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Assessment of the Role of Oxytocin Receptors in Phenylpropanolamine-Induced Anorexia in Rats

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McMAHON, L. R. AND PAUL J. WELLMAN. *Assessment of the role of oxytocin receptors in phenylpropanolamine induced anorexia in rats.* PHARMACOL BIOCHEM BEHAV **57**(4) 767–770, 1997.—The anorexic effects of phenylpropanolamine (PPA) have been attributed to activation by PPA of $\alpha 1$-adrenoceptors within rat hypothalamic paraventricular nucleus (PVN). The PVN, however, is a nexus for a number of ascending and descending fibers systems that release transmitters and modulators known to inhibit appetite. The focus of the present study was to assess the possibility that oxytocin activity might play a role in the anorexic action of PPA. The present study therefore examined the effects of systemic administration of the oxytocin antagonist L-366,948 on PPA-induced anorexia. Adult male rats ($n = 10$ per group) were pretreated (IP) with either 0, 1, or 2 mg/kg L-366,948 15 min prior to treatment injections (IP) of either 0, 5, 10 and 15 mg/kg PPA. Food and water intakes were recorded for a 30 min period (1600 h) starting 30 min after the treatment injection. Rats pretreated with vehicle and then treated with PPA exhibited a dose-dependent suppression of feeding with a maximal effect evident at 15 mg/kg PPA. Pretreatment with 1 or 2 mg/kg L-366,948 alone did not alter feeding nor did these doses alter the anorexia induced by PPA. These results suggest that direct or indirect oxytocin activity is not a factor in the anorexic action of PPA, a finding that further strengthens the notion that PPA inhibits food intake via activation of $\alpha 1$-adrenoceptors. © 1997 Elsevier Science Inc.

L-366,948 $\alpha 1$-Adrenergic receptors   Feeding

FEEDING in rats is suppressed by systemic administration of phenylpropanolamine (PPA), a variant of the phenethylamine molecule (9,10,20). The anorexic action of PPA is thought to result from its efficacy as an $\alpha 1$-adrenergic receptor agonist (25). Pharmacological studies have demonstrated that PPA binds to and activates $\alpha 1$-adrenergic receptors (12,14). Dose-dependent suppression of feeding is observed in rats following systemic administration of a variety of $\alpha 1$-adrenoceptor agonists including PPA (26), cirazoline (5), amidephrine (13) and SKF-89748 (13). Several lines of evidence suggest that PPA may act within the hypothalamic paraventricular nucleus (PVN) in rats to reduce food intake (20). Binding studies have localized $\alpha 1$-adrenoceptors within rat PVN (28,29). Moreover, intra-PVN injections of a variety of $\alpha 1$-adrenoceptor agonists including PPA, cirazoline, methoxamine and phenylephrine suppress food intake in rats (5,6,13,24). Finally, the anorexic action of systemically administered PPA is reversed by intra-PVN pretreatment with the $\alpha 1$-adrenoceptor antagonist benoxathian (23). These findings strongly support the notion

that PPA acts within the PVN via stimulation of $\alpha 1$-adrenoceptors to suppress food intake.

Yet, a measure of caution is in order prior to concluding that activation of $\alpha 1$-adrenoceptors mediates the anorexic property of PPA. A number of neurotransmitters and neuromodulators with inhibitory effects on feeding are localized within the PVN. Oxytocin (OT) cells are localized within the magnocellular and parvocellular regions of the PVN (15). Intracerebroventricular (ICV) infusions of OT suppress food intake (1,15) and this effect is reversed by ICV pretreatment with an OT-antagonist (16). OT cells within the PVN project to the pituitary and diffuse throughout the brain. Olson et al. (16) suggest that parallel activation of the OT pathways may reduce food intake and suppress gastric motility with the former reflecting pituitary secretion of OT and the latter an action of diffuse OT pathways on brainstem vagal sites. Interestingly, PPA reduces feeding and inhibits gastric emptying (21).

To evaluate the possibility that PPA might directly or indirectly interact with OT receptors to suppress food intake, the
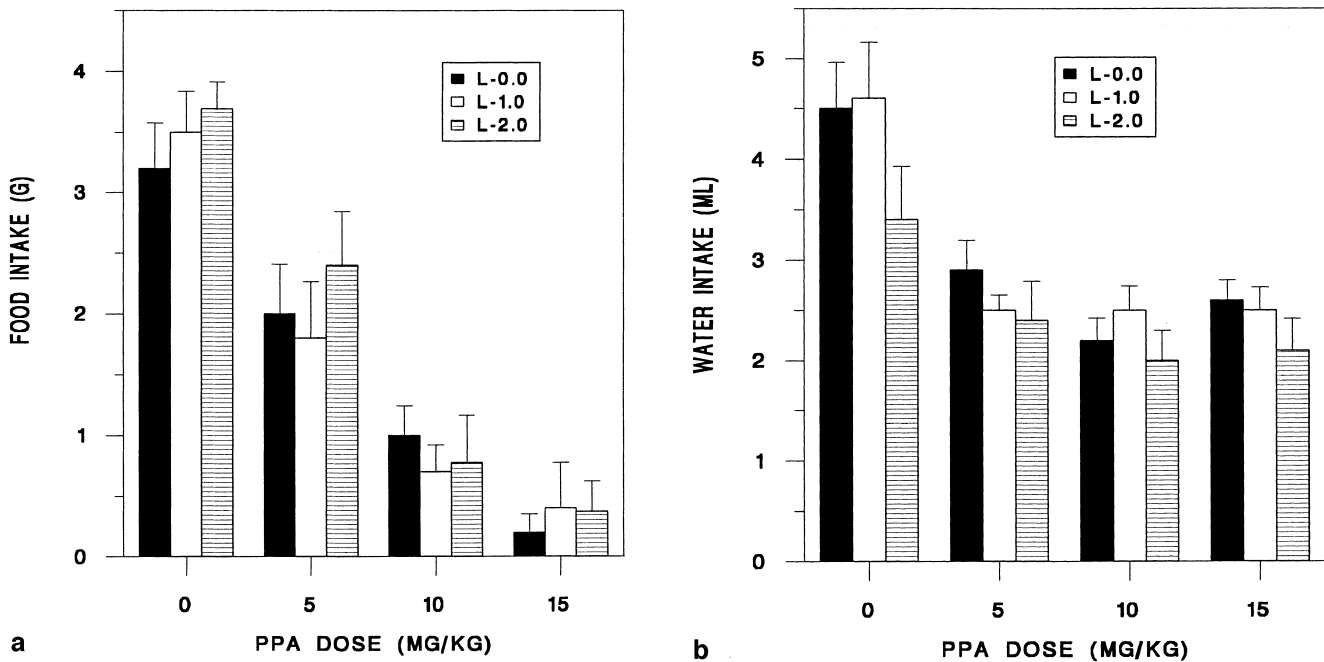

FIG. 1. (a) Mean group pellet intake (g) during a 30 min period for rats pretreated (at −45 min) with either 0 (vehicle), 1 or 2 mg/kg L-366,948 (L), and then treated (at −30 min) with either 0, 5, 10 or 15 mg/kg PPA. The lines above and below each bar represent the standard error of the mean. (b) Mean group water intake (ml) during a 30 min period for rats pretreated (at −45 min) with either 0, 1 or 2 mg/kg L-366,948 (L), and then treated (at −30 min) with either 0, 5, 10 or 15 mg/kg PPA. The lines above and below each bar represent the standard error of the mean.

present study examined the effects on PPA anorexia of pretreatment with the OT antagonist L-366,948. This novel cyclic hexapeptide is known to selectively and potently antagonize oxytocin receptors (4,17). Systemic injections of L-366,948, at a dose of 1 mg/kg, effectively antagonize central OT receptors (3). In the present study, rats were pretreated with 1 or 2 mg/kg L366,948 15 minutes prior to administration of either 0, 5, 10 or 15 mg/kg PPA. In vitro studies reveal a rapid onset of action of L-366,945 with a duration of action persisting for at least 3 h (4) whereas PPA is effective within 5 min of IP administration and has a duration of action exceeding an hour (9,11,23,26).

METHODS

Subjects

The subjects were 30 male Sprague–Dawley viral-free albino rats (obtained from Harlan Industries; Houston, TX) weighing approximately 300 g at the beginning of the study. The rats were housed individually in standard plastic rodent cages in a colony room maintained at 21 ± 1°C under a 12h/12h illumination schedule (lights on at 0700). The rats were provided with continuous access to tap water and rodent pellets (Teklad) except as described in the experimental protocol. The rats were maintained in the colony room for 7 days prior to experimentation to acclimate them to daily handling and routine colony procedures.

Drugs

Solutions of L-366,948 were prepared by dissolving L-366,948 (kindly donated by Dr. Roger Freidinger, Merck Research Laboratories) into a sterile 0.9% saline solution at a concentration of 1 and 2 mg/ml. The phenylpropanolamine solutions (5, 10, and 15 mg/ml) were prepared by dissolving phenylpropanolamine hydrochloride ((±)-norephedrine: Sigma) into a sterile 0.9% saline solution. All drug solutions were calculated as the weight of the salt in the vehicle solution and were prepared fresh prior to each injection trial.

Procedure

Following the seven-day acclimation period, each rat underwent a series of 9 baseline ingestion trials. Beginning each day at 1100 h, feeders and water bottles were removed from each cage for the implementation of a 3 h food and water deprivation period. Beginning each day at 1400 h, each rat was weighed, handled, and transferred to a clean home cage outfitted with a wire grid floor. A cardboard pad positioned under the grid floor was used to collect food spillage. Approximately 20 g of the pellet diet was placed on the grid floor of each cage and each cage was provided with a water bottle. After 30 min access, the remaining food and spillage was removed from the cage and food intake was recorded to the nearest 0.1 g (corrected for spillage). Water intakes were recorded to the nearest 0.1 mL. The rats were then allowed free access to food and water until the next day at 1100 h.

For baseline days 8 and 9, mock injections of the vehicle solution were given (IP) to acclimate the rats to the injection procedure. This mock injection procedure consisted of giving each rat two injections of vehicle solution: a mock pretreatment injection followed by a mock treatment injection at 45 and 30 min, respectively, prior to the start of the ingestion trial.

Prior to drug testing, the rats were separated into three groups (n = 10 each) based on comparable distributions of food and water intake scores (means and standard deviations)

as recorded during the final two baseline feeding trials. One group was designated to receive pretreatment injections of the vehicle solution, while the other groups were designated to receive pretreatment injections of either 1 or 2 mg/kg L-366,948. Treatment injections of the different doses of phenylpropanolamine (PPA: 0, 5, 10, and 15 mg/kg) were then randomly assigned to the rats in each pretreatment group. During the drug trials (D1–D4), rats in the vehicle pretreatment group received a pretreatment injection of the vehicle solution followed by a treatment injection of one of the doses of PPA. Rats in the 1.0 mg/kg L-366,948 pretreatment group received a pretreatment injection of the 1.0 mg/kg L-366,948 solution followed by a treatment injection of one of the doses of PPA. Similarly, rats in the 2.0 mg/kg L-366,948 pretreatment group received a pretreatment injection of the 2.0 mg/kg L-366,948 solution followed by a treatment injection of one of the doses of PPA. During the drug trials (D1–D4), food and water intakes were measured as outlined above for the baseline ingestion procedures. To minimize drug-carryover effects, each drug trial was separated by 2 ingestion trials in which no injections were given.

Statistical Analyses

The design of this experiment represents a split-plot factorial with a between group factor of oxytocin antagonist (0, 1 or 2 mg/kg) and a within-group factor of PPA dose (0, 5, 10, and 15 mg/kg). Separate ANOVAs were computed for the food and water intake data of this experiment. Significant main effects were further examined using the Newman–Keuls procedure (SigmaStat). Difference probabilities less than 0.05 were deemed statistically significant. Data from one rat was discarded whose baseline intakes were low due to illness, resulting in a total of 29 rats for this experiment.

RESULTS

Figure 1a (top panel) depicts the effects on food intake of pretreatment with either 0, 1 or 2 mg/kg L-366,948 and treatment with various doses of PPA. Under these testing conditions, rats pretreated with vehicle and then treated with vehicle consumed 3.2 grams of food in 30 min. Treatment with PPA in a group of rats pretreated with vehicle produced dose-dependent suppressions of feeding with a maximal effect evident at 15 mg/kg. These effects of PPA on food intake were confirmed by a significant ANOVA main effect of PPA dose ($F(3, 78) = 58.3$, $p < 0.0001$). Pretreatment with either 1 or 2 mg/kg L-366,948 only slightly increased baseline food intakes (3.4 and 3.6 g, respectively) in rats treated with vehicle, and did not substantially alter the anorexic effects of 5–15 mg/kg PPA. ANOVA revealed no significant effect of L-366,948 dose (0, 1 or 2 mg/kg) on food intake ($F(2, 26) = 0.33$, $p < 0.7163$)

nor was there a significant interaction between the factors of L-366,948 pretreatment dose and PPA treatment ($F(3, 78) = 0.43$, $p < 0.8522$).

Figure 1b (bottom panel) depicts the effects on water intake of the joint pretreatments and treatments of this experiment. Vehicle-pretreated rats treated with vehicle consumed 4.5 mls of water. PPA significantly reduced water intake in this experiment ($F(3, 78) = 21.3$, $p < 0.001$). The inhibitory effects of PPA on water intake, however, were not a function of dose but rather each dose of PPA suppressed water intake by about 45%. Newman–Keuls contrasts revealed that each dose of PPA significantly differed from the vehicle dose but no dose of PPA was significantly different from any other PPA dose. Moreover, pretreatment with either 1 or 2 mg/kg L366,948 did not alter water intake ($F(2, 26) = 2.8$, $p < 0.08$) nor was there a significant interaction between L366,948 pretreatment dose and the effect of PPA on water intake ($F(3, 78) = 0.62$, $p < 0.7152$).

DISCUSSION

The present study sought to examine a potential interaction between PPA and OT systems in the modulation of feeding suggested by simlar actions on feeding and gastric motility between OT and PPA treatments. Rats were pretreated with either 1 or 2 mg/kg L-366,948, an OT receptor antagonist. This drug is known to antagonize central OT activity following systemic administration at a dose level of 1 mg/kg (3,17). In the present experiment, pretreatment with either dose of L-366,948 did not alter either basal food or water intake nor was there a significant effect of this OT antagonist on the inhibitory effect of PPA on food intake or on water intake.

PPA has been in use for decades as an over-the-counter appetite suppressant by persons wanting to lose modest amounts of weight (8,10). Recent studies have documented a substantial decrease in health risks associated with obesity following weight losses as small as 5–10% (2). The efficacy of PPA to induce modest weight loss has been established (8,10), but only recently has a distinct mechanism been proposed to account for the anorexic property of PPA (25). The mechanism notes that PPA is an agonist at α1-adrenoceptors and suggests that activation of these adrenoceptors results in suppression of feeding. Earlier studies have failed to implicate dopamine systems (19), cholecystokinin systems (27) or serotonin systems (11) in the inhibitory effects of PPA on food intake. The present study extends that line of research to OT systems and further strengthens the notion that PPA acts via stimulation of α1-adrenoceptors.

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