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Effects of the α_{1a}-Adrenoceptor Antagonist RS-17053 on Phenylpropanolamine-Induced Anorexia in Rats

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WELLMAN, P. J., L. R. McMAHON, T. GREEN AND A. TOLE. *Effects of the α_{1a}-Adrenoceptor antagonist RS-17053 on phenylpropanolamine-induced anorexia in rats.* PHARMACOL BIOCHEM BEHAV **57**(1/2) 281–284, 1997.—Activation of α1-Adrenergic receptors via systemic administration of drugs such as phenylpropanolamine (PPA) and cirazoline results in the suppression of feeding in rats. Whether PPA acts via activation of the three currently identified α1-Adrenoceptor subtypes is unknown. The intent of the present study was thus to examine the effects of systemic administration of the novel α_{1a}-Adrenoceptor antagonist RS-17053 on PPA-induced anorexia. Adult male rats (n=6 to 8 per group) were pretreated (IP) with either 0, 0.1, 0.5, 2.5, or 10.0 mg/kg RS-17053 or with 2.0 mg/kg of the prototypical α1-Adrenoceptor antagonist prazosin. Five minutes later, each rat was treated (IP) with either 0, 5, 10 or 15 mg/kg PPA. Food and water intakes were recorded for a 30 min period starting 10 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 the 15 mg/kg dose of PPA. Pretreatment with 2.0 mg/kg prazosin reversed the anorexic activity of PPA. Pretreatment with RS-17053 (0.1–2.5 mg/kg) did not alter either baseline feeding or the anorexic action of PPA. These results suggest that PPA does not act via the α_{1a}-Adrenergic receptor subtype to suppress food intake. © 1997 Elsevier Science Inc.

Phenylpropanolamine Anorexia RS-17053 α1-Adrenergic receptors Prazosin

PHENYLPROPANOLAMINE (PPA) has been used for decades as an over-the-counter drug intended to suppress appetite in persons wanting to lose moderate amounts of weight. The anorexic efficacy of PPA has been established (8,11,16, 18), but only recently has a distinct mechanism been proposed to account for the anorexic property of PPA. The anorexic action of PPA is thought to result from its efficacy as an α1-Adrenergic receptor agonist (18). Pharmacological studies have demonstrated that PPA binds to and activates α1-Adrenergic receptors (12,14). Systemic administration of other α1-Adrenergic agonists including cirazoline (3), amidephrine (13) or SKF 89748 (13) suppresses food intake in rats. Finally, the anorexic action of systemically administered PPA or cirazoline is reversed by systemic pretreatment with the general α1-Adrenoceptor antagonist prazosin (17).

The aforementioned findings support the notion that PPA suppresses food intake via activation of α1-Adrenoceptors, but do not indicate whether PPA acts via one of the currently identified α1-Adrenoceptor subtypes. The α1-Adrenoceptor is comprised of three subtypes, labelled α_{1a}, α_{1b}, and α_{1d}. (5,9). The α1-Adrenoceptor antagonist prazosin does not parse the receptor subtypes in that each exhibits high affinity for this general α_1-antagonist. Yet, the subtypes can be distinguished on the basis of function, relative binding of pharmacological probes, and cloning sequences (5,6,9). A limited number of probes have been identified that selectively antagonize the α_{1a}-Adrenoceptor subtype. The novel compound RS-17053, for example, has been recently shown to exhibit selectivity for the α_{1a}-Adrenoceptor subtype (6) over the α_{1b}- and α_{1d}-adrenoceptor subtypes (6,7), and to functionally antagonize α_{1a}-Adrenoceptors located within rat kidney (6).

Whether the anorexic action of PPA is specifically associated with activity at one or more of the α1-Adrenoceptor subtypes is unknown. The present study therefore sought to determine whether pretreatment with the α_{1a}-antagonist RS-17053 (0.1–10 mg/kg) would reverse the anorexic action of systemically administered PPA (5–15 mg/kg). Doses of RS-17053 and of PPA were chosen to encompass a full dose range (1,10,16). In addition, 2.0 mg/kg prazosin (IP) served as a positive control condition, in that this prazosin dose was noted

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in an earlier study to reverse the anorexic action of systemically administered PPA (17).

METHODS

Subjects

The subjects were 185 male Sprague–Dawley viral-free albino rats (obtained from Harlan Industries; Houston, TX) weighing approximately 275–300 g at the beginning of the study. The rats were housed individually in standard plastic rodent cages in a colony room maintained at 21.0 ± 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 solution was prepared using Tween 80 (3 drops per ml) dissolved in sterile distilled water. RS-17053 solutions were prepared by dissolving RS-17053 (RS: 0.1, 0.5, 2.5 and 10 mg/ml) into the vehicle. RS-17053 was kindly donated by Dr. David E. Clarke of Roche Bioscience Corporation (Palo Alto, CA). The phenylpropanolamine solutions (5, 10 and 15 mg/ml) were prepared using PPA hydrochloride (±-norephedrine: Sigma Chemical) dissolved in the vehicle. A solution of prazosin hydrochloride (2 mg/ml: Sigma Chemical) was similarly prepared. All solutions were prepared as the weight of the base and salt in vehicle solution.

Procedure

The rats were maintained in the colony room for a minimum of one week prior to the start of the experiment to acclimate them to daily handling and routine colony procedures. Each rat underwent a series of baseline feeding trials. Beginning at 1500 h each day, each rat was provided with a clean cage with a cardboard pad placed beneath a metal grid floor. The feeders and water bottles were removed, and each rat was weighed prior to the start of its feeding trial. One hour later, approximately 12 g of the pellet diet was placed on the grid floor of each home cage and each water bottle was weighed and returned to the home cage. Each rat was allowed 30 min access to the pellet diet and water. Following each feeding trial, the remaining food and spillage was removed from the cage, and weighed to determine individual food intake (to the nearest 0.1 g). Water intakes were measured to the nearest 0.1 ml. Rats had continuous access to food and water until the start of the next ingestive trial on the following day.

On days 8, 9 and 10, each rat received separate vehicle injections (1.0 ml/kg, IP), with one injection given at 15 min prior to and the second injection given at 10 min prior to the feeding trial. These vehicle injections served to adapt the rats to the injection protocol used during the drug trial.

The baseline intake trials on days 8–10 were averaged for each rat and were used to assign the rats to experimental conditions. The design of this experiment was a completely between-group factorial consisting of pretreatment and treatment combinations yielding 24 groups. The pretreatment factor consisted of vehicle, 0.1 mg/kg RS, 0.5 mg/kg RS, 2.5 mg/kg RS, 10 mg/kg RS, or the positive control of 2 mg/kg prazosin. The treatment factor was vehicle, 5 mg/kg PPA, 10 mg/kg PPA, or 15 mg/kg PPA. The rats were randomly assigned to one of these twenty-four pretreatment-treatment combinations. On the drug day, the rats were injected with their respective pretreatment condition at −15 min and their treatment condition at −10 min prior to the start of the 30 min trial. Food and water intakes were recorded to the nearest 0.1 g and 0.1 ml for each rat as noted above.

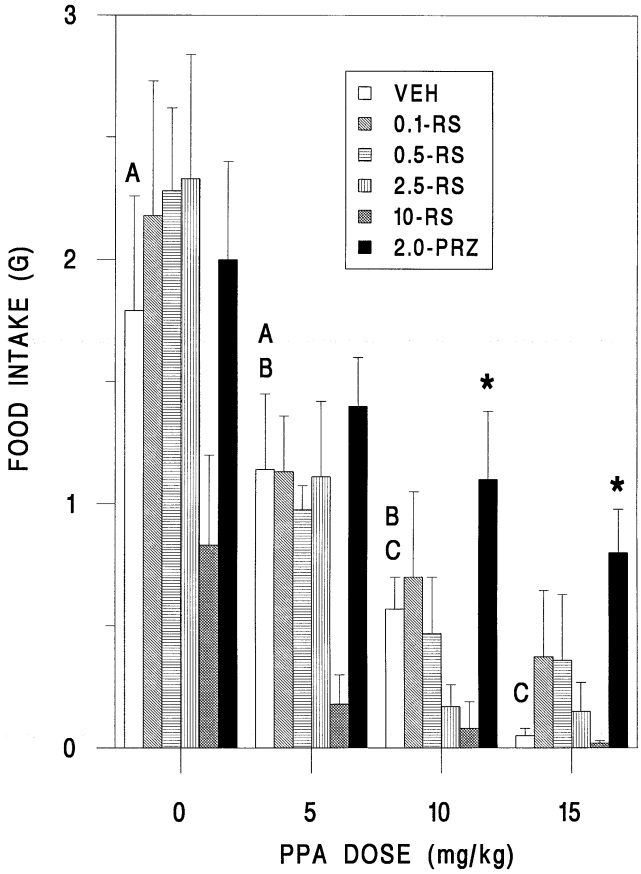

FIG. 1. Mean group pellet intake (g) during a 30 min period for rats pretreated (−15 min) with either vehicle, various doses of RS-17053 (RS: 0.1–10.0 mg/kg) or 2 mg/kg prazosin (PRZ) and then treated (−10 min) with either 0, 5, 10 or 15 mg/kg PPA. The lines above each bar represent the standard error of the mean. Different letters above the bars indicate a significant difference (bars with a common letter are not significantly different); $p < 0.05$. Asterisks above the bars indicate a significant difference from the vehicle pretreatments within the PPA treatment dose; $p < 0.05$.

The pretreatment and treatment intervals were chosen on the basis of the extant literature relating the onset and duration of action of PPA and of RS-17053. PPA and RS-17053 exhibit similar profiles with a rapid onset of action (less than 5 min) following systemic administration, with a duration of action exceeding 60 min (D. E. Clarke, personal communication, May 15, 1996; 16,19).

Data Analyses

The food intake and water intake data of this experiment were analyzed using two separate designs. The primary design was a 5×4 factorial with a between-group factor of DRUG PRETREATMENT (vehicle, 0.1 RS, 0.5 RS, 2.5 RS, or 10 mg/kg RS) and a between-group factor of PPA DOSE (vehicle, 5, 10, or 15 mg/kg PPA). The secondary design was a 2×4 factorial with a between-group factor of DRUG PRETREAT-

MENT (vehicle, 2.0 mg/kg prazosin) and a between-group factor of PPA DOSE (vehicle, 5, 10, or 15 mg/kg PPA). Separate General Linear Model (GLM) analyses (2) were computed using SAS for the dependent variables of food intake and water intake. Additional contrasts were made using Duncan's Multiple Range procedure. Difference probabilities less than 0.05 were deemed statistically significant.

RESULTS

Figure 1 depicts the changes in food intake associated with the RS-17053 and prazosin pretreatments and the PPA treatments of this experiment. A GLM analysis revealed a significant effect of the PPA treatment condition [$F(3, 132) = 29.74$, $p = 0.0019$]. Post-hoc analyses using Duncan's procedure ($\alpha = 0.05$, df = 132, MSE = 0.588) revealed that the vehicle treatment condition was significantly different from the 10 mg/kg and 15 mg/kg PPA treatment conditions and that the vehicle and 5 mg/kg PPA treatment conditions were significantly different from the 15 mg/kg PPA treatment condition. The GLM analysis revealed that RS-17053 pretreatment significantly altered food intake [$F(4, 132) = 6.28$, $p < 0.0001$]. Further contrasts using Duncan's procedure ($\alpha = 0.05$, df = 132, MSE = 0.557), revealed that 10 mg/kg RS-17053 significantly suppressed food intake. However, informal observation of the rats after injection suggested that the locomotion was impaired at the 10 mg/kg RS-17053 dose. In contrast, no significant differences were noted between vehicle and the 0.1-2.5 mg/kg doses of RS-17053. Thus, the sole effect of RS-17053 on feeding in this experiment was an inhibitory one, but was only evident at a high dose that also impaired locomotion. There was no significant interaction between RS-17053 pretreatment conditions and PPA treatment conditions [$F(12, 132) = 1.58$, $p < 0.1049$]. Thus, pretreatment with RS-17053, at doses that alone did not alter feeding, had no impact on anorexia induced by PPA.

Prazosin pretreatment at 2 mg/kg reversed the anorexic action of systemic PPA. A GLM analysis revealed a significant effect of prazosin pretreatment [$F(1, 63) = 7.34$, $p = 0.0090$], but no significant interaction of prazosin pretreatment conditions with PPA treatment conditions [$F(3, 63) = 0.78$, $p = 0.5096$].

Figure 2 depicts the changes in water intake associated with the RS-17053 and prazosin pretreatment conditions and the PPA treatment conditions of this experiment. A GLM analysis revealed a significant effect of the PPA treatment condition [$F(3, 132) = 15.1$, $p < 0.0001$] on water intake. Post-hoc analyses using Duncan's procedure ($\alpha = 0.05$, df = 132, MSE = 1.0377) revealed that water intakes after each of the three PPA treatment doses were significantly reduced relative to water intake after vehicle treatment, but these doses were not different from one another. The RS-17053 pretreatment conditions did not significantly alter water intake [$F(4, 132) = 0.54$, $p = 0.7055$], nor was there evident a significant interaction between RS-17053 pretreatment and PPA treatment conditions [$F(12, 132) = 0.49$, $p = 0.9136$]. Finally, there was no effect of prazosin pretreatment on water intake [$F(1,54) = 0.11$, $p = 0.7372$] nor was there a significant interaction between prazosin pretreatment conditions and PPA treatment conditions [$F(3, 54) = 1.55$, $p = 0.2113$].

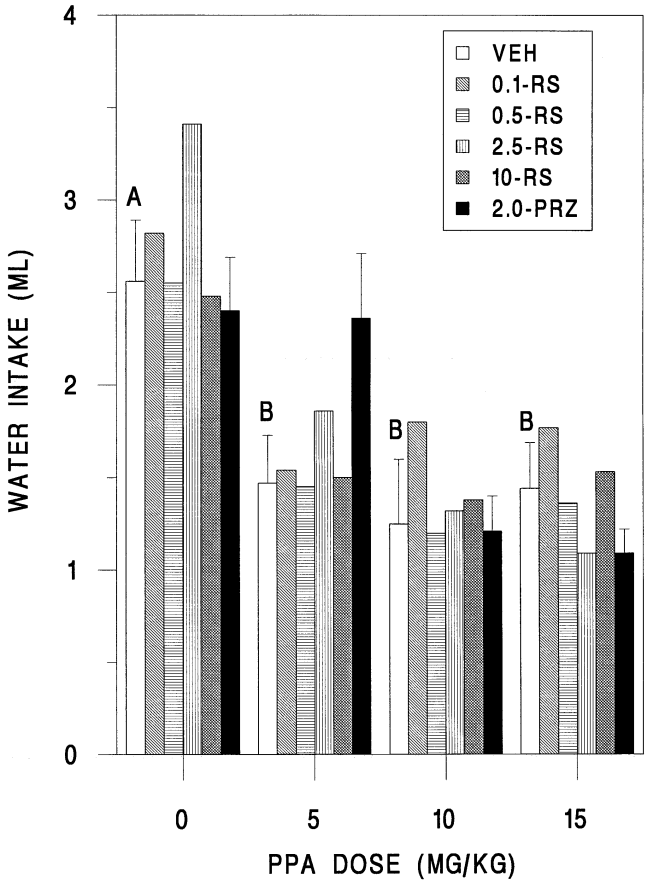

FIG. 2. Mean group water intake (ml) during a 30 min period for rats pretreated (−15 min) with either vehicle, various doses of RS-17053 (RS: 0.1–10.0 mg/kg) or 2 mg/kg prazosin (PRZ) and then treated (−10 min) with either 0, 5, 10 or 15 mg/kg PPA. The lines above each bar represent the standard error of the mean. Different letters above the bars indicate a significant difference (bars with a common letter are not significantly different); $p < 0.05$.

DISCUSSION

The α1-Adrenoceptor hypothesis suggests that PPA suppresses feeding via activation of α1-Adrenoceptors (18). The current study examined whether PPA anorexia results from the selective activation by PPA of a specific subtype of α1-Adrenoceptors. The ligand chosen in the current study was RS-17053, a recently identified compound known to selectively inactivate α_{1a}-Adrenoceptors (6,7). RS-17053 has a rapid onset of action, and a duration of action exceeding 60 min (D. E. Clark, personal communication, May 15, 1996). Except at a very high dosage of 10 mg/kg, RS-17053 did not alter food intake or water intake, nor did this antagonist reverse the anorexia induced by PPA doses ranging from 5-15 mg/kg. This finding suggests that PPA does not act via the α_{1a} subtype to suppress feeding.

Prazosin has been noted to bind with equal affinity to each of the three α_{1a} subtypes (5,7), and is thus a non-selective antagonist for α1-Adrenoceptors. In the present study, a single dose of prazosin (2 mg/kg) was noted to reliably antagonize the anorexia induced by PPA doses ranging from 5–15 mg/kg. The present results are similar to those obtained in our earlier study in which systemic prazosin reversed the anorexic action of PPA (17). The effect of prazosin was restricted to the anorexic activity of PPA. Water intake was suppressed by PPA, but this effect was not a function of dose, as was the case for the effect of PPA on food intake. Antagonism of

α1-Adrenoceptors using prazosin did not alter the action of PPA on water intake. This outcome also confirms our earlier study in which systemic prazosin did not alter the hypodipsic activity of PPA.

The present findings suggest that although PPA anorexia is reversed by systemic administration of prazosin, PPA is unlikely to suppress feeding via the α_{1a}-Adrenoceptor subtype. Whether the α_{1b}- or α_{1d}-Adrenoceptor subtypes may play a role in PPA anorexia is an open question due to the lack of suitable selective receptor subtype antagonists (9). Yet, there are hints in the literature that may point to the α_{1d}-Adrenoceptor subtype as playing a role in mediating PPA anorexia. Oshita, Kigoshi, and Muramatsu (15) noted that the α1-Adrenoceptor agonist methoxamine exerted significant activity at α_{1d}-Adrenoceptors in rabbit aorta, but was without effect at α_{1b}-Adrenoceptors in rabbit aorta. Methoxamine may thus represent a pharmacological probe by which to dissociate α_{1d}-Adrenoceptors and α_{1b}-Adrenoceptors. Interestingly, anorexia is noted in rats following microinjections of methoxamine into the paraventricular hypothalamic nucleus (PVN: 4), a brain region containing α1-Adrenoceptors (20). Although no study to date has compared the relative distribution of α_{1d}- and α_{1b}-Adrenoceptors within the PVN, each receptor subtype has been localized within rat brain (5). These preliminary studies hint that PPA may act via the α_{1d}-adrenoceptor subclass, but a firm conclusion awaits future studies that examine the effects on feeding in rats of administration of selective α_{1d}-adrenoceptor agonists and antagonists.

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