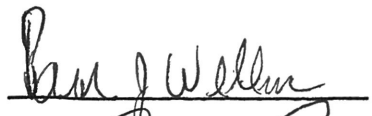
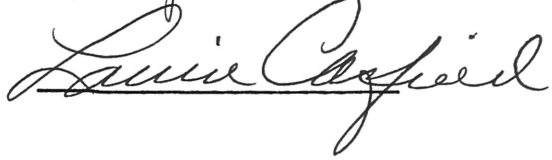
A COMPARISON OF ISOMERS AND CONGENERS OF PHENYLPROPANOLAMINE (PPA) AND NORPSEUDOEPHEDRINE ON GASTRIC RETENTION AND FOOD INTAKE IN RATS

Theresa M. Jones
University Undergraduate Fellow, 1986-1987
Department of Psychology
Texas A&M University

APPROVED

Fellows Advisor: 

Honors Director: 

A Comparison of Isomers and Congeners of Phenylpropanolamine (PPA) and Norpseudoephedrine on Gastric Retention and Food Intake in Rats

Theresa M. Jones
Department of Psychology
Texas A&M University

Running head: COMPARISON OF ISOMERS AND CONGENERS

Table of Contents

Abstract

The purpose of this study was to compare the action of various drugs on gastric retention (Experiment 1) and food intake (Experiment 2). The subjects were 79 Sprague-Dawley male albino rats. (Experiment 1 = 40), (Experiment 2 = 39). In Experiment 1, the rats consumed a pellet meal and were then injected (IP) with saline or either (30 mg/kg) of l-PPA, d-PPA, l-norpseudoephedrine or d-norpseudoephedrine. The rats treated with saline, d-norpseudoephedrine, l-norpseudoephedrine and d-PPA exhibited a marked clearance of food. The rats treated with l-PPA detained a large portion of the test meal, indicating that l-PPA is indeed effective in the inhibition of gastric emptying. In Experiment 2, the rats were first injected (IP) with saline or either (30 mg/kg) of l-PPA, d-PPA, l-norpseudoephedrine or d-norpseudoephedrine. After injection, the rats consumed a pellet meal. The rats treated with d-PPA exhibited a normal amount of grams consumed. The rats treated with l-norpseudoephedrine and d-norpseudoephedrine displayed a small but not significant decline in food intake. The l-PPA drug group demonstrated a significant reduction in food intake. This data concludes that l-PPA is not only effective in gastric inhibition, but effective in the reduction of food intake and that the former may mediate the latter as well.

The drug phenylpropanolamine (PPA) is commonly found in many over-the-counter diet pills, antihistamines and in many widely prescribed decongestants (Physicians' Desk Reference, 1987). The many effects of PPA include a reduction in food intake and body weight (Wellman and Sellers, 1981), as well as a reduction in white adipose tissue. PPA has also been reported to elevate blood pressure in human subjects as demonstrated by Goodman, Wright, Barlascini, McKenney and Lambert (1986). Lake, Alagna, Quirk, Moriarty and Reid (1985) found a significant relationship between psychosis in humans and the use of PPA. further investigation into PPA is necessary in better understanding its many effects.

Phenylpropanolamine is a mixture of two norephedrine isomers, d-norephedrine and l-norephedrine, that induce anorexia and produce a feeling of satiety. It is unclear as to whether PPA acts via the brain or on some peripheral mechanism, such as the stomach in particular. Davies, Rossi, Pankseep, Bean and Zolovick (1983) have reported that fenfluramine, another anorexic drug, controls food intake by short term signals relating to food in the gastro-intestinal tract. PPA may act on the liver, nerves in the gut or perhaps on the vagus nerve which would relay a message to the brain. Another possibility concerning the mechanism by which PPA reduces food intake, is taste aversion and suppression of water (Wellman, Malpas & Wikler, 1981). Perhaps PPA produces feelings of illness therefore reducing food intake.

In the present study the effects of d-PPA, l-PPA, d-Norpseudoephedrine and l-Norpseudoephedrine on gastric retention were investigated. Norephedrine and norpseudoephedrine are often

confused due to similar chemical structures, compounds and molecular weights as demonstrated by Eisenberg, Silverman and Maher (unpublished findings). As reported by Morgan (1985) norephedrine and norpseudoephedrine are indeed distinct isomers despite the confusion. Due to the common confusion concerning these two isomers, norpseudoephedrine was used as a basis to compare the effects of PPA on gastric retention and food intake. In a follow up experiment, the effects of d-PPA, l-PPA, d-Norpseudoephedrine and l-Norpseudoephedrine on food intake were evaluated. The rationale of these two experiments was to determine if a correlation exists between the effects of these isomers on gastric retention and food intake.

EXPERIMENT 1

Method

Animals

The animals were 40 male Sprague-Dawley albino rats (Timco Inc., Houston, Texas) weighing 200-240 grams at the beginning of the experiment. The rats were maintained in a temperature-controlled (23.0°C) colony room under a 24 hour illumination schedule and were individually housed in plastic rodent cages. (Lab Products; Bryan, Texas).

Drugs

The phenylpropanolamine (PPA) and norpseudoephedrine solutions (30 mg/ml) were prepared prior to injection by dissolving d- and l-PPA and d- and l-Norpseudoephedrine hydrochloride into sterile distilled water. A 0.9% saline solution was also prepared. The dosage of sodium pentobarbitol used was (50 mg/ml) and was obtained from Sigma Chemical Company. The l-PPA was obtained from Roehr Chemicals and the d-PPA obtained from Aldrich Chemical Company. The l-Norpseudoephedrine was also obtained from Aldrich Chemical Company and the d-Norpseudoephedrine was obtained from Merz and Co.

Design and Procedure

The rats were maintained in the colony for 2 weeks prior to the start of the experiment. The rats were handled daily and given continous access to pellets (Teklad) and tap water. On Day 0 (Sunday), the rats were deprived of food at 1500 hours but, allowed continuous access to tap water. On Day 1 at 1500 hours

the rats were individually weighed and then placed into individual plastic feeding cages. At 1530 hours each rat was given a 1 hour access to 10 grams of pellets (Teklad). On Day 1 during the 1 hour access to food the rats were not allowed water. After the 1 hour access to food, all of the rats were returned to the home cages and the remaining food left in the feeding cages was weighed. The difference between the weight of the pellets put into the individual feeding cages and the weight of the unconsumed food left over in the feeding cages was taken as the amount eaten in grams. On Days 2 thru 6, the schedule remained the same as on Day 1, except that starting on Day 2 the rats were allowed continuous access to tap water during their daily 1 hour feeding periods to facilitate the consumption of the dry pellet diet. On Day 7 (the Test Day) 5 gram portions of pellets were preweighed. The test day began at precisely 1500 hours with 15 minute intervals between access to food and injection time for each rat. At 1500 hours rat number 1 was placed in an individual feeding cage, with 5 grams of food and given continuous access for 15 minutes. A calibrated water bottle containing tap water was placed in each feeding cage to allow access to water while feeding. At 1515 hours rat number 1 was taken out of the feeding cage and injected with either l-PPA, d-PPA, l-Norpseudoephedrine, d-Norpseudoephedrine (30 mg/kg) or saline (1.0 ml/kg) and returned back to the home cage with no access to food or water. At 1505 hours the second rat was given 15 minutes access to food. Each rat was separated by a 5 minute interval and the schedule for feeding time and injection were in the same manner as for rat

number 1. The rats were weighed prior to the 15 minute feeding period. By weighing the rats for the six days prior to the test day, a baseline was established. Due to the fact that all of the rats were approximately of the same weight they were assigned to the treatment groups and control group by randomization. The number of rats per each of the 4 drug groups and 1 saline group was eight. Two hours following injection the rats were sacrificed with an overdose of sodium pentobarbitol (50 mg/ml, ip). The first rat was sacrificed at 1715 hours, the second at 1720 and so on. The stomach was dissected free, blotted dry and weighed to the nearest .01 gram. The stomach contents were then removed and placed in a petri dish, which was then weighed to the nearest .01 gram. The food remaining in the individual feeding cages was weighed. Food intake for the test day was the difference between the weights of the food in and food out. Gastric retention was defined as the proportion between the dry stomach content and the amount of food consumed by each rat.

Results

Figure 1 shows the results of the effects of the isomers of PPA and of Norpseudoephedrine on gastric retention. As demonstrated by this figure there was virtually no statistical difference between d- and l-norpseudoephedrine in their effect on gastric retention ($\underline{f}$ (4,35) = 0, $\underline{P}$ < 0.2). As also indicated by the figure, d- and l-Norpseudoephedrine had only a minimal effect on gastric retention quite comparable to that of saline. In the comparison between d- and l-PPA is where the most interesting difference between the drug groups is found. As shown by the

figure, l-PPA had a tremendous inhibitory effect on gastric retention ($f(4,35) = 2.78, P < 0.01$), indicating that l-PPA is indeed more potent than d-PPA or the other drug groups. There did not appear to be any significant difference between the effect of d-PPA and saline on gastric retention. Neither one of these drugs appeared to inhibit gastric emptying. The most significant difference obtained was between l-PPA and saline, ($f(4,35) = 3.08, P < .01$). It appears that the l-PPA treatment group did indeed retain a sizeable amount of the meal that was consumed.

Discussion

In the present study, the rats treated with saline, l-Norpseudoephedrine, d-Norpseudoephedrine and d-PPA exhibited a normal clearance of the consumed test meal. The rats treated with l-PPA showed the most interesting results with significant gastric retention of the consumed meal.

A data set comparable to that of Experiment 1 does not exist for the action of these drugs on food intake. It was therefore of great interest to evaluate the effects of l-PPA on food intake and to compare the effects with those of l-PPA on gastric retention.

individual home cages. Each home cage contained a wire floor and DACB pad which was changed prior to the 1 hour access to food. At 1430 hours each rat was given a 1 hour access to 10 grams of pellets (Teklad) and a continuous access to tap water during this period. After the 1 hour access to food, the wire floors and DACB pads were taken out of each cage and the remaining food left in the cages was weighed. The difference between the weight of the pellets put into the cages and the weight of the unconsumed food left over after the 1 hour access to food was taken as the amount eaten in grams. On Days 2 thru 7 the schedule remained the same. By weighing the rats for the 7 days prior to the test day, as well as on the test day a baseline was established. The rats were randomly assigned to the 4 treatment groups and 1 vehicle control group. On Day 8 the test day 15 gram portions of pellets were preweighed. The test day began at precisely 1330 hours. All of the rats were weighed prior to the experiment and the cages were cleaned and provided with a fresh DACB pad. The first rat was injected at 1330 with saline (1.0 ml/kg) of either l-PPA, d-PPA, l-Norpseudoephedrine or d-Norpseudoephedrine (30 mg/kg). The next rat was injected at 1332, there was a 2 minute interval between successive injections. At 1400 hours the first rat was given access to food and tap water, the second rat at 1432 and the schedule followed with 2 minute access inervals. There was a 30 minute interval between injection time and access to food. Each rat was given a 2 hour access to food with a continuous access to tap water. Following the experiment the remaining food left in the cages was taken out and weighed. Food

EXPERIMENT 2

Method

Animals

The animals were 39 male Sprague-Dawley albino rats (Timco Inc., Houston, Texas) weighing 200-250 grams at the beginning of the experiment. The rats were maintained in a temperature-controlled (23.0°C) colony room under a 24 hour illumination schedule and were individually housed in plastic rodent cages. (Lab Products; Bryan, Texas).

Drugs

The phenylpropanolamine (PPA) and Norpseudoephedrine solutions (30 mg/kg/ml) were prepared prior to injection by dissolving d- and l-PPA and d- and l-Norpseudoephedrine hydrochloride into sterile distilled water. A 0.9% saline solution was also prepared. The l-PPA was obtained from Roehr Chemicals and the d-PPA obtained from Aldrich Chemical Company. The l-Norpseudoephedrine was also obtained from Aldrich Chemical Company and the d-Norpseudoephedrine was obtained from Merz and Co.

Design and Procedure

The rats were maintained in the colony room for 1 week prior to the start of the experiment. The rats were handled daily and given continuous access to pellets (Teklad) and tap water. On Day 0 (Sunday) the rats were deprived of food at 1400 hours but, allowed continuous access to tap water. On Day 1 at 1400 hours the rats were individually weighed and then placed back into the

intake was defined as the difference between the weight of the food put into each individual cage at the beginning of the 2 hour access and the weight of the remaining food left in the cages following the experiment. Food intake is the amount of food consumed in grams.

Results

Figure 2 depicts the effects of the treatment groups on mean group food intake in grams. As indicated by the figure, the saline group and the d-PPA treatment group exhibited a normal amount of grams consumed. The d-Norpseudoephedrine and l-Norpseudoephedrine treatment groups demonstrated a small decline in food intake but these differences were not significant. The most interesting difference found was between the saline group and l-PPA group, ($t(13) = 2.2, \underline{P} < .05$). As shown by the figure, the l-PPA treatment group exhibited a significant reduction in food intake, indicating that l-PPA's effect on food intake is indeed inhibitory.

In the calculation of the data of both Experiments 1 and 2 an Analysis of Variance and individual T-tests were used.

Discussion

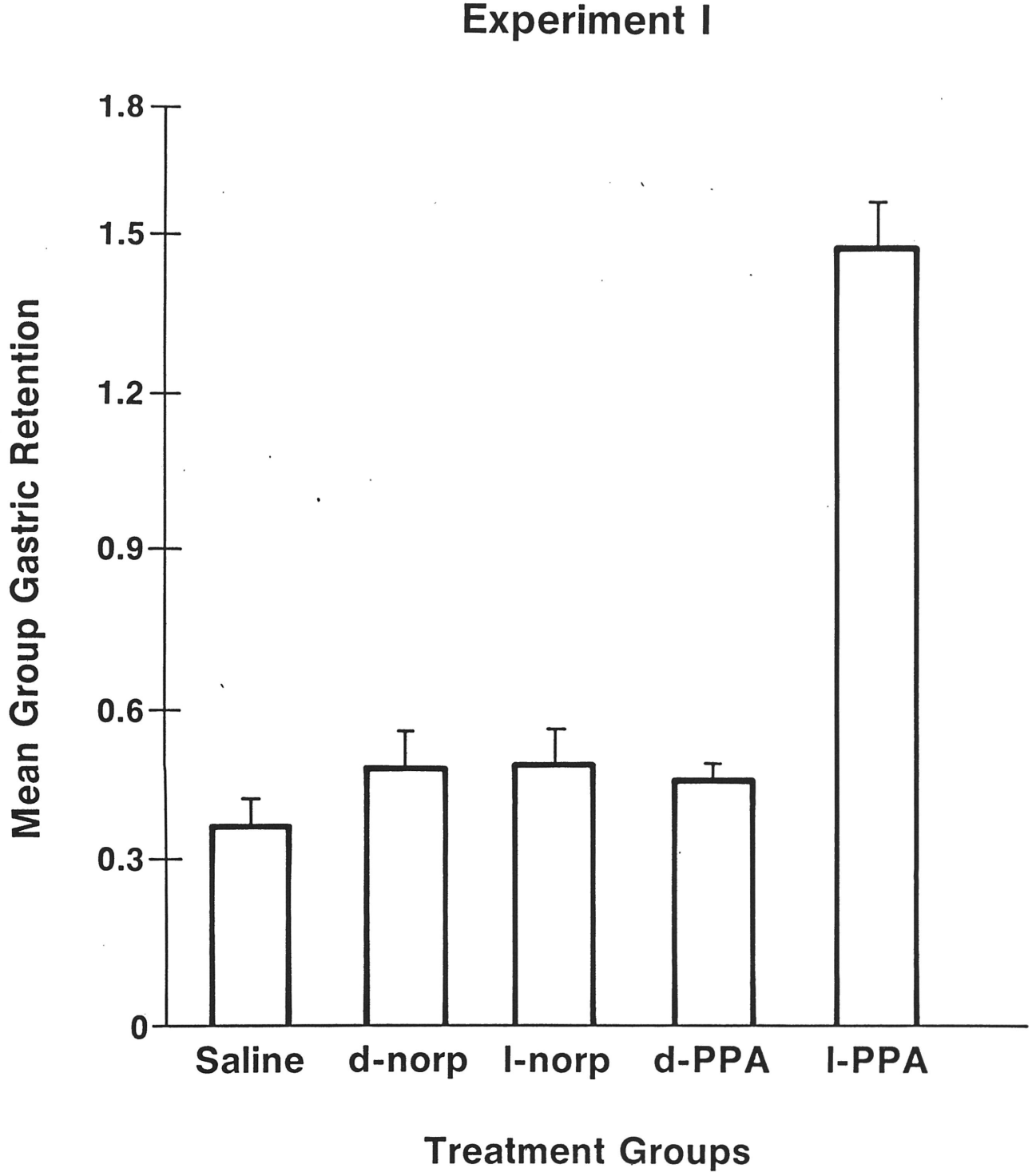
In Experiment 2 the rats treated with d-PPA exhibited a normal amount of food consumption, comparable to that of the saline group. The d-Norpseudoephedrine and l-Norpseudoephedrine treatment groups did not demonstrate a significant decline in food intake. The l-PPA treatment group exhibited a significant reduction in food intake.

In Experiment 1 d-PPA had virtually no effect on gastric retention and as demonstrated by Experiment 2 d-PPA had virtually no effect on food intake. The Norpseudoephedrine treatment groups in Experiment 1 did not show a marked decline in gastric retention nor did they exhibit a marked decline of food intake in Experiment 2. In both Experiments 1 and 2 the most interesting differences found were between the saline groups and the l-PPA groups. Both experiments are consistent with one another in l-PPA's potent effect on gastric retention and reduction of food intake. The data from both of these experiments suggests that there is a relationship between the action of these drugs on gastric retention and food intake. The results of these two experiments is conclusive with the hypothesis that a correlation between the effects of these isomers on gastric retention and food intake does indeed exist. The results of these two experiments are consistent with the study done by Eisenberg, Silverman and Maher (unpublished findings) that demonstrates the different clinical and pharmacological effects of Norephedrine and Norpseudoephedrine. The findings of these two experiments support Morgan's (1985) report on the pharmacologically and clinically distinct isomers of PPA. Experiment 1 (gastric retention) and Experiment 2 (food intake) confirms the notion that isomers and congeners have different clinical effects.

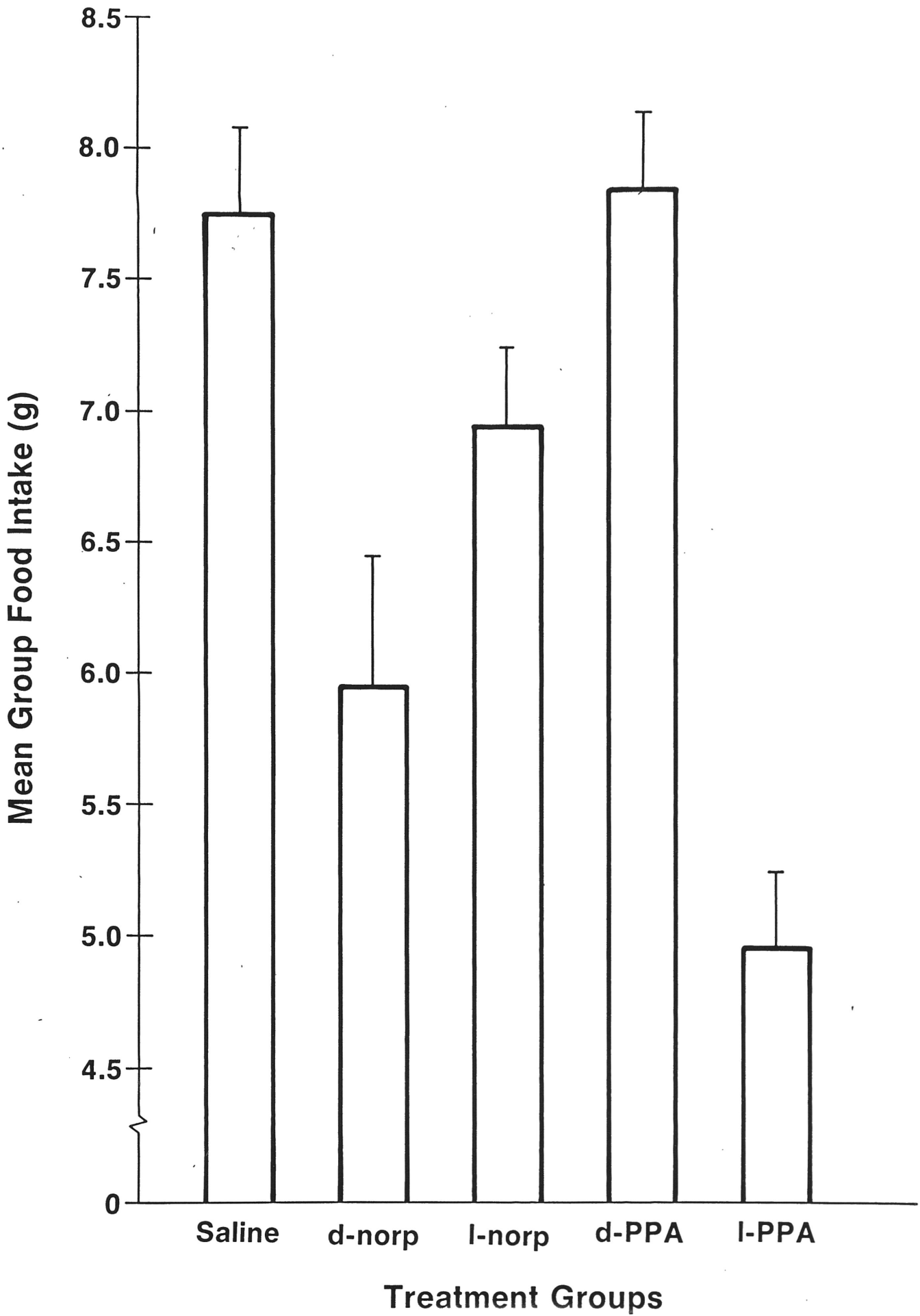
FIGURE CAPTIONS

Figure 1. Mean group gastric retention levels between reatment groups in rats treated with either 1.0 ml/kg saline or 30 mg/kg of l-PPA, d-PPA, l-Norpseudoephedrine or d-Norpseudoepehdrine.

Figure 2. Mean group food intake levels in grams consumed between treatment groups in rats treated with either 1.0 ml/kg saline or 30 mg/kg of l-PPA, d-PPA, l-Norpseudoephedrine or d-Norpseudoephedrine.


Experiment I
1.8
1.5
1.2
0.9
0.6
0.3
0
Mean Group Gastric Retention
Saline
d-norp
l-norp
d-PPA
l-PPA
Treatment Groups

Experiment II



References

Bridges, J. W.; Dent, J. G.; and Johnson, P. (1975). The effect of some pharmacologically active amines on the rate of gastric emptying in rats. Life Sciences, 18, 97-108.

Cole, S. O. (1978). Interaction of food deprivation with different measures of amphetamine effects. Pharmacology Biochemistry and Behavior, 10, 235-238.

Davies, R. F.; Rossi III, J.; Pankseep, J.; Bear, N. J.; and Lolovick, A. J. (1983). Fenfluramine anorexia: a peripheral locus of action. Physioloy and Behavior, 30, 723-730.

Eisenberg, M. S.; Silverman, H. I.; and Maher, T. J. (unpublished findings). A comparison of the effects of phenylpropanolamine, d-amphetamine and d-norpseudoephedrine on green-field locomotion and food intake in the rat.

Goodman, R. P.; Wright, J. T.; Barlascini, C. O.; McKenney, J. M.; and Lambert, R. N. (1986). The effect of phenylpropanolamine on ambulatory blood pressure. Clinical Pharmacology Therapy, 40, 144-147.

Grinker, J. A.; Drewnowski, A.; Enns, M.; and Kissilef, H. (1979). Effects of d-amphetamine and fenfluramine on feeding patterns and activity of obese and lean Zucker rats. Pharmacology Biochemistry and Behavior, 12, 265-275.

Kararek, R. B.; Ho, L.; and Meade, R. G. (1980). Sustained decrease in fat consumption produced by amphetamine in rats maintained on a dietary self-selection regime. Pharmacology Biochemistry and Behavior, 14, 539-542.

Lake, C. R.; Alagna, S. W.; Quirk, R. S.; Moriarty, K. M.; and Reid, A. A. (1985). Does phenylpropanolamine cause psychiatric disorders? Clinical Pharmacology and Therapeutics Series, 5, 285-314.

Morgan, J. P. (1985). Norephedrine and Norpseudoephedrine: Pharmacologically and Clinically Distinct Isomers of Phenylpropanolamine. Clinical Pharmacology and Therapeutics Series, 5, 180-208.

Rowland, N. and Carlton, J. (1984). Inhibition of Gastric Emptying by Peripheral and Central Fenfluramine in Rats: Correlation with Anorexia. Life Sciences, 34, 2495-2499.

Wellman, P. J.; Arasteh, K.; Ruddle, J. R.; and Strickland, M. (1986). Effects of Phenylpropanolamine on Gastric Emptying in the Adult Rat. Brain Research Bulletin, 2-10.

Wellman, P. J.; Malpas, P. B.; and Wikler, K. C. (1981). Conditioned taste aversion and unconditioned suppression of water intake induced by phenylpropanolamine in rats. Physiological Psychology, 9, 203-207.

Wellman, P. J. and Sellers, T. L. (1985). Weight Loss Induced by Chronic Phenylpropanolamine: Anorexia and Brown Adipose Tissue Thermogenesis. Pharmacology Biochemistry and Behavior, 24, 605-611.