related inhibition of the ketocyclazocine-induced hyperphagia.

The mechanism involved in PPA's inhibition of 24 hr of FD-, 2-DG- and insulin-induced eating must be further investigated since different systems (e.g., opioid or dopaminergic) have been reported to be involved in these types of hyperphagias. Naloxone and naltrexone (opioid antagonists) and haloperidol (a dopamine antagonist) have been shown to decrease FD- and 2-DG-induced eating [2, 3, 7, 11, 12, 14, 17]. On the other hand, insulin-induced eating has been shown not to be as sensitive to opioid receptor blockade [11,17], and may involve both opioid and non-opioid related mechanisms [15]. Moreover, the mechanism of insulin-induced hyperphagias has been correlated more with striatal dopamine activity [13,15]. Therefore, we conclude that when hyperphagias were induced by FD, 2-DG or insulin, PPA suppressed eating in a dose-related fashion by mechanisms not yet fully understood, although substantial evidence has implicated the role of catecholamines in the lateral hypothalamus [8]. These results demonstrate that PPA is effective in reducing food intake in rats made hyperphagic by various stimuli that act via different neurochemical mechanisms. The mechanism(s) by which PPA suppresses food intake remains unresolved.

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