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The Effects of Phenylpropanolamine on Zucker Rats Selected for Fat Food Preference

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Treatments of human and rodent obesity frequently involve administration of amphetamine derivatives, much like phenylpropanolamine, which suppress food intake. The Zucker rat is a commonly employed model of youth-onset obesity in which the homozygous genotype manifests hyperphagia as well as other characteristics that parallel human obesity. Using a macronutrient selection procedure, we examined phenylpropanolamine's differential actions in controlling dietary intake, spontaneous open-field activity, and regional hypothalamic neurotransmitter levels in obese female Zucker rats of varying fat food preference. We hypothesized that phenylpropanolamine would alter hypothalamic monoamine levels differently in low-fat preferring and high-fat preferring Zucker rats, and hence affect feeding behavior and activity differently in these two groups. It was found that in high-fat preferring animals, phenylpropanolamine significantly decreased spontaneous open-field activity, decreased only carbohydrate caloric intake, and increased serotonin and 5-HIAA levels in the paraventricular nucleus (PVN). In low-fat preferring animals, phenylpropanolamine decreased carbohydrate, protein, and total caloric intake, had no significant effect of spontaneous activity, and increased serotonin and 5-hydroxyindole acetic acid levels in the PVN. Inherent and induced physiological differences of low-fat and high-fat preferring animals are discussed as well as phenylpropanolamine's potential in combination drug therapy for the treatment of human hyperphagic obesity.

Keywords: Appetite; Macronutrients; Obesity; PPA; Zucker

INTRODUCTION

Obesity has long been recognized as a contributing factor to many age-related diseases, such as hypertension and diabetes, as well as a significant social problem that extends beyond the consequences of health (Warwick and Schiffman, 1992; Schwartz, 1997). Many strategies have been employed to either prevent or diminish obesity including exercise regimens, restrictive diets and appetite suppressants. The anorectic method for the treatment of obesity has been the most readily accepted and represents the administration of a variety of amphetamine derivatives, including phenylpropanolamine (PPA) (Hoebel, 1978).

It is very clear that three key areas of the regional hypothalamus are involved in the central regulation of obesity in rats (Bray and York, 1998). The paraventricular nucleus (PVN) and ventromedial hypothalamus (VMH), if lesioned, can cause obesity. Adrenalectomy can reverse or ameliorate these obese states. Likewise, the lateral hypothalamus (LH) is an area involved in regulation of caloric intake and the central sympathetic nervous system (McMahon and Wellman, 1996). Drugs which act via serotonergic and/or catecholaminergic pathways are certain to exert some of their action by altering monoamines in one or more of these regions (Davies *et al.* 1993; McMahon and Wellman, 1996). In addition, amphetamine derivatives not only affect feeding behavior and consumption, but they also influence activity levels (e.g. spontaneous locomotion, Wellman, 1990), which might indirectly influence appetite and/or weight gain.

The beta-phenethylamine PPA is a racemic mixture of D- and L-norephedrine that suppresses food intake in both murine models and humans. Once believed to have appetite suppressive action within the LH

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(Hoebel *et al.*, 1975; Hoebel, 1978), numerous studies with male Sprague-Dawley albino rats now suggest that PPA's primary anorectic actions take place within the hypothalamic PVN (Wellman and Davies, 1991b; Wellaman *et al.* 1993). Intra-PVN microinjections of PPA (Wellman and Davies, 1990) as well as the inactivation of α1-adrenoceptors within the PVN (Wellman and Davies, 1991a) provide strong evidence for PPA's α1-adrenergic agonist activity within the rat PVN. Recently, the use of PPA-containing over-the-counter diet medications has been prohibited by the Federal Drug Administration for human use, although the basis for this recall is still equivocal. Research on this pharmaceutical agent clearly demands more attention.

While it has been established that PPA is an effective appetite suppressant in the albino rat and mouse, no evidence exists for PPA's effects in the genetically obese rat. The Zucker rat is a frequently used model of youth-onset obesity in which the homozygous genotypes manifests hyperphagia, increased adiposity, hyperinsulinism, hypertriglyceridemia, low growth hormone, hyperleptinemia and an abnormal sympathetic nervous system (Svec and Porter, 1996; Bray and York, 1998). This animal model provides for a unique way to test the effects of drugs that are known to alter the central autonomic nervous system neurotransmitters or monoaminergic neurotransmitters (Svec and Porter, 1996; 1997). Our own laboratory has been very active in demonstrating the neuroactive action of dehydroepiandrosterone (DHEA), fenfluramine, and other hormonal and pharmacological anorexiants in an attempt to understand better the factors controlling appetite suppression and food selection (Svec *et al.*, 1994; Hargrave *et al.*, 1997; Gillen *et al.*, 1999). Our specific approach has been to present Zucker rats with separate bowls of nearly pure macronutrients and allow them to choose *ad libitum* (Mullen and Martin, 1992; Abadie *et al.* 1993). This approach provides a means of monitoring the effects of exogenous agents on macronutrient selection as well as caloric intake and open-field activity. Animal's fat food preferences can be determined, allowing us to correlate the inherent and induced feeding behavior with the hypothalamic nonoamine levels of obese and lean, low-fat preferring, and high-fat preferring animals.

Those who consume high-fat diets are at the greatest risk for gaining weight and thus, are most susceptible to the health problems that result from obesity (Warwick and Schiffman, 1992; Schwartz, 1997). When provided with a choice between fats, proteins, and carbohydrates, Zucker rats, like most humans, tend to consume substantial amounts of fat and thus, gain more weight (Warwick and Schiffman, 1992; Svec *et al.*, 1994). As we have clearly demonstrated in the past (Svec *et al.*, 1994; Svec and Porter, 1996; 1997), these animals indeed demonstrate particular preferences for different amounts of fat in their diets. By determining those animals that prefer very high-fat diets as well as those that do not, we can more appropriately test the effects of exogenous agents on diet consumption and the neurotransmitters levels that influence diet selection and consumption.

High and low fat preference goes across genotypes of Zucker rats. The aim of this present study is to examine the role of PPA in the alteration of macronutrient intake in obese female Zucker rats in a meal fed paradigm. Rats were selected for fat preference by screening for high and low fat preferences before beginning the experiments. PPA clearly interacts with catechoaminergic systems and these systems are clearly related to the control of fat intake. Using a high fat diet and macronutrient selection procedures, PPA's differential actions in controlling dietary intake, spontaneous open-field activity, and regional hypothalamic neurotransmitters levels in obese female Zucker rats were examined. We hypothesize that PPA, an alpha adrenergic agonist, can decrease catecholamine levels by receptor autofeedback mechanisms. In addition serotonin or its metabolites may be increased by the alpha adrenergic agonist by a direct in series connection to catechoaminergic neurons. Since these monoaminergic systems affect feeding behavior, we hypothesize that feeding behavior and spontaneous activity differently in low-fat preferring and high-fat preferring Zucker rats may be low in low fat preferring rats compared to high fat preferring rats. It is proposed that these studies will help elucidate the actions of PPA in the genetically obese rat that prefers high fat over low fat.

MATERIALS AND METHODS

Animals

Zucker rats were obtained from our breeding colony maintained in the Department Physiology at the LSU Dental School. All animals were five to seven month old obese females (fa/fa) derived from crosses of obese males (fa/fa) and heterozygous lean females (FA/fa). All animals were housed under controlled lighting (12 h light/dark schedule) and temperature (22 ± 1°C) conditions. Each was fed Purina laboratory chow in its pelleted form before beginning all experiments and water was provided *ad libitum*. During each experiment animals were housed in individual wire mesh cages. Animal weights were determined via a triple beam balance and used as the basis for the amount of any drug given. All animal use was pre-approved by the Animal

Care and Use Committee of Louisiana State University Health Sciences Center.

Reagents

Phenylpropanolamine hydrochloride (±-Norephedrine; PPA) was obtained from Sigma Chemical Co., St. Louis, MO. Each solution of PPA was obtained by dissolving the corresponding amount of PPA salt into sterile 0.9% sodium chloride (Baxter Healthcare Corp., Deerfield, IL), resulting in a solution of milligrams of PPA salt per milliliter of vehicle. A new sterile solution was utilized in each of the experiments. Injections were given into the peritoneal cavity (ip) as mg of PPA per kg of rat body weight.

Diets

Purina Rodent Laboratory Chow #5001 (Ralston-Purina, St. Louis, MO) was the diet consumed by all rats prior to all experiments. The caloric equivalent of this diet, according to the manufacturer, is 3.3 cal/gm (59.4% of calories from carbohydrate, 28.4% from protein and 12.3% from fat). This standard chow diet was used in part of experiment three. During all experiments, water was provided *ad libitum*.

A high fat diet was used in experiments one, two and three. The diet was prepared according to the method of Grossman *et al.* (1998) as adapted from Warwick and Schiffman (1992). The caloric density of this diet is 5.62 cal/gm and the percentages by kilocalories for protein, carbohydrate and fat are 12.3, 11.5 and 76.1, respectively.

For experiments four and six each rat was given a three bowl macronutrient selection diet. The three macronutrients included a bowl of mostly carbohydrate (90% carbohydrate, 5% fat, 5% protein), fat (90% fat, 5% carbohydrate, 5% protein) or protein (90% protein, 5% carbohydrate, 5% fat). Each was fortified with vitamins and minerals. The specific composition of this diet has been published previously (Abadie *et al.*, 1993). The individual feeding bowls were fastened to one side of the wire mesh cages via springs in order to prevent spillage. Bowls containing powdered diet (e.g. carbohydrate and protein) were also equipped with lids with one hole in the middle through which the animals could readily access the food. This method has been used extensively in our lab and elsewhere (Mullen and Martin, 1992; Abadie *et al.*, 1993) and significantly minimizes spillage. If spillage did occur, the food was collected off the paper sheets underneath the hanging cages and was accounted for in the final caloric intake measures.

Experiment 1—Caloric Intake of PPA-treated Rats on High Fat Diet

Twenty-four obese female Zucker rats were accommodated to the single bowl high fat diet for one week. Prior to the experiment the animals were fasted overnight, weighed and divided randomly into either saline (control) or PPA treatment groups. The animals were then given the single bowl, high fat diet. The intervals between injection and initial food bowl presentation (Post injection time), were 15, 30, 45 and 60 min. Two control rats received saline ip and four rats received 10 mg/kg PPA ip for each represented post injection time. The dosage of 10 mg/kg PPA was selected for use in this experiment based on our laboratory experience in which 10 mg/kg PPA was an effective dosage well tolerated by the animals. The specific dose-response of PPA in female Zucker rats is examined in experiment 2. All food bowls were weighed prior to the experiment and total caloric intake was measured over 4 × 15 min intervals (60 min total) by weighing each food bowl, recording the grams consumed, and calculating the caloric equivalent for the high fat diet.

Experiment 2—Dose Response of Rats on High Fat Diet

Twenty animals were accommodated to the high fat diet for 4 days. Prior to the experiment the animals were fasted overnight, weighed and divided randomly into five treatment groups according to dosage of PPA ($n = 4$ for each dosage) given ip. The dosages used were 0, 0.1, 1.0, 5.0 and 10.0 mg/kg. A 15-min time interval between injection and food presentation was used for all animals based on the results of experiment 1. All food bowls were weighed prior to the experiment and total caloric intake measured after 15 min by weighing each food bowl, recording the grams consumed and calculating the caloric equivalent for the high fat diet.

Experiment 3—Activity of Rats on either High Fat Diet or Chow as a function of PPA Dose

Twenty animals were accommodated to the high fat diet for 4 days. Prior to the experiment the animals were fasted and divided randomly into five treatment groups according to dosage of PPA ($n = 4$ for each dosage). Animals were weighed, accommodated to the experiment room, injected ip with a post injection time of 15 min (determined from experiment 1), and allowed to accommodate to the soundproof activity chamber for 5 min before recording spontaneous activity as described previously (Nguyen *et al.*, 1999). An accommodation time of 5 min was used based on previous experience

that more than 5 min of accommodation does not significantly alter results (Nguyen *et al.*, In press). Post injection time interval represents time interval between injection and initial activity measurement. Open-field spontaneous activity was recorded for 15 min. The same 20 rats were next placed on the standard chow diet for 4 days, a length of time found to be more than adequate for re-adjusting to the diet based on previous experience in our laboratory (Abadie *et al.*, 1993). Thus, no temporal effect due to diet differences was expected. The experiment was then repeated with the 20 animals as described above.

Experiment 4—PPA's Effects on Macronutrient Caloric Intake in High- vs. Low-fat Preferring Animals

The fat food preference of 20 obese female zucker rats was determined via macronutrient selection procedures over the course of one week. Three food dishes corresponding to the three macronutrient components were attached to the cage via springs to prevent spillage. Each bowl was weighed prior to being put in the animal's cage. The rats were allowed to feed for 1 h a day after which the three bowls were weighed and the differences recorded in grams consumed. These amounts were then converted into caloric equivalent of each macronutrient (protein = 3.7 cal/g; carbohydrate = 3.8 cal/g; fat = 6.8 cal/g) (Abadie *et al.*, 1993). Linear regression analysis was used to correlate % fat of total calories for days two and three of the selection and resulted in a correlation coefficient (R^2) value of 0.80. The 5 animals that consistently chose the highest and 5 that consistently chose the lowest percentage of fat were selected for further use. Animals were maintained on the macronutrient diet until being fasted overnight before the experiment. Animals were weighed and injected ip with 5.0 mg/kg PPA (the minimum effective dosage determined in experiments 2 and 3). Fifteen minutes later food bowls were given. All bowls were weighed prior to the experiment and fat, carbohydrate, protein and total caloric intake was measured after 15 min by weighing each food bowl, recording the grams consumed, and calculating the caloric equivalent for each macronutrient.

Experiment 5—Activity of High- vs. Low-fat Preferring Rats on Macronutrient Diet

The same 5 high and 5 low fat-preferring animals utilized in experiment 4 were evaluated for activity levels. The same rats were utilized because their particular preferences for fat-intake were definitively known from the previous experiment. Animals were subsequently maintained on macronutrient diet until fasted overnight before the experiment. Animals were accommodated to the experiment room, weighed, injected with saline control, and allowed to accommodate to the soundproof activity chamber for 5 min as described previously from our lab (Nguyen *et al.*, 1999). Open-field spontaneous activity was then recorded for 15 min. A 15-min post injection time interval was used for all animals. These 10 animals were next maintained on the macronutrient diet for 4 days, after which the experiment was repeated using 5.0 mg/kg PPA ip instead of saline. Drug order effects were assumed to be non-significant owing to the fact that the animals first received only saline ip, followed 4 days later by PPA. This particular design was felt to be most appropriate as it would allow for any wash-out of potential temporal effects of saline injection.

Experiment 6—Neurotransmitter Studies

The fat food preference of 40 animals was determined via macronutrient selection procedures over the course of one week. Linear regression analysis was used to correlate % fat of total calories for days 2 and 3 and resulted in a R^2 value of 0.83. The 15 animals, which consistently chose the highest and 15 which consistently chose the lowest fat food preference were selected for use. Of the 15 highest fat-preferring animals, 5 animals were chosen at random for either 5.0 mg/kg PPA or saline control (two groups of 5; total of 10 animals). The same procedure was used for the low-fat preferring animals (two groups of 5; total of 10 animals). Animals were maintained on the macronutrient diet until fasted overnight before the experiment. The next morning the animals were injected ip. Fifteen minutes later the animals were sacrificed via rapid decapitation. Immediately following decapitation, the brains were removed and frozen whole in liquid nitrogen. Two 1-mm coronal sections were made posterior to the optic chiasm according to the method of McLaughlin *et al.* (1986) as modified in our laboratory (McLaughlin *et al*. 1986; Abadie *et al.*, 1993). Punches of the rostral and caudal sections corresponding to the following hypothalamic regional nuclei and terminal fields were used: PVN, LH and VMH. Areas corresponding to the Raphe were also isolated in each animal.

Each tissue sample was weighed to the nearest 0.1 mg and homogenized in a mobile phase buffer of 0.1 M citrate, 10% ethanol and 250 mg/l sodium octyl sulfate at pH4. Each homogenate was centrifuged at 12000 rpm in a RC2B Sorvall centrifuge. The supernatant was then frozen at 80° until assayed. Dual Ranin HP pumps provided a flow rate of 1.5 ml/min, sample injection was accomplished using an Alcott model 728 autosampler, and electrochemical detection of chromatographically

separated monoamines was carried out by an ESA model 5100 Coulchem multielectrode system as described in previous publications (Hargrave *et al.*, 1997; Porter and Svec, 1998). Amounts of norepinephrine (NE), epinephrine (EPI), dopamine (DA), 5-hydroxytryptamine (5HT-serotonin) and 5-hydroxyindoleacetic acid (5-HIAA) were determined for each section and expressed as ng of monoamine per mg of tissue wet weight. Chromatograms were reprocessed using Rainin Dynamax software for Macintosh.

Statistical Analyses

Overall significance ($p < 0.05$) as well as treatment and phenotype effects on group differences were assessed utilizing the program SuperAnova or Statview (Abacus Concepts, Berkeley, CA) by one-way and multiple analyses of variance (ANOVA) and Fisher's Protected LSD, respectively. All values reported are group averages ± SEM.

RESULTS

Experiment 1

The total caloric intake of 24 animals, receiving either saline (control) or 10 mg/kg PPA, on the high fat diet was measured to determine optimal post injection time and feeding interval length. Of the total 1 h feeding period measured in 4 × 15 min intervals, all animals consumed the overwhelming majority of their total calories within the first 15 min (results not shown). Figure 1 shows only this first 15 min feeding period. Those animals treated with 10 mg/kg PPA demonstrated significantly lower total caloric intake relative to their saline control counterparts at each respective post injection time interval. The most notable effects of PPA in decreasing total caloric intake took place with a 15-min post injection time. However, PPA had a significant effect in reducing total caloric intake all four post injection time intervals.

Experiment 2

The dose response of PPA was determined with 20 obese animals on the high fat diet after a 15-min post injection time and a 15-min feeding period. Figure 2 shows that animals receiving either 5 or 10 mg/kg PPA ($n = 5$ for each group) had significantly lower total caloric intake than those animals receiving saline vehicle. There was no significant difference between those animals receiving either 5 or 10 mg/kg nor was there a significant difference between those animals receiving 0, 0.1, or 1.0 mg/kg. Based on this evidence, the minimum effective dose of PPA in

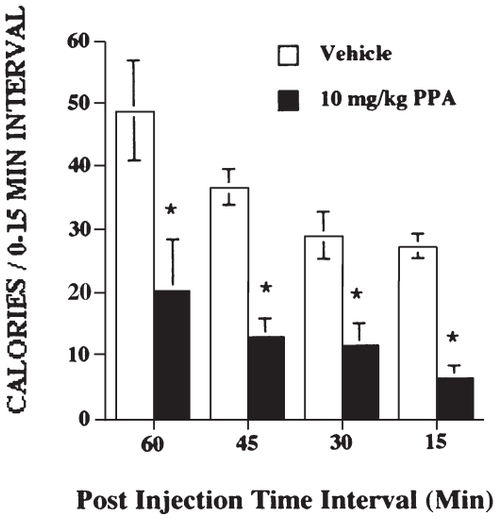

FIGURE 1 Caloric intake of obese female Zucker rats in the first 15 min feeding interval as a function of post injection time. Twenty-four obese female Zucker rats, six to seven months old, were placed on the high fat diet one week. Animals were fasted overnight and then divided into either saline control or treatment groups (10 mg/kg PPA ip). Total caloric intake was measured over four 15 min feeding intervals (60 min total). Results are presented for only the first 15 min feeding interval. Post injection time (min) is the time interval between injection and bowl presentation. Values represent mean total calories ± standard error of mean for each group. Asterisk (*) indicates significant different from same vehicle control group at $p < 0.05$.

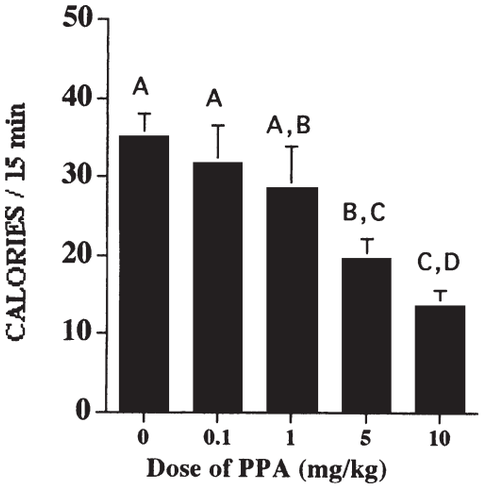

FIGURE 2 Dose response of PPA (ip) in obese female Zucker rats on the high fat diet. Twenty obese female Zucker rats, five to six months old, were placed on the high fat diet for 4 days. Animals were fasted overnight and then divided into five treatment groups according to dosage of PPA ($N = 4$ each dosage). Total caloric intake was measured over the first 15 min. A 15-min post injection time interval was used for all animals. Post injection time (min) is the time interval between injection and initial bowl presentation. Values represent mean total calories ± standard error of mean for each group. Columns sharing the same letter are not significantly different at a level of $p < 0.05$.

obese female Zucker rats to decrease significantly caloric intake when on a high fat diet was determined to be 5.0 mg/kg. Therefore, this dosage was the only one used throughout the remaining experiments (with the exception of experiment 3).

Experiment 3

The open-field spontaneous activities of obese female animals on either the high fat or standard chow diets were determined following injection of varying doses of PPA. The top graph of Fig. 3 shows that, on a high fat diet, animals that received either 5.0 or 10.0 mg/kg had significantly lower activities relative to the animals that received either 0, 0.1, or 1.0 kg/mg. The bottom of Fig. 3 shows that these same animals, when on a standard chow diet, have the same response to PPA as they did on the high fat diet. Diet did not significantly alter the dose-response of PPA in any of the animals. Activity was not significantly lower from baseline (saline control) until 5.0 or 10.0 mg/kg PPA was administered. This confirms the results from experiment 2 that suggest that 5.0 mg/kg PPA is the minimum effective dosage and thus, the only dosage used in the remaining experiments.

Experiment 4

PPA's effects on fat, carbohydrate, protein and total caloric intake was recorded in both low and high fat-preferring obese female Zucker rats on a macronutrient selection diet. Figure 4 shows that, for low fat-preferring animals, those that received 5.0 mg/kg PPA ip had significantly lower total, carbohydrate, and protein caloric intake relative to those low-fat preferring animals that received only saline. There was no difference in the amount of fat calories consumed. For high-fat preferring animals, those animals that received 5.0 mg/kg PPA ip had significantly lower carbohydrate caloric intake only; total, fat and protein caloric intakes were the same between the treatment and control groups.

Experiment 5

The open-field spontaneous activity of both low and high fat-preferring animals was determined following injection of 5.0 mg/kg PPA or vehicle only. Figure 5 shows that PPA did not significantly reduce activity level in low fat-preferring animals, but did in high fat-preferring animals. There was no significant difference between the activity levels of low or high fat-preferring animals treated with PPA.

Experiment 6

The effects of PPA on the neurotransmitter levels of both low- and high- fat preferring animals were determined. Table I demonstrates that, in the LH, those low fat-preferring animals that received saline control had, on average, significantly lower levels of DA than the high fat-preferring animals that received saline control. Further, those high fat-preferring animals that received PPA also had significantly lower DA levels than their control counterparts (high fat-preferrers that received saline control).

In the VMH, those low fat-preferring animals that received PPA had significantly higher levels of serotonin (5HT) than the high fat-preferring animals

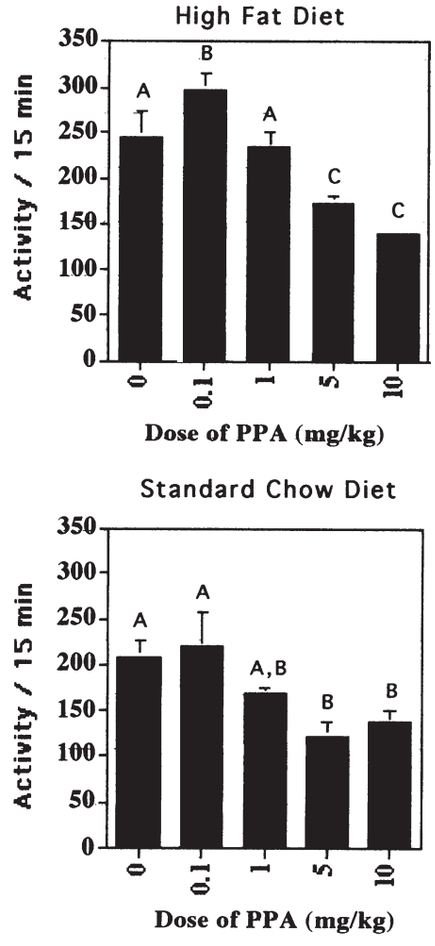

FIGURE 3 Open-field spontaneous activity of obese female Zucker rats on either the high fat or standard chow diets as a function of PPA dosage. Twenty obese female Zucker rats, five to six months old, were placed on the high fat diet for 4 days. Animals were fasted overnight and then divided into five treatment groups according to dosage of PPA ($N = 4$ for each dosage). After accommodating to the sound-proof activity chamber, the animals were measured for their open-field spontaneous activity for 15 min. The same 20 animals were next placed on standard chow diet for 4 days and the experiment was repeated. A 15-min post injection time interval was used for all animals in both experiments. Post injection time (min) is the time interval between injection and initial recording of spontaneous activity. Values represent total activity for 15 min ± standard error of mean for each group. Columns sharing the same letter are not significantly different at a level of $p < 0.05$.

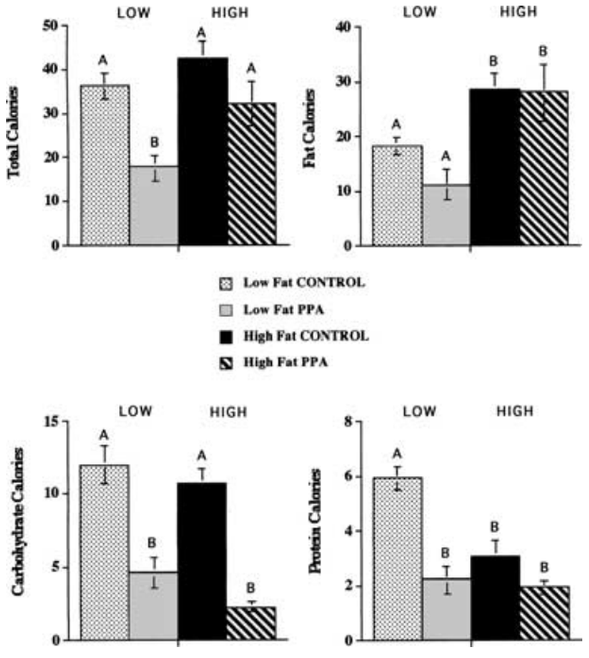

FIGURE 4 Different effect of 5.0 mg/kg PPA (IP) in high vs. low fat-preferring obese female Zucker rats on total, fat, carbohydrate and protein caloric intake. The fat food preference of 20 obese female Zucker rats, five to six months old, was determined through macronutrient selection procedures. The 5 animals, which consistently chose the highest and the 5 that consistently chose the lowest fat food preference were selected for use. The animals were fasted overnight, injected ip with 5.0 mg/kg PPA, and then measured for total, fat, carbohydrate and protein caloric intake for 15 min. A 15-min post injection time interval was used for all animals. Post injection time (min) is the time interval between injection and initial bowl presentation. Values represent mean total calories ± standard error of mean for each group. Columns sharing the same letter are not significantly different at a level of $p < 0.05$.

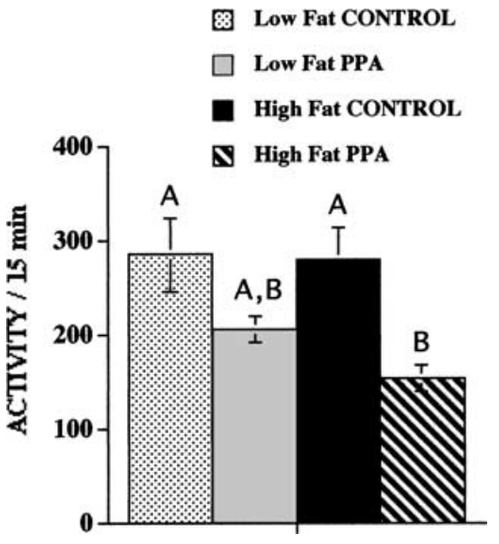

FIGURE 5 Open-field spontaneous activity of high and low fat-preferring obese female Zucker rats on macronutrient diet. The fat food preference of 20 obese female Zucker rats, five to six months old, was determined through macronutrient selection procedures. The 5 animals, which consistently chose the highest and the 5 that consistently chose the lowest fat food preference were selected for use. The animals were fasted overnight and allowed to accommodate to the sound-proof activity chamber prior to the experiment. Open-field spontaneous activity was measured for 15 min. The experiment was then repeated five days later with the same 10 animals after receiving 5.0 mg/kg PPA (IP). A 15-min post injection time interval was used for all animals. Post injection time (min) is the time interval between injection and initial recording of spontaneous activity. Values represent total activity for 15 min ± standard error of mean for each group. Columns sharing the same letter are not significantly different at a level of $p < 0.05$.

that received only saline control. In the PVN, those low-fat preferring animals which received PPA had significantly higher serotonin and 5-HIAA levels than those high-fat preferring animals which received only saline control. Those high-fat preferring animals that received PPA also had significantly higher serotonin and 5-HIAA levels than those high-fat preferring animals which received only saline control.

There were no significant ($p < 0.05$) differences in NE or Epi levels in animals of either high or low-fat preference nor were there any differences in control (saline) vs. PPA-treated animals. Further, monoamine levels in the raphe did not differ between any fat-preference/treatment combination. Thus, NE, EPI and raphe data are not presented in Table I.

DISCUSSION

Obesity is the second most important modifiable health risk in the US; only cigarette smoking is more dangerous. Thus an understanding of the pathophysiology and treatment of obesity is of major public health importance. The Zucker rat presents a model for youth-onset obesity in which the homozygous genotype manifests hyperphagia and other conditions associated with an abnormal sympathetic nervous system, making this model a particularly appropriate one for studying human obesity (Bray, 1977).

Our laboratory has explored extensively the use of a macronutrient meal paradigm in which animals are allowed *ad lib* food selection from separate bowls of nearly pure macronutrients (Abadie *et al.*, 1993; Svec and Porter, 1996). Besides allowing us to monitor the effects of drugs on diet selection, this approach allows us to examine inherent and induced differences between animals preferring a high-fat diet vs. those that prefer a low-fat diet. Prior to all experiments and since weaning, all animals were fed a standard, high-carbohydrate rodent chow that contains only 12.3% of its calories from fat. Our previous studies have shown that when allowed to feed from this macronutrient selection diet, all animals prefer to have a higher intake of fat (average around 50%) (Abadie *et al.*, 1993). Like these rats,

TABLE I Effects of PPA (5.0 mg/kg IP) on neurotransmitter levels in both high- and low-fat preferring animals on macronutrient diet

Brain region	Fat reference	Treatment	Dopamine	Serotonin	5HIAA
LH	Low	Control	**0.164 ± 0.020d**	0.681 ± 0.071	1.022 ± 0.126
		PPA	0.184 ± 0.033	0.756 ± 0.048	1.057 ± 0.049
	High	Control	0.241 ± 0.021	0.751 ± 0.049	0.980 ± 0.060
		PPA	**0.171 ± 0.016c**	0.683 ± 0.055	0.947 ± 0.082
VMH	Low	Control	0.811 ± 0.098	0.398 ± 0.054	0.612 ± 0.075
		PPA	0.864 ± 0.103	**0.449 ± 0.036e**	0.947 ± 0.082
	High	Control	0.817 ± 0.220	0.329 ± 0.030	0.485 ± 0.042
		PPA	0.846 ± 0.128	0.343 ± 0.026	0.510 ± 0.035
PVN	Low	Control	0.130 ± 0.018	0.450 ± 0.054	0.722 ± 0.093
		PPA	0.128 ± 0.007	**0.567 ± 0.025e**	**0.835 ± 0.035e**
	High	Control	0.137 ± 0.028	0.371 ± 0.067	0.579 ± 0.091
		PPA	0.146 ± 0.015	**0.563 ± 0.058c**	**0.898 ± 0.073c**

The fat food preference of 40 obese female Zucker rats, five to seven months old, was determined through macronutrient selection procedures. The 15 animals which consistently chose the highest and the 15 which consistently chose the lowest fat preference were selected. Of the 15 highest fat-preferring animals, 5 animals were chosen at random for either 5.0 mg/kg ip injection or saline control (two groups of 5; total of 10 animals). The same procedure was used for the low fat-preferring animals control (two groups of 5; total of 10 animals). Animals were fasted overnight and a 15-min post injection time was used for all animals. Animals were sacrificed by rapid decapitation and the following regional brain areas were immediately removed, weighed and homogenized: paraventricular nucleus (PVN), ventromedial hypothalamus (VMH), lateral hypothalamus (LH) and the raphe (not reported here). Automatic HPLC electrochemical analysis was performed to determine relative concentrations of norepinephrine (NE), epinephrine (EPI), dopamine (DA), 5-hydroxyindolacetic acid (5HIAA) and 5-hydroxytryptamine (5-HT or serotonin). NE and EPI values are not reported here due to non-significant differences in these values between any combination of diet-preference/treatment comparisons. Values represent mean ng/mg sample weight ± standard error of mean for each group. "**c**" indicates significant different between treatment and control groups of same fat preference; "**d**" indicates significant difference between control groups of opposite fat preference; "**e**" indicates significant different between PPA treatment group of low-fat preference and control group of high-fat preference, all at $p < 0.05$.

humans also prefer a high percentage of fat in their total diet, once again making the obese Zucker rat model appropriate for study.

Both total caloric intake and specific diet selection depend upon the hypothalamus (Bray and York, 1998). It is believed that hypothalamic monoamine levels control feeding behavior and diet selection. Drugs which act via serotonergic (e.g. fenfluramine) and catecholaminergic pathways exhibit some of their action by altering these monoamines in one or more of the hypothalamic regions (Hoebel, 1978; Wellman, 1990; Wellman and Davies, 1990; 1991a). In particular, PPA is an anorectic agent that has been studied for its actions within the LH and the hypothalamic PVN of albino and Sherman rats (Hoebel *et al.*, 1975; Wellaman *et al.* 1993). While such actions are identified in the male Sprague-Dawely albino rat, nothing is known about their effects in the Zucker rat model, one that seems to reflect some of the characteristics of human obesity.

We have demonstrated in the preceding experiments that PPA alters feeding behavior in obese female Zucker rats. This adds to the current evidence that PPA is an effective anorectic agent in rodents (Hoebel *et al.*, 1975; Wellman, 1990). In female Sprague-Dawley rats on a self-selection diet, Schwartz and Hoebel (1989) demonstrated that varying doses of PPA significantly decreased all three macronutrients equally. However, at lower doses (2.5 and 5.0 mg/kg), PPA decreased protein and fat intake more so than carbohydrates. Using female obese Zucker rats on high fat (Fig. 2) and self-selection diets, this present study has confirmed that, as originally demonstrated by Schwartz and Hoebel (1989), PPA has an effect on total caloric intake in a dose response manner (Fig. 2). More importantly, PPA differentially alters specific macronutnent intake levels in animals *pre-selected for fat food preference.* In low-fat preferring animals, 5.0 mg/kg PPA decreased carbohydrate, protein and total caloric intake, whereas in the high-fat preferring animals, PPA decreased only carbohydrate caloric intake. Although PPA did not affect fat caloric intake in either group, it clearly affected animals that preferred low-fat diets much more than the high-fat preferring counterparts (Fig. 4).

Previous reports have demonstrated that low doses of PPA ip significantly reduce non-spontaneous locomotor activity in male Long-Evans rats (Mittleman *et al.*, 1993). We have also demonstrated that PPA exhibits differential effects on the behavior of obese female Zucker rats. When animals are placed on a high fat diet, they exhibit the same decrease in open-field spontaneous activity when given varying dosages of PPA as they do when fed the standard rodent chow and given the same doses of PPA. However, when allowed to feed from a macronutrient selection paradigm, the animals which were high-fat preferrers demonstrated this decrease in spontaneous activity while animals which were low-fat preferrers did not, indicating a differential effect of PPA on activity depending upon animal fat preference. Based on these results, we conclude that PPA's effects on activity and food intake are different for high- and low-fat preferring phenotypes of obese female Zucker rats and that, because of these disconnected effects, a decrease in activity is not necessarily the cause of decreased caloric intake.

It has been hypothesized that the anorectic actions of PPA are mediated by the alteration of monoamines in one or more of these regions of the rat hypothalamus (Hoebel *et al.*, 1975; Hoebel, 1978; Wellman and Davies, 1991a; Davies *et al.*, 1993; Wellaman *et al.*, 1993). McMahon and Wellman (1996) have demonstrated that even 20 mg/kg PPA ip did not significantly alter extracellular levels of serotonin in the male Sprague-Dawley albino rat PVN. Thus, PPA possesses α1-adrenergic agonist activity within the albino rat PVN (Wellman and Davies, 1991a), but does not cause appetite suppression via 5-HT release in the PVN (McMahon and Wellman, 1996). However, these conclusions have been made using non-obese animals consuming standard rodent pellets (TekLab). This present study attempted to determine the effects of PPA on the neurotransmitter levels of both high-fat and low-fat preferring obese female Zucker rats in order to examine the inherent and induced monoamine levels of these two phenotypes.

We demonstrate that low-fat preferring animals have significantly lower levels of DA than high-fat preferring animals. PPA induced higher levels of PVN serotonin and 5-HIAA in low and high fat preferring rats. This is in contrast with that of McMahon and Wellman (1996) who found that PPA did not significantly alter extracellular levels of serotonin in the PVN of male Sprague-Dawley albino rats on standard rodent chow. In addition, low-fat preferring animals that received PPA demonstrate a significant increase in serotonin levels relative to those high-fat preferring animals that received only saline control. Therefore, we conclude that, in addition to the inherent monoamine differences between low-fat and high-fat preferring animals in the DA levels of the LH, PPA increases serotonin and 5-HIAA levels in the PVN of both low-fat and high fat-preferring animals. However, this increase in 5HT and 5-HIAA levels is more dramatic in the high-fat preferring animals. Although no conclusion can be drawn from this current study regarding the potential release or re-uptake inhibition of serotonin or its metabolite following PPA administration, others have found that PPA does not significantly alter extracellular levels of serotonin in the albino rat (McMahon and Wellman, 1996). However, no evidence yet exists for PPA's influence on serotonin or 5-HIAA levels in the PVN of obese Zucker rats outside of this current study, suggesting a range of potential questions yet to be answered regarding the neuropharmacology of PPA in this genetically obese model.

The significance of these findings may lie in their application to human obesity. These results indicate that PPA is differentially effective in altering activity and feeding behavior based on diet preference. Currently, studies involving the treatment or prevention of human obesity do not attempt to account for such underlying differences within the study population. Thus, potentially responsive patients may become lost in the statistical analyses of a larger group.

Our laboratory has shown that DHEA tends to have a greater effect on fat food intake in Zucker rats (Svec *et al.*, 1994; Svec and Porter, 1997). Combined with this current study involving PPA's effects in both high- and low-fat preferring animals, these results suggest a unique combination of agents that may be beneficial in treating obesity: PPA to decrease carbohydrate intake and DHEA to decrease fat intake. We have already explored the combination of DHEA and fenfluramine and have found this to be very effective (Gillen *et al.*, 1999). Clearly, more studies must involve such combinations in an attempt to differentially treat those suffering from hyperphagic obesity with the lowest likelihood of causing side effects.

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