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Isobolographic Assessment of the Effects of Combinations of Phenylpropanolamine and Fenfluramine on Food Intake in Rats

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WELLMAN, P. J., S. TOW AND L. McMAHON. *Isobolographic assessment of the effects of combinations of phenylpropanolamine and fenfluramine on food intake in rats.* PHARMACOL BIOCHEM BEHAV **50**(2) 287-291, 1995.—Phenylpropanolamine (PPA) suppresses appetite in rats via activation of α_1-adrenergic receptors within the paraventricular hypothalamus (PVN). The serotonergic (5-HT) agonist fenfluramine (FEN) is thought to suppress appetite via stimulation of 5-HT release within the PVN rather than activation of adrenergic receptors. Whether a mixture of these neurochemically distinct anorexic drugs will serve as an effective appetite suppressant is unknown. In the present experiment, drug-drug interactions between PPA and FEN were explored using an isobologram technique. Fixed doses of PPA (0 vs. 5 mg/kg) were combined with various doses of FEN (1.25, 2.5, and 5.0 mg/kg) and fixed doses of FEN (0 vs. 2.5 mg/kg) were combined with various doses of PPA (0, 5, 10, and 15 mg/kg). Drug combinations were injected IP 30 min before a 1-h feeding trial in 16-h food-deprived rats. PPA and FEN were dose-additive in this paradigm, an outcome that supports the feasibility of a new appetite suppressant composed of a mixture of PPA and FEN.

Phenylpropanolamine Feeding Anorexia Fenfluramine Serotonin α_1-Adrenoceptors Paraventricular hypothalamus

PHENETHYLAMINE drugs such as phenylpropanolamine (PPA) are thought to suppress appetite (8,16,22) via activation of α_1-adrenergic receptors within the hypothalamic paraventricular nucleus (PVN). The argument for an adrenergic mechanism of action of PPA is based on several converging lines of evidence including: a) the identification of α_1-adrenergic receptors within the PVN (10,11); b) electrophysiologic studies that reveal that feeding-relevant cells in the PVN are excited by α_1-adrenergic receptor agonists (7); c) the observation of suppression of appetite after intra-PVN microinjection of α_1-adrenergic agonists such as phenylpropanolamine, methoxamine, phenylephrine, and cirazoline (2,4,20,22); and d) studies in which α_1-adrenergic receptor antagonists such as benoxathian or prazosin reverse the anorexia induced by systemic injections of either PPA or cirazoline (19,21).

Also localized within the PVN is a serotonergic system similarly involved in the suppression of appetite. Intra-PVN injections of either serotonin (5-HT) or the 5-HT agonist fenfluramine (FEN) suppress feeding (5,9,13), whereas lesions of the PVN slightly attenuate the anorexic action of fenfluramine (15). Fenfluramine anorexia is apparently related to the release of 5-HT from PVN cells that subsequently suppress appetite.

It is interesting that PPA and FEN suppress appetite via activation of neurochemically distinct cells within the PVN, with PPA activating α_1-adrenoceptors and with FEN releasing 5-HT to suppress appetite. The issue of whether PPA and fenfluramine act via completely distinct mechanisms is related to potential future combinations of these drugs as appetite suppressants. An emerging trend in the pharmacotherapy of obesity is the rational combination of anorexiants that act via independent neurochemical mechanisms to suppress food intake and body weight. Weintraub et al. (14), for example, reported significant weight loss in obese humans receiving a combination of fenfluramine and phentermine. Similarly, if PPA does not act via 5-HT mechanisms to suppress feeding, then it may be possible to combine low doses of α_1-adrenergic

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agonists (such as PPA) with serotonergic agonists (such as FEN) to provide for more effective anorexic agents with lower associated risks. The present experiment therefore provides a bidirectional isobologram analysis of the effects of combining a fixed dose of PPA with various doses of FEN (as well as a fixed dose of FEN combined with various PPA doses) on feeding behavior using the methods outlined by Wessinger (23). An isobologram analysis determines whether drug-drug mixtures exert effects that are additive or effects that diverge from dose-additive. Of particular concern is whether PPA and fenfluramine combinations might be supra-additive (that is, the combination of these two drugs exerts anorexic effects that are greater than those predicted from their separate effects). Half of a group of rats (n = 10) received a fixed dose of PPA (0 or 5.0 mg/kg, IP) in combination with various doses of FEN (0, 1.25, 2.5, and 5.0 mg/kg, IP), whereas the remaining rats (n = 10) received a fixed dose of FEN (0 and 2.5 mg/kg) in combination with various doses of PPA (0, 5, 10, and 15 mg/kg, IP). Combinations were injected IP 30 min before a 60-min test of feeding in 16-h food-, but not water-deprived rats.

METHOD

Animals

The animals were 20 male Sprague-Dawley albino rats (obtained from Harlan Industries, Houston, TX) weighing 291-335 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 h/12 h illumination schedule (lights on at 0700 h). The rats were provided limited access to tapwater and rodent pellets (Teklad, Winfield, IA) in the home cage from approximately 1000-1800 h throughout the experiment.

Drugs

A sterile saline solution was prepared using sterile distilled water and 0.9% (wt./vol.) sodium chloride. Solutions of phenylpropanolamine hydrochloride (Sigma Chemical Co., St Louis, MO: ±-norephedrine; 5, 10, and 15 mg/ml) and of D,L-fenfluramine hydrochloride (A.H. Robins, Richmond, VA: 1.25, 2.5, and 5.0 mg/ml) were prepared by dissolving each dose into sterile saline. All drug solutions were calculated as the salt of drug per milliliter of vehicle.

Procedure

Baseline 60-min food intakes were recorded for each rat on 12 consecutive days. The rats were deprived of food and water at about 1800 h of the previous day. At about 0930 h of the test day, each rat was transferred to a clean cage outfitted with a cardboard pad positioned beneath each wire floor. On days 8-12, the rats were given a daily IP injection of saline (1 ml/kg each). Thirty minutes later, approximately 20 g of food pellets were placed in each cage and a water bottle was provided to each rat. Food intake was recorded to the nearest 0.1 g after correction for food spillage (collected on a paper pad positioned beneath each cage floor). Water intake was recorded to the nearest 1.0 ml (data not presented). The rats had continuous access to pellets and tapwater during the 7-h period after each intake test (1100-1800 h). At 1800 h, food, but not water, was removed from the home cage.

Two groups of rats (n = 10 each) were formed on the basis of comparable average food intake during the last 4 days of the baseline phase. Each group was randomly assigned to receive a pretreatment consisting of either a fixed dose of PPA (5 mg/kg, IP) vs. vehicle or a fixed dose of FEN (2.5 mg/kg, IP) vs. vehicle. Rats in the PPA pretreatment condition received one of eight drug combinations on each drug trial. These consisted of either vehicle pretreatment combined with various doses (IP) of FEN (0, 1.25, 2.5, or 5.0 mg/kg) or 5 mg/kg PPA pretreatment combined with various doses (IP) of FEN (0, 1.25, 2.5, or 5.0 mg/kg). In the FEN pretreatment condition, rats received a combination of either vehicle pretreatment with one of four PPA doses (0, 5, 10, or 15 mg/kg) or 2.5 mg/kg FEN with one of four PPA doses (0, 5, 10, or 15 mg/kg). Each rat in a pretreatment condition (PPA or FEN) received each of the eight pretreatment-treatment combinations. Half of the rats in each pretreatment condition received the vehicle pretreatment in combination with the four drug treatments followed by a block of tests in which the drug pretreatment was given in combination with the four drug treatments. Within each drug treatment block, the four doses of drugs were given in random order. The drug test sequence thus consisted of eight trials conducted using the baseline procedures. The trials consisted of alternating drug (D) and vehicle (V) trials conducted on successive days. On the drug days, the rats received successive injections (separated by 15 s) of the pretreatment (PPA or vehicle; FEN or vehicle) in combination with one of the four drug treatment conditions. On the vehicle trials, the procedures were followed as before except that the rats were injected with vehicle (1.0 ml/kg) 30 min before the feeding test. The vehicle test interpolated 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 7 h after the end of each drug trial, an interval that allowed each rat to recover from any marked anorexic effects induced during the drug trials.

Data Analysis

Separate analyses of variance (ANOVA) (1) were computed for the combination of 5 mg/kg PPA with fenfluramine and 2.5 mg/kg fenfluramine with PPA. Each analysis of food intake was computed using a repeated measures design with two factors. The first factor was pretreatment (vehicle or 5 mg/kg PPA; vehicle or 2.5 mg/kg FEN); the second factor was dose (PPA: 0, 5, 10, and 15 mg/kg; FEN: 0, 1.25, 2.5, and 5.0 mg/kg). ANOVAs were followed by computation of ED_{50} values for each drug with separate calculations by pretreatment condition.

RESULTS

After preinjection of vehicle or 2.5 mg/kg FEN, PPA produced a dose-dependent suppression of feeding behavior (Fig. 1, top panel). Injection of 5, 10, and 15 mg/kg PPA in the vehicle pretreatment condition produced suppressions of feeding of 21, 32, and 44%, respectively. These effects were confirmed by ANOVA, which revealed a significant effect of PPA dose [$F(3, 27) = 16.4, p < 0.0001$]. A regression analysis, computed using the data from the vehicle pretreatment condition, revealed that the ED_{50} for PPA in vehicle-pretreated rats was 20.4 mg/kg. PPA also produced a dose-dependent suppression of feeding in rats pretreated with 2.5 mg/kg FEN. Pretreatment alone reduced food intake by approximately 3.0 g. ANOVA also revealed a significant effect of pretreatment [$F(1, 9) = 163.2, p < 0.0001$], but no signifi-

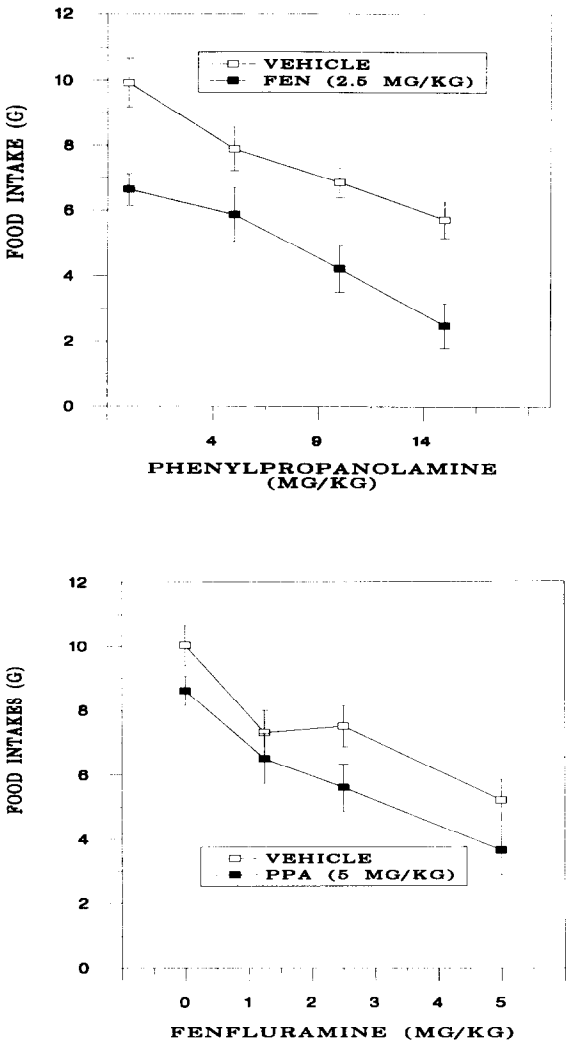

FIG. 1. (Top panel). Mean group food intakes from rats injected with either vehicle (□) or 2.5 mg/kg fenfluramine (■) in combination with 0, 5, 10, and 15 mg/kg (IP) phenylpropanolamine. The lines above each symbol represent the standard error of the mean. (Bottom panel). Mean group food intakes from rats injected with either vehicle (□) or 5 mg/kg phenylpropanolamine (■) in combination with 0, 1.25, 2.5, and 5.0 mg/kg (ip) fenfluramine. The lines above each symbol represent the standard error of the mean.

cant interaction between the factors of pretreatment and PPA dose [$F(3, 27) = 0.51, p < 0.68$]. Further regression analysis revealed that pretreatment with a fixed dose of FEN at 2.5 mg/kg reduced the PPA ED_{50} to 13.3 mg/kg.

After preinjection with either vehicle or 5 mg/kg PPA, fenfluramine also produced a dose-dependent suppression of feeding (Fig. 1, bottom panel). Injection of 1.25, 2.5, and 5 mg/kg FEN in the vehicle pretreatment condition produced suppressions of feeding of 27, 26, and 49%, respectively. These effects were confirmed by ANOVA, which revealed a significant effect of FEN dose [$F(3, 27) = 17.5, p < 0.0001$]. A regression analysis, computed for the vehicle pretreatment condition, revealed that the ED_{50} for FEN in vehicle-pretreated rats was 6.6 mg/kg. FEN also produced a dose-dependent suppression of feeding in rats pretreated with 5.0 mg/kg PPA. PPA pretreatment alone reduced food intake by approximately 1.5 g. ANOVA also revealed a significant effect of pretreatment [$F(1, 9) = 12.0, p < 0.0001$], but no significant interaction between the factors of pretreatment and FEN dose [$F(3, 27) = 0.19, p < 0.89$]. Further regression analysis revealed that pretreatment with a fixed dose of PPA at 5 mg/kg reduced the FEN ED_{50} to 5.0 mg/kg.

Figure 2 depicts an isobologram summarizing the overall findings of the experiment. Depicted are the respective ED_{50} values for the dose-response curves of PPA (computed using the vehicle-pretreatment data) and of FEN (similarly computed using the vehicle-pretreatment data). The theoretical dose-addditive line is indicated by a solid line joining the ED_{50} values of PPA on the vertical axis and of FEN on the horizontal axis. To evaluate whether the fixed combinations of 5 mg/kg PPA with FEN and of 2.5 mg/kg FEN with PPA exerted dose-additive effects on feeding (23), the standard error of the mean of the respective ED_{50} values was computed and multiplied by 1.96 to provide a 95% confidence interval. The upper bound of the confidence interval values are shown in Fig. 2 as a dotted line lying above the theoretical dose-additive line. The logic here was that if the ED_{50} value of the drug combination was within the boundary of the dose-additive line and the confidence interval boundary, then that combination of PPA and of FEN exerted an effect that was dose-additive.

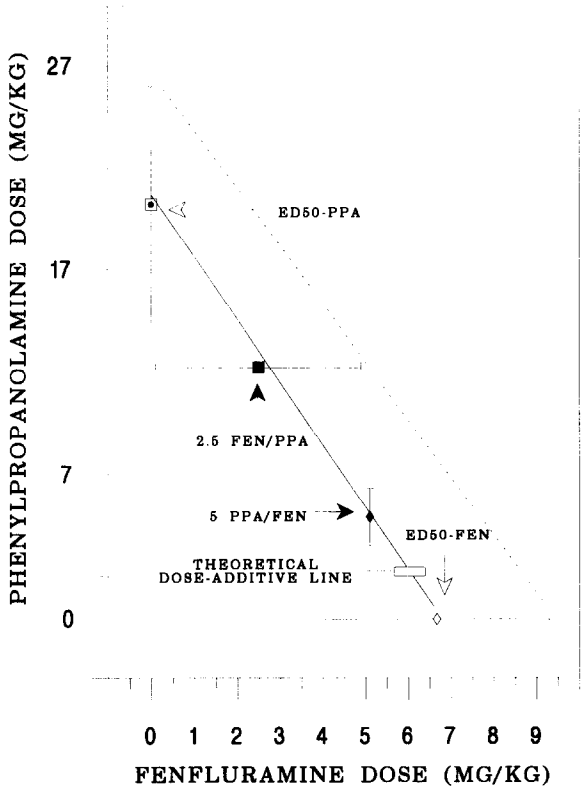

FIG. 2. An isobologram of the effects of various combinations of PPA and of fenfluramine on food intake.

As shown in Fig. 2, the combinations of a fixed dose of 5 mg/kg PPA with FEN and that of a fixed dose of 2.5 mg/kg FEN with PPA produced anorexic effects judged to be dose-additive.

Thus, in rats pretreated with vehicle, both PPA and FEN alone produced a dose-dependent suppression of feeding. After vehicle pretreatment, the ED_{50} for PPA anorexia was estimated to be 20.4 mg/kg, whereas the ED_{50} for FEN anorexia was estimated to be 6.6 mg/kg. When 5 mg/kg PPA was combined with FEN, the FEN ED_{50} value was reduced to 5 mg/kg. When 2.5 mg/kg FEN was combined with PPA, the PPA ED_{50} value was reduced to 13.3 mg/kg. An isobolographic analysis showed that the combined effects of PPA and FEN were dose-additive under the doses and conditions of this study.

DISCUSSION

The present results suggest that combinations of PPA, an α_1-adrenergic agonist, and of fenfluramine, an indirect 5-HT agonist, are dose-additive. The results are consistent with a body of literature that suggests that these two anorexic agents act via independent neurochemical mechanisms within the PVN. Indeed, in a series of studies, we have examined the possible interactions between PPA and various serotonin systems. The concern was prompted by two sets of earlier pharmacologic studies. In the first, Innes and Kohli (6) evaluated the effect of various sympathomimetic amines on contraction and relaxation of guinea pig ileum, which is contracted by activation of 5-HT receptors. PPA induced a mixed pattern of predominantly relaxation (and some contraction) whereas α_1-adrenergic agonists such as phenylpehrine and methoxamine produced consistent contraction similar to that induced by 5-HT. The results of second study suggested that PPA might act partially to stimulate 5-HT receptors in that both fenfluramine and PPA produced slowing of gastric emptying (12,18). Yet, to date, we have found no evidence that anorexia produced by PPA is mediated in part by activity of the serotonin system within the PVN. PPA anorexia is not altered by pretreatment with 8-OH-DPAT (which reduces 5-HT release via activation of the $5\text{-}HT_{1a}$ autoreceptor), nor does PPA increase extracellular 5-HT within the PVN as measured by microdialysis (McMahon and Wellman, unpublished data).

Taken as a whole, these studies suggest that a promising strategy for new appetite suppressants is to take advantage of our knowledge of existing appetite-suppressing drugs. When two compounds act via dissimilar neurochemical mechanisms, as is the case for PPA and FEN, it may be possible to effectively mix the two drugs while retaining the desired final effect of suppressing food intake. The advantage of the mixture is that if there are untoward effects of each drug, which are a function of dose for each compound, it may be possible to reduce the respective dose of each compound, and therefore to reduce the potential of side effects while retaining the desired effect on food intake. The present results suggest that it may be possible to effect a mixture of phenylpropanolamine and of fenfluramine that will require smaller doses of each to suppress appetite.

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