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Effects of d-Amphetamine and Fenfluramine on Feeding Patterns and Activity of Obese and Lean Zucker Rats[1,2]

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GRINKER, J. A., A. DREWNOWSKI, M. ENNS AND H. KISSILEFF. *Effects of d-amphetamine and fenfluramine on feeding patterns and activity of obese and lean Zucker rats.* PHARMAC. BIOCHEM. BEHAV. 12(2)265–275, 1980.—The effects of two doses of d-amphetamine and fenfluramine on male Zucker rats maintained ad lib on solid and liquid diets were investigated using the technique of meal pattern analysis. Amphetamine-induced anorexia was of short duration in both obese and lean rats. In the lean rats, anorexia was followed by rebound feeding resulting in little or no reduction in total daily intake. The drug reduced meal sizes of obese but not lean rats and caused a transient decrease in meal frequency. Increased spontaneous activity paralleled the decreased food intake. In contrast, anorexia following fenfluramine was greater, more prolonged and of equivalent magnitude in obese and in lean rats. No rebound feeding was observed. Reduction in intake was achieved primarily by changes in meal size rather than in meal frequency. These data demonstrate that food intakes of genetically obese Zucker rats are more susceptible to the action of d-amphetamine than those of lean rats, and are consistent with reports of differential neurotransmitter levels in the obese and lean rats.

d-Amphetamine Fenfluramine Zucker rat Meal patterns Activity

IN most studies of anorectic drugs, the reduction in food consumed within a fixed time interval has served as the principal measure of the drug's effectiveness. However, such discrete measures of intake tend to mask the temporal profile of drug action and are not sensitive to the drug's effects on the principal parameters of feeding behavior [5,14]. We have therefore employed computerized, on-line data recording techniques [1,14] to examine the effects of two prototypic anorectic drugs: d-amphetamine and fenfluramine, on continuous feeding and activity patterns of Zucker rats.

Our research had a twofold theoretical aim. The primary aim was to establish a unique profile of action for each drug with regard to its temporal effects on food and water intakes, meal parameters and patterns of activity. Previous research [4,5] has suggested that the two drugs have different durations of action and may suppress feeding in one of two ways: by suppressing appetite or by promoting satiety. The technique of meal pattern analysis allows us to investigate the effects of each drug on meal frequency and meal size as well as the patterns of drug action in rats maintained on diets of different nutrient composition and different caloric density.

Our second aim was to use the two drugs as pharmacological probes to study the nature of mechanisms underlying the control of feeding behavior in genetically obese and lean Zucker rats. Although the genetically obese Zucker rat is generally regarded as one of the most appropriate models of juvenile onset human obesity [23], little is known about its response to the commonly used anorectic drugs. Results of one study [8] suggest that the obese rat adapted to a 5 hr feeding schedule may be more resistant than the lean to the action of amphetamine. However, deprived obese Zucker rats have been reported to be as responsive as lean rats to low doses of amphetamine [21]. Further evidence suggests that Zucker obese rats are slower than the lean to recover from chronic drug-induced anorexia [14] and to adapt to a calorically more dilute diet [10]. We wished to test the hypothesis that control of intake in the genetically obese Zucker rat is weaker or less precise than that in the lean rat, and that the control system is accordingly more susceptible

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to external perturbation and slower to recover from its effects.

EXPERIMENT 1

We first investigated the effects of two doses of amphetamine and fenfluramine on the feeding patterns of Zucker rats maintained on a solid diet of 45 mg Noyes pellets and water. In addition to measuring the effects of each drug on daily food and water consumption, we monitored the temporal profiles of drug action on food intakes and on the principal parameters of feeding behavior, including meal frequency and meal size. Furthermore, since amphetamine is reported to be a stimulant and fenfluramine a sedative [13, 16, 17], we monitored the rats' concomitant running-wheel activity.

METHOD

Animals

Six genetically obese (fa/fa) and five lean (Fa/−) male Zucker rats were obtained from the breeding colony at the Biology Department at Vassar College. Obese rats weighed a mean of 729.3 g (range 667–803 g) and lean rats weighed a mean of 414.0 g (range 367–438 g). Prior to the experiment, the rats had been accustomed to a diet of 45 mg Noyes pellets and water (see [14]). This diet has a caloric concentration of 3.6 kcal/g, and its nutrient composition, according to the manufacturer's specifications is 52.9% carbohydrate, 23.9% protein and 5.6% fat. The rats had free access to water at all times.

Apparatus

The rats were individually housed in LC34 Wahmann activity wheels and cages, placed in noise-attenuating chambers, under a computer-controlled light/dark cycle (lights on 6 a.m. to 6 p.m.) at a constant temperature of 68°F. Throughout the experiment two different food delivery systems were used in conjunction with the standard Grayson-Stadler pellet dispenser: photobeam-triggered eatometers, in which each new pellet was delivered one second following pellet removal [18], and contact-activated BCI food cups: shallow chambers into which a pellet was dispensed when the animal touched a metal baffle hanging in front of the chamber. The delivery systems were connected to a PDP-8 computer via a solid state digital I/O interface constructed at the Rockefeller University. The total number of pellets consumed was measured daily, and water intakes were read from 100 ml graduated cylinders. The delivery of each pellet and each revolution of the activity wheel were continuously monitored on an on-line computer. Daily records of six animals (3 obese and 3 lean) were recorded at one time. Subsequent analyses of the temporal course of intake and the parameters of feeding behavior were carried out on a PDP-10 computer at the University of Pennsylvania [2,18].

Data Collection

The data collection criteria were set as low as possible to prevent elimination of potentially valuable data prior to inspection. For data collection purposes only, a minimum of one pellet dispensed during one minute constituted the threshold criterion for a bout of feeding. The feeding bout was regarded as terminated when no pellets had been dispensed for at least one minute. Similarly, the threshold criterion for a bout of activity was set at one revolution of the running wheel occurring within one minute and the bout was regarded as terminated when no revolutions occurred for at least one minute.

Subsequent analyses of meal parameters, however, are based on the definition of a meal and thus involve a combination of empirical and theoretical considerations (see [1, 14, 18]). Some investigators [2,18] have argued that small bouts of feeding separated by short pauses are to be regarded as single meals, and that meals should be separated from each other by intermeal intervals (IMI) of at least 15 minutes. We have obtained qualitatively similar results using both the 15 minute and the 2 minute IMI criteria [14], with the shorter (2 minute) criterion being more sensitive to drug-induced shifts in meal frequency. Consequently, following a preliminary inspection of the data, we eliminated from consideration all meals below 5 pellets (.22 g) and we used both the 2 and the 15 minute IMI criteria for the subsequent calculations of meal parameters.

Drugs

Both d-amphetamine and fenfluramine were dissolved in sterile 0.9% saline in volumes of 5 mg/ml and 20 mg/ml, respectively. Two doses of d-amphetamine sulphate (1.5 and 3.0 mg/kg) and two doses of fenfluramine hydrochloride (3.0 and 5.0 mg/kg) were used, with the rats receiving equivalent volumes of saline (lean: 0.4 ml; obese: 0.6 ml) on saline control days. The animals were injected between 5:45 and 6:00 p.m. at the beginning of the dark cycle.

Procedure

Following a six day baseline period during which no injections were given, the rats were injected intraperitoneally (IP) with saline for two consecutive days. The rats first received amphetamine and then fenfluramine, with six to 10 days intervening between the two drugs. On the first drug day, the rats received one of the two doses of amphetamine followed by three to five days of recovery during which all rats received saline injections. After recovery, the rats received the second dose of amphetamine. A similar design was used for fenfluramine injections. The order of presentation of drug doses was counterbalanced across rats, with half of the rats receiving the higher dose of each drug first, and half receiving the lower dose first. We used an analysis of variance with repeated measures and with genotype (obese vs lean), condition (saline and two drug doses) and time (8 consecutive 3-hour time periods) as the main variables. Because of the unequal numbers of obese and lean rats, one obese rat of median food intake was dropped from the statistical analysis. Wilcoxon's signed ranked tests were used in analyzing activity data because individual activity levels were highly variable.

RESULTS

Food and Water Intakes

An analysis of intake during baseline and saline conditions showed that saline injections did not significantly affect the temporal pattern of food intake as compared to the baseline condition, $F(2,16)=0.52$; n.s. In addition, during both baseline and saline conditions obese rats ate more than lean rats (genotype, $F(1,8)=22.80$; $p<0.01$) and both sets of

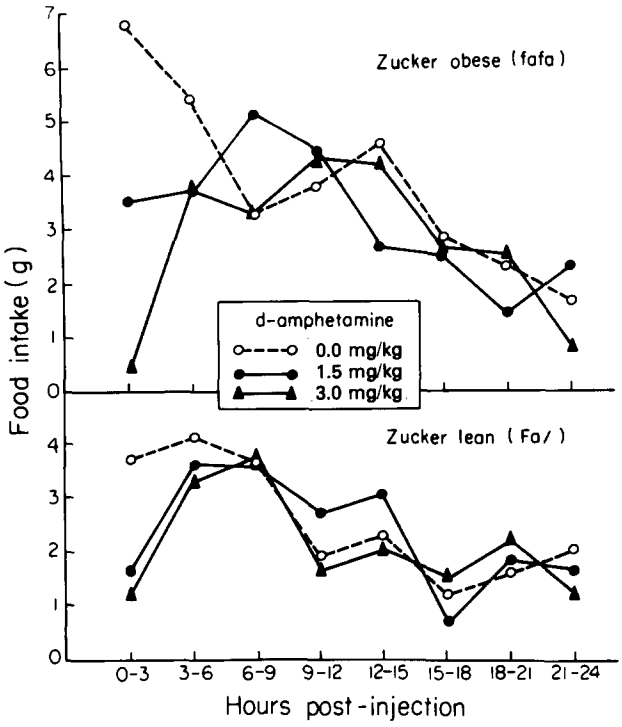

FIG. 1. Temporal effects of administration of d-amphetamine (0.0, 1.5, 3.0 mg/kg) on food intakes of obese (n=6) and (n=6) lean Zucker rats in Experiment 1. Eight consecutive 3-hr periods are shown.

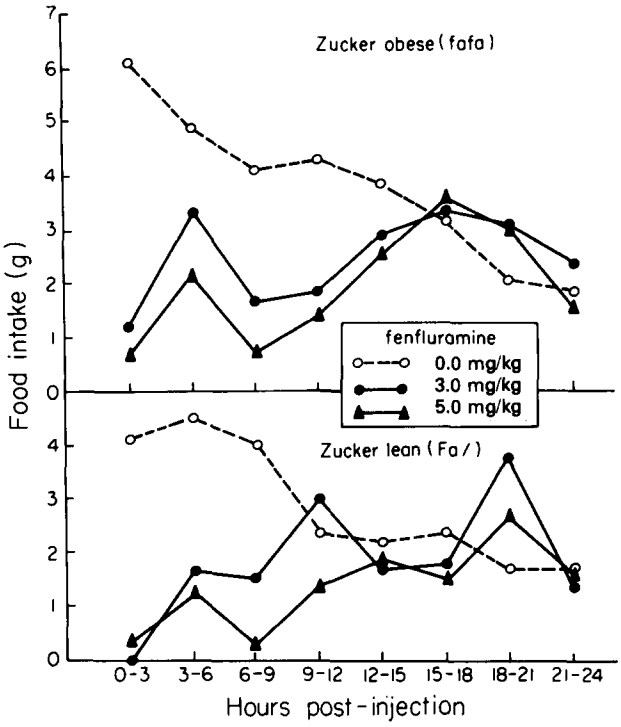

FIG. 2. Temporal effects of administration of fenfluramine (0.0, 3.0, 5.0 mg/kg) on food intake of obese (n=6) and lean (n=6) Zucker rats in Experiment 1. Eight consecutive 3-hr periods are shown.

rats showed a circadian distribution of intake, consuming approximately 65% of their daily intakes during the dark cycle. Either condition thus provides an appropriate control for drug injections, and saline controls were used in Experiment 1.

Amphetamine (averaged over drug dose) reduced the daily food intake of obese rats by 23.0% and that of the lean by 13.8%. Fenfluramine, averaged over drug dose, reduced the intake of obese rats by 40.1% and that of the lean rats by 44.5%. Daily water intakes (averaged over drug dose) were not reduced by amphetamine (7.4% decrease for obese rats; 2.3% increase for lean rats). However, fenfluramine, $F(2,27)=9.00$; $p<0.01$, reduced the daily water intake in a dose-dependent manner consistent with reductions in food intake (obese: 31.4% low dose, 45.1% high dose; lean: 23.7% low dose; 40.3% high dose).

The temporal profile of drug action on food intake was established by measuring the rats' food consumption during eight consecutive three-hour periods following administration of each drug. The temporal course of intake following amphetamine is shown in Fig. 1. The effects of the drug were immediate but relatively short-lived, and full recovery of intake occurred within six hr postinjection. Analysis of variance of intake scores showed significant main effects of drug dose, $F(2,16)=20.75$; $p<0.01$, genotype, $F(1,8)=11.63$; $p<0.01$, and time, $F(7,56)=4.71$; $p<0.01$, as well as a significant drug dose by time interaction, $F(14,112)=4.93$; $p<0.01$. Subsequent planned comparison tests (Dunnett's t [24]) confirmed that a significant suppression in intake occurred only within the initial three hr postinjection ($p<0.05$).

The temporal course of intake following fenfluramine is shown in Fig. 2. Suppression of intake was prolonged and the effects of the drug persisted for up to 12 hr postinjection. Analysis of variance showed significant main effects of drug dose, $F(2,16)=46.08$; $p<0.01$, and genotype, $F(1,8)=9.48$; $p<0.01$, as well as a significant drug dose by time interaction, $F(14,112)=6.77$; $p<0.01$. Subsequent planned comparison tests (Dunnett's t) confirmed that a significant suppression in intake lasted for at least nine hr (12 hr in the obese and nine hr in the lean, $p<0.05$).

Meal Parameters

Because conventional analyses of meal parameters over 12 hr periods may obscure subtle changes occurring during that time, we measured meal frequencies and meal sizes at consecutive three hr intervals following drug administration. Temporal changes in meal frequency and in meal size (2 min IMI) following injections of amphetamine are shown in Figs. 3 and 4, respectively. Obese and lean rats ate approximately equal numbers of meals per day: the main effect of genotype, $F(1,8)=1.07$; n.s., was not significant. The drug transiently decreased the number of meals during the initial three hr postinjection, with recovery occurring between three and six hr. There was no main effect of drug dose, $F(2,16)=2.81$; n.s., but the effect of time, $F(7,56)=4.36$; $p<0.01$, and the drug dose by time interaction, $F(14,112)=3.51$; $p<0.01$, were both significant. The same results were obtained using meal frequencies established on the basis of a 15 min IMI: time,

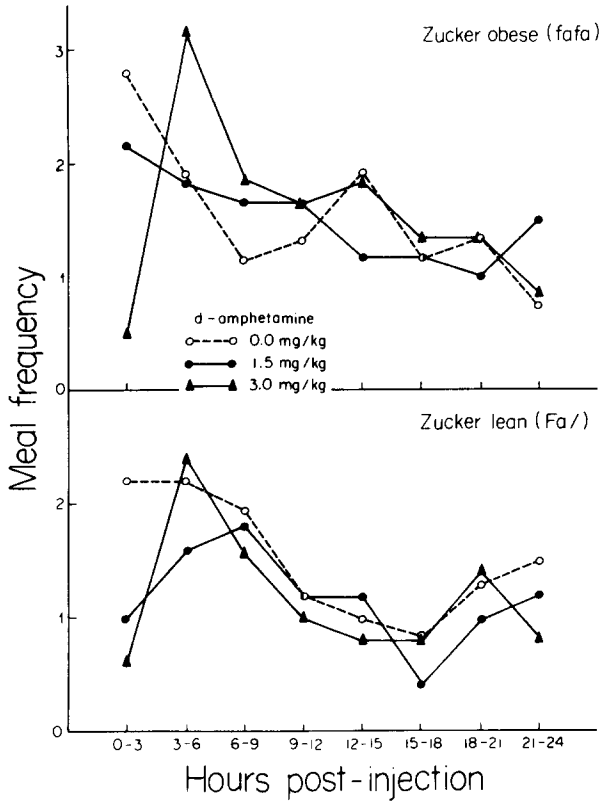

FIG. 3. Temporal effects of d-amphetamine (0.0, 1.5, and 3.0 mg/kg) on meal frequency (2 min IMI) of obese and lean Zucker rats in Experiment 1. Eight consecutive 3-hr periods are shown.

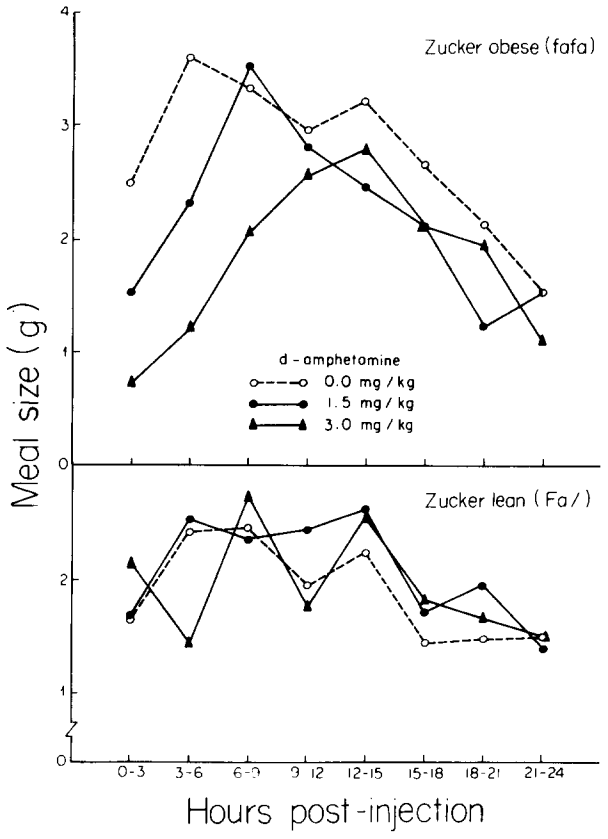

FIG. 4. Temporal effects of d-amphetamine (0.0, 1.5, and 3.0 mg/kg) on average meal size (g) (2 min IMI) of obese and lean Zucker rats in Experiment 1. Eight consecutive 3-hr periods are shown.

$F(7,56)=3.46$; $p<0.01$; drug dose by time, $F(14,112)=3.55$; $p<0.01$.

Data in Fig. 4 show that although amphetamine had little effect on meal sizes of lean rats, its effects on meal sizes of obese rats were both substantial and prolonged. Analysis of variance of meal sizes (2 min IMI) showed significant main effects of drug dose, $F(2,16)=7.83$; $p<0.01$, and time, $F(7,56)=7.54$; $p<0.01$, as well as the genotype by drug dose, $F(2,16)=10.38$; $p<0.01$ and drug dose by time, $F(14,112)=3.31$; $p<0.01$ interactions. A significant second order interaction between genotype, drug dose and time, $F(14,112)=2.79$; $p<0.01$ indicated that the temporal effects of the drug were more pronounced for the obese rats. A corresponding analysis of meal sizes established on the basis of a 15 min IMI produced parallel results.

The effects of fenfluramine on the temporal profiles of meal frequencies (2 min IMI) are shown in Fig. 5. The drug suppressed the meal frequencies of the lean rats for at least 6 hr but failed to suppress meal frequencies of the obese rats. The main effect of drug dose, $F(2,16)=6.10$; $p<0.01$, the dose by time interaction, $F(14,112)=2.81$; $p<0.01$, and the genotype by dose by time interaction, $F(14,112)=2.22$; $p<0.05$, were all significant. Analysis of variance of meal frequencies at 15 min IMI also showed significant main effects of drug dose, $F(2,16)=6.88$; $p<0.01$, as well as dose by time, $F(14,112)=3.08$; $p<0.01$, and genotype by dose by time, $F(14,112)=1.78$; $p<0.05$, interactions.

Data in Fig. 6 show that fenfluramine exerted its principal effect through a large and prolonged (9–12 hr) suppression of meal size. Analysis of variance (2 min IMI) showed significant main effects of drug dose, $F(2,16)=17.52$; $p<0.01$ and time, $F(7,56)=5.86$; $p<0.01$ as well as a significant drug dose by time interaction, $F(14,112)=7.57$; $p<0.01$. Corresponding analyses of meal sizes established with the 15 min IMI criterion were equivalent.

Onset and size. The effects of the two drugs on the latency and size of the first meal following drug injections are shown in Table 1 for both obese and lean rats. Amphetamine significantly delayed the onset, $F(2,18)=18.79$; $p<0.01$, and reduced the size of the first meal, $F(2,18)=6.40$; $p<0.01$. Both doses of fenfluramine delayed the onset of the first meal in lean rats and the high dose delayed the first meal in the obese. The effects of genotype, $F(1,9)=13.11$; $p<0.01$, drug dose, $F(2,18)=17.63$; $p<0.01$, and the genotype by drug dose interaction, $F(2,18)=4.47$; $p<0.05$, were all significant. Fenfluramine also reduced the size of the first meal in both obese and lean rats, $F(2,18)=6.77$; $p<0.01$.

Activity

Table 2 shows the effects of the two drugs on the total number of revolutions of the running wheel and on the number of running bouts during the first 6 hr postinjection. Amphetamine increased the number of running bouts within

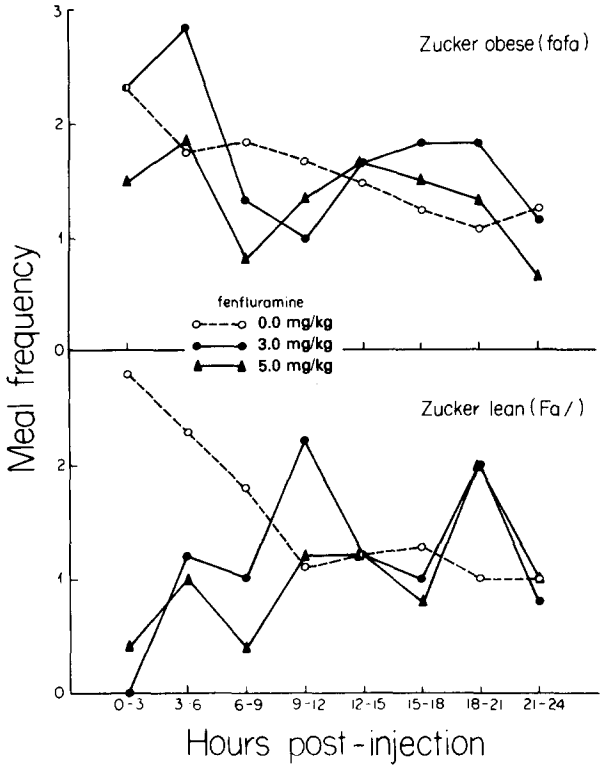

FIG. 5. Temporal effects of administration of fenfluramine (0.0, 3.0, 5.0 mg/kg) on meal frequency of obese and lean Zucker rats in Experiment 1. Eight consecutive 3-hr periods are shown.

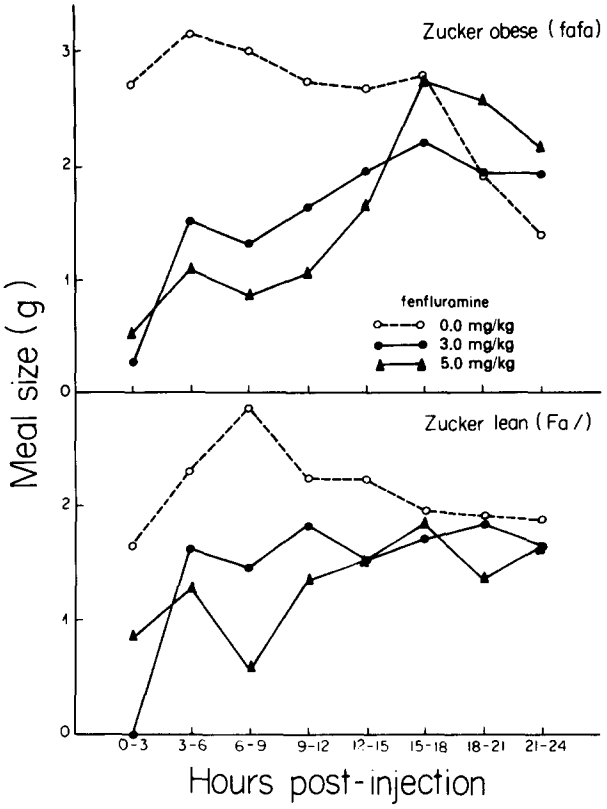

FIG. 6. Temporal effect of administration of fenfluramine (0.0, 3.0, 5.0 mg/kg) on average meal size (g) (2 min IMI) of obese and lean Zucker rats in Experiment 1. Eight consecutive 3-hr periods are shown.

the first three hr. Only the number of running bouts during the first 3 hr differed significantly from saline control levels (Wilcoxon's matched-pairs signed-ranks test pooled over genotype and dosage: $p<0.01$). The amphetamine-induced pattern of behavior was thus characterized by more frequent though short bursts of running-wheel activity.

In contrast, fenfluramine suppressed running-wheel activity by reducing the total number of revolutions of the running wheel during both the first ($p<0.05$) and second ($p<0.01$) 3-hr periods, while the number of running bouts remained constant. The opposite effects of amphetamine and fenfluramine on the rats' activity patterns as well as the prolonged effects of fenfluramine are consistent with previous findings [13, 16, 17].

DISCUSSION

The two drugs, amphetamine and fenfluramine had different behavioral profiles. Percent suppression of intake following amphetamine was greater for obese than for lean rats.

TABLE 1

EFFECTS OF ANORECTIC DRUGS ON THE MEAN LATENCY AND SIZE (± SEM) OF THE FIRST MEAL IN EXPERIMENT 1

	Zucker obese (fa/fa)		Zucker lean (Fa/−)	
	Latency (min)	Size (g)	Latency (min)	Size (g)
d-amphetamine				
0.0 mg/kg	14.9 (6.6)	2.2 (0.5)	32.6 (27.1)	1.7 (0.2)
1.5 mg/kg	103.5 (19.2)	1.1 (0.3)	153.2 (55.6)	1.2 (0.3)
3.0 mg/kg	167.8 (18.9)	0.6 (0.2)	165.6 (18.5)	1.3 (0.5)
fenfluramine				
0.0 mg/kg	32.4 (11.6)	2.3 (0.5)	35.4 (20.2)	1.6 (0.4)
3.0 mg/kg	93.0 (38.6)	1.6 (0.7)	266.0 (23.9)	1.5 (0.4)
5.0 mg/kg	137.0 (33.3)	0.4 (0.1)	326.2 (70.2)	0.8 (0.2)

TABLE 2
TEMPORAL PROFILE OF ACTION OF ANORECTIC DRUGS ON RUNNING-WHEEL ACTIVITY IN EXPERIMENT 1

Hours postinjection	Zucker obese (fa/fa) bouts 0–3	bouts 3–6	revolutions 0–3	revolutions 3–6	Zucker lean (Fa/–) bouts 0–3	bouts 3–6	revolutions 0–3	revolutions 3–6
d-amphetamine								
0.0 mg/kg	2.4	3.7	27.5	31.2	3.8	5.7	27.6	78.7
1.5 mg/kg	11.0	2.3	44.7	6.5	17.2	5.8	49.4	102.2
3.0 mg/kg	19.0	4.2	52.7	8.0	17.2	10.0	43.8	97.4
fenfluramine								
0.0 mg/kg	2.2	3.8	46.4	78.8	5.1	7.3	34.5	110.7
3.0 mg/kg	5.0	1.8	25.0	21.5	3.6	4.0	7.2	15.4
5.0 mg/kg	1.3	1.5	2.2	2.8	3.4	4.4	4.4	18.6

The pattern of amphetamine action was characterized by an immediate suppression of intake followed by an equally rapid (3–6 hr) recovery to control levels. The drug transiently reduced the mean number of meals eaten by obese and lean rats within three hr postinjection, as well as the average meal size of obese rats within nine hr postinjection. The drug also delayed the onset of feeding and reduced the size of the first meal. The activity profile included more frequent though shorter bursts of running-wheel activity during the initial three hr.

Percent suppression of intake following fenfluramine was equal for obese and lean rats. The pattern of fenfluramine action was characterized by an immediate and more pronounced suppression of intake that was followed by slow and incomplete recovery. Fenfluramine reduced the average meal size of obese and lean rats for up to 12 hr postinjection and reduced the number of meals eaten by the lean rats within the first six hr. The drug delayed the onset of feeding, reduced the size of the first meal, and led to shorter bouts of running-wheel activity.

The present data are consistent with earlier reports [5] that amphetamine delays the onset of feeding in rats and reduces meal frequency, whereas fenfluramine reduces the size of the first meal as well as the average meal size. Such data have been interpreted as showing that amphetamine affects meal initiation and therefore appetite, whereas fenfluramine affects meal termination and therefore satiety [3,5]. However, the present data show that amphetamine also reduced meal sizes of obese rats, while fenfluramine reduced meal frequencies of lean rats. Both drugs delayed the onset of feeding and reduced the size of the first meal. We are therefore unable to postulate that direct and mutually exclusive effects on appetite and satiety are the basis of amphetamine and fenfluramine action: rather, it appears that meal frequency and meal size are affected to a different extent by each drug.

It may be that the observed effects on meal parameters are the result of the differential impact of pharmacological intervention on the circadian pattern of feeding. This experiment differs from previous studies in that the rats were fed ad lib and were injected immediately prior to the dark phase at 6:00 p.m. In previous research, rats which had been deprived for 16 hr were injected with drugs during the light phase [5]. Slightly different effects on meal frequency and meal size might therefore be expected for each drug depending on the time of day and the nutritional status of the animal.

Another possibility is that the effects of the two drugs on meal parameters vary qualitatively as well as quantitatively as a function of drug dose and that increasing doses of each drug would be expected to affect both meal frequency and meal size. For example, the observed greater effects of fenfluramine than of amphetamine on meal parameters might be ascribed to the higher dose levels used. However, the doses of the two drugs were directly comparable to doses used by previous investigators which had been established on the basis of ED50 for two-hr food intakes in deprived animals [4,5]

EXPERIMENT 2

In the present experiment we examined the effects of amphetamine and fenfluramine on feeding and activity patterns of Zucker rats maintained on a liquid diet of sweetened condensed milk. We wished to establish that the temporal pattern and the mode of drug action observed in Zucker rats on a solid diet and water could be generalized to a dilute liquid diet. Liquid diets have been frequently employed in studies of meal patterns since the time and size of each meal can be precisely and easily measured. There can be no hoarding of food and the time of each lick can be accurately detected [1]. Since the two doses of fenfluramine in Experiment 1 affected meal size for a maximum duration of 9 hr, we used a higher dose to extend the anorectic effect into the light phase and to examine the effect of the higher dose on meal frequency.

METHOD

Animals

Five genetically obese (fa/fa) and six lean (Fa/–) male Zucker rats, approximately eight months old, were obtained from the Biology Department at Vassar College. Obese rats weighed a mean of 808.6 g (range 786–864 g) and lean rats weighed a mean of 489.5 g (range 417–536 g). For two weeks prior to the experiment, the rats had been accustomed to a liquid diet of Borden's sweetened condensed milk, diluted 3:1 with water and supplemented with vitamins and minerals [1,14]. The nutrient composition of this diet (percent of calories) based on the manufacturer's specifications is 77% carbohydrate, 10.8% protein and 11.9% fat (dry weight). Its

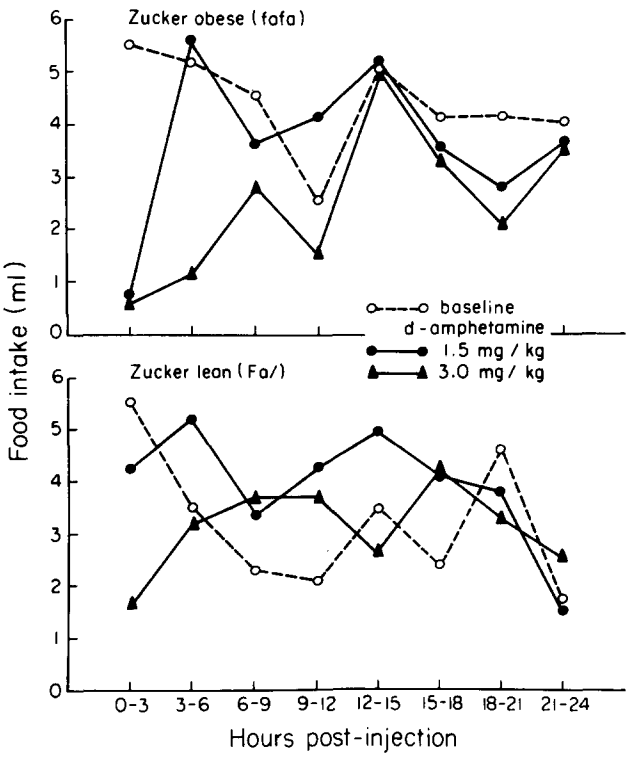

FIG. 7. Temporal effects of administration of d-amphetamine (1.5, 3.0 mg/kg) on food intakes of obese (n=5) and lean (n=6) Zucker rats in Experiment 2. Eight consecutive 3-hr periods are shown.

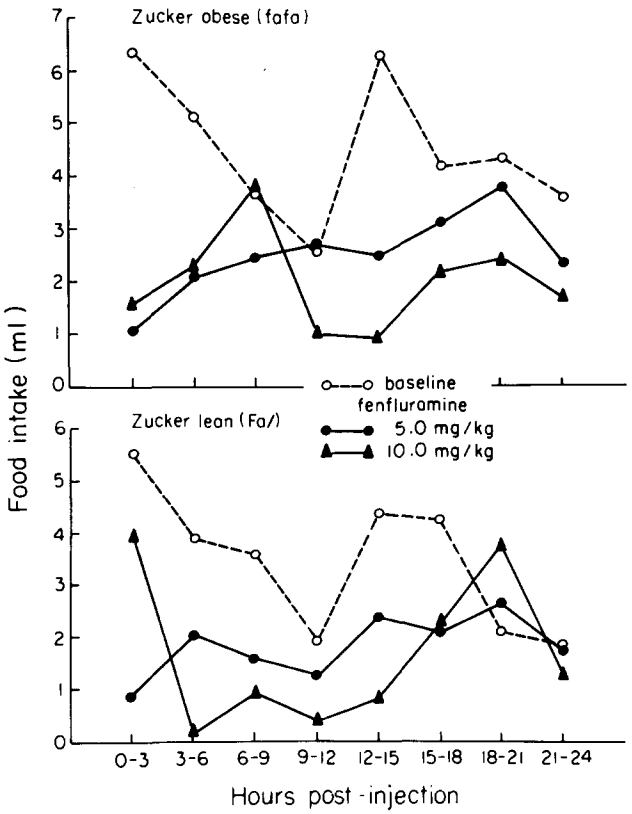

FIG. 8. Temporal effects of administration of fenfluramine (5.0 and 10.0 mg/kg) on food intakes of obese (n=5) and lean (n=6) Zucker rats in Experiment 2. Eight consecutive 3-hr periods are shown.

caloric density is 3.2 kcal/ml. The rats had free access to water at all times.

Apparatus

The same apparatus and light-dark schedule were used as in Experiment 1. Liquid diet was dispensed through a feeding tube connected to a drinkometer circuit, which was in turn interfaced with a PDP-8 computer. The rats' total food and water intakes were recorded daily. Water intakes with liquid diet were consistently low (<10 ml/day) and were not appreciably affected by either drug. Consequently, they will not be reported here.

Data Collection

We set the threshold criterion for the initiation of a feeding bout or meal at 20 licks occurring within one min. The intermeal interval (IMI) criterion for the termination of a meal was the lack of a feeding response within two min. Similarly, the threshold criterion for the initiation of a bout of activity was two revolutions of the running-wheel occurring within one min, and the interbout criterion for its termination was the lack of a response within two min. During subsequent analyses and following a preliminary examination of the data, we eliminated from consideration as random responses or noise all meals below 0.1 ml, and we used a more conservative IMI criterion of 15 min for additional estimates of meal frequency and meal size [1,14].

Drugs

The two drugs, amphetamine and fenfluramine were dissolved in sterile 0.9% saline in volumes of 10 mg/ml and 20 mg/ml, respectively. Two doses of d-amphetamine sulphate (1.5 mg/kg and 3.0 mg/kg) and two doses of fenfluramine hydrochloride (5.0 and 10.0 mg/kg) were used for intraperitoneal injections. In the saline control conditions, the rats were injected with equivalent volumes of isotonic saline. All rats were injected between 5:45 and 6:00 p.m. at the start of the dark cycle.

Design

Following a baseline period of four days of no injections, the first group of rats was injected with each of the two doses of amphetamine or with isotonic saline over three consecutive days. The order of injections was counterbalanced in a Latin Square design. Following another baseline period of no injections, the same rats were injected with each of the two doses of fenfluramine or with isotonic saline, with drug doses and saline again counterbalanced in a Latin Square design.

The same procedure was followed with the second group of rats, except that the sequence of drug administrations was reversed so that the rats were first exposed to fenfluramine and then to amphetamine. The data for the two groups of rats were pooled during analysis.

RESULTS

Food Intakes

Both amphetamine and fenfluramine suppressed food intake in obese rats, particularly during the initial twelve hours. However, following the low dose (1.5 mg/kg) of am-

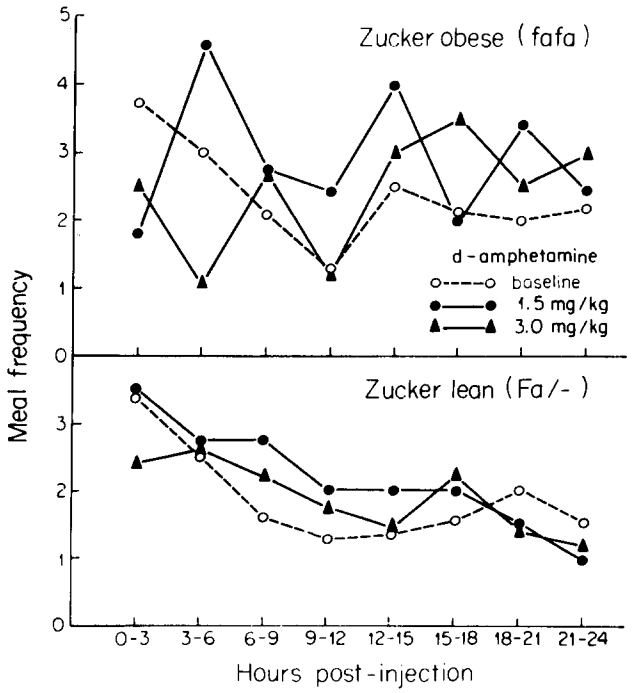

FIG. 9. Temporal effects of administration of d-amphetamine (1.5 and 3.0 mg/kg) on meal frequency (2 min IMI) of obese (n=5) and lean (n=6) Zucker rats in Experiment 2. Eight consecutive 3-hr periods are shown.

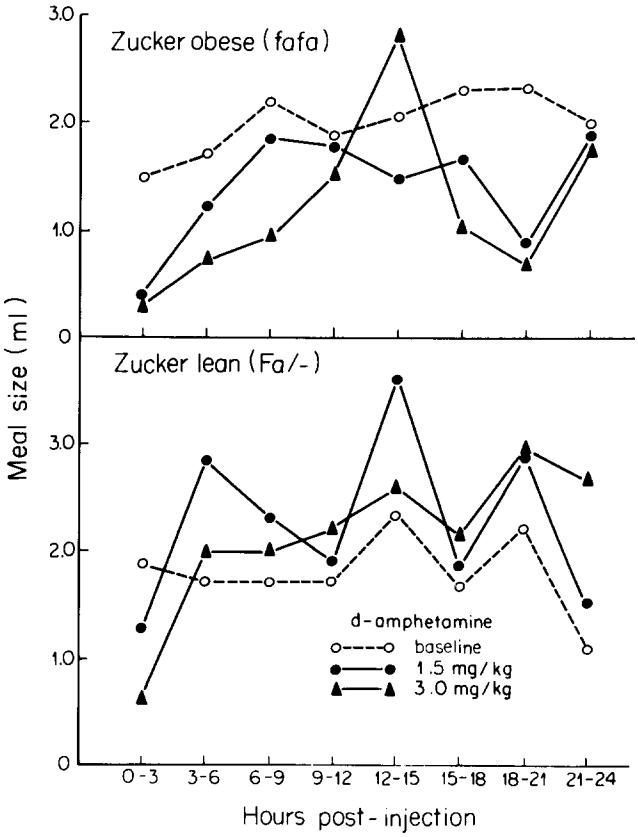

FIG. 10. Temporal effects of administration of d-amphetamine (1.5 and 3.0 mg/kg) on average meal size (ml) (2 min IMI) of obese (n=5) and lean (n=6) Zucker rats in Experiment 2. Eight consecutive 3-hr periods are shown.

phetamine, the daily food intake of lean rats actually increased relative to baseline. The effect of amphetamine averaged over both drug doses was to increase the food intake of lean rats by 12.0% and to reduce food intake of obese rats by 29.7%. Average percentage reduction in intake caused by fenfluramine was 45.6% for the obese rats and 50.7% for the lean.

Temporal profiles of suppression and recovery of food intake following amphetamine injections are shown in Fig. 7. Amphetamine caused an initial suppression in intake that was followed by a rapid dose-dependent recovery and in the case of lean rats by rebound feeding. The main effect of drug dose, $F(2,16)=10.82$; $p<0.01$, and the drug dose by time interaction, $F(14,112)=2.39$; $p<0.01$, were significant, reflecting rapid recovery from the effects of the drug. A significant drug dose by genotype interaction, $F(2,16)=7.81$; $p<0.01$, reflected the greater overall suppression of intake in the obese rats.

Temporal profiles of suppression and recovery following fenfluramine are shown in Fig. 8. Fenfluramine produced a prolonged suppression of feeding, with recovery occurring only toward the end of 24 hr. The main effects of drug dose, $F(2,16)=9.89$; $p<0.01$, and time, $F(7,56)=2.55$; $p<0.05$, were both significant. A significant drug dose by time interaction, $F(14,112)=2.82$; $p<0.01$ reflected the rats' eventual recovery following fenfluramine administration.

Meal Parameters

The effects of amphetamine on the temporal course of meal frequency (2 min IMI) are shown in Fig. 9. A small and nonsignificant reduction in the number of meals during the first three hr was later followed by compensatory feeding. There was no significant effect of drug dose, $F(2,16)=3.27$; n.s., and no drug dose by time, $F(14,112)=1.93$; n.s., interaction.

The reduction in meal size following amphetamine is shown in Fig. 10. This reduction was prolonged in obese rats. The main effect of time, $F(7,56)=3.87$; $p<0.01$, and the genotype by drug dose interaction, $F(2,16)=6.98$; $p<0.01$ were both significant. Amphetamine reduced meal sizes of obese animals but had virtually no effect on meal frequency.

The effects of fenfluramine on the temporal course of meal frequency (2 min IMI) are shown in Fig. 11. The drug reduced meal frequencies of both obese and lean rats. The main effect of time, $F(7,56)=4.88$; $p<0.01$, and the drug dose by time interaction, $F(14,112)=2.91$; $p<0.01$ were both significant. The reduction in meal size as shown in Fig. 12 was both greater and longer lasting than the reduction in meal frequency. Analysis of meal size data showed significant effects of drug dose, $F(2,16)=4.25$; $p<0.05$, and time, $F(7,56)=4.07$, $p<0.01$; and no significant interactions.

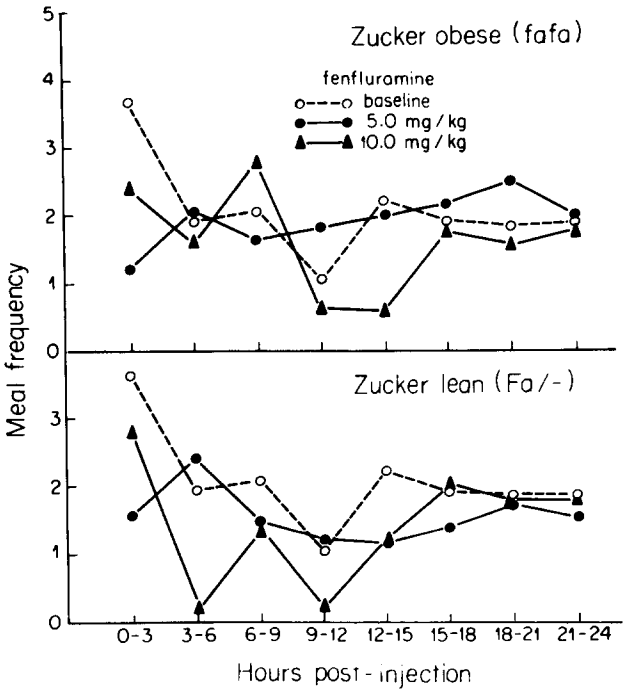

FIG. 11. Temporal effects of administration of fenfluramine (5.0 and 10.0 mg/kg) on meal frequency (2 min IMI) of obese (n=5) and lean (n=6) Zucker rats in Experiment 2. Eight consecutive 3-hr periods are shown.

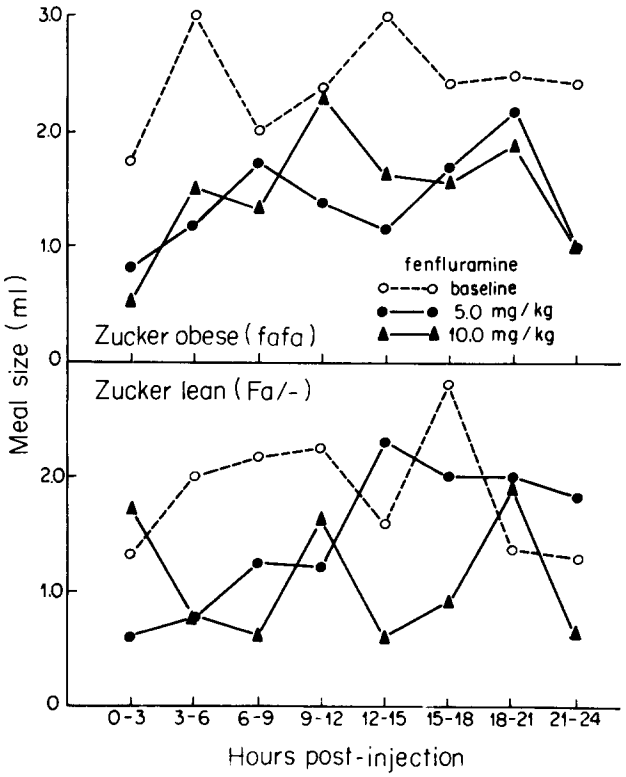

FIG. 12. Temporal effects of administration of fenfluramine (5.0 and 10.0 mg/kg) on average meal size (ml) (2 min IMI) of obese (n=5) and lean (n=6) Zucker rats in Experiment 2. Eight consecutive 3-hr periods are shown.

Onset and Size

The two drugs had different effects on the latency and size of the first meal. The high dose of amphetamine significantly delayed the onset of the first meal in the obese and lean rats (Wilcoxon's matched pairs, signed ranks test; $p<0.05$; lean: 41.5 min vs 132.2 min; obese: 16.1 min vs 83.0 min). However, meal size was unchanged. In contrast, both the low and high doses of fenfluramine, while not increasing the latency, reduced the size of the first meal in both lean and obese rats (Wilcoxon's test: $p<0.05$; lean: 1.83 ml vs 0.81 ml, 0.50 ml; obese: 2.40 ml vs 0.68 ml, 1.34 ml).

Activity

Effects of the two drugs on two measures of activity, the number of running bouts and the number of revolutions of the running wheel, are summarized in Table 3. During the baseline period all rats ran more at night than during the day, and lean rats ran both more often and for longer periods of time than did obese rats. Although baseline levels of activity were low, the two drugs had opposite effects on patterns of activity: amphetamine acted as a stimulant, whereas fenfluramine acted as a mild sedative. The number of running bouts and the total number of revolutions in the dark

TABLE 3

EFFECTS OF ANORECTIC DRUGS ON RUNNING WHEEL ACTIVITY IN EXPERIMENT 2

	Zucker obese (fa/fa)				Zucker lean (Fa/−)			
	bouts		revolutions		bouts		revolutions	
	Dark	Light	Dark	Light	Dark	Light	Dark	Light
d-amphetamine								
baseline	2.8	1.3	11.6	5.6	15.0	4.0	135.1	46.7
1.5 mg/kg	10.3	1.0	62.0	2.7	18.0	6.0	179.0	46.0
3.0 mg/kg	11.3	4.3	40.7	12.0	21.0	6.5	186.6	37.8
fenfluramine								
baseline	3.0	2.2	10.9	5.3	16.2	4.2	142.7	20.4
5.0 mg/kg	1.7	3.7	7.3	10.0	10.8	6.7	53.3	51.2
10.0 mg/kg	2.0	1.7	6.7	9.3	13.2	5.0	73.3	35.7

following amphetamine treatments (averaged over drug dose and pooled over obese and lean rats) were significantly above the baseline condition (Wilcoxon's matched-pairs signed ranks test: $p<0.05$). In contrast, fenfluramine treatment significantly reduced the number of running bouts in the dark ($p<0.05$) and slightly reduced the total number of revolutions of the running wheel.

DISCUSSION

The two drugs suppressed the rats' intakes of liquid food, although the present effects were more variable than those obtained with solid food in Experiment 1. The two drugs had different effects on obese and lean rats. Fenfluramine treatment reduced the daily intake of both obese and lean rats equally by up to 50%. Amphetamine treatment reduced the daily intake of obese rats by 16% (low dose) and 43% (high dose). The low dose of amphetamine actually increased the daily food intake of lean rats, while the high dose had no effect. These data are consistent with the results of Experiment 1 showing that amphetamine had a greater effect on intakes of obese than of lean rats.

The analysis of temporal profiles of drug action showed that the effects of amphetamine were more fleeting than those of fenfluramine, in agreement both with previous reports [4,5] and our own observations in Experiment 1. The two drugs had different courses of action on meal parameters and patterns of activity. Amphetamine reduced meal sizes of obese rats and transiently reduced meal sizes of lean rats. There was no significant change in meal frequency. However, the high dose of amphetamine did delay the onset of the first meal as observed previously [5]. Fenfluramine reduced both meal frequencies and meal sizes of obese and lean rats and reduced the size but not the onset of the first meal. Both meal frequency and meal size were thus affected to a different extent by each drug. The two drugs also had opposite effects on the rats' running wheel activity.

Additional behavioral data [3] suggest that amphetamine administration results in faster, frequent meals whereas fenfluramine administration results in slower, shorter meals. The rate of eating, however, may simply be a function of the stimulating and sedating properties of the two drugs.

One interpretation of these data is that the obese rats are more susceptible to the anorectic action of amphetamine than are lean rats. Although this interpretation contrasts with previous reports that obese rats are equally susceptible [21] or even more resistant to the action of amphetamine [8], it is consistent with our hypothesis that the control of food intake in obese rats may be more vulnerable to disruption than that in lean rats. Our data showed that amphetamine had greater suppressive effects on the intake of obese than of lean rats within three hr postinjection. The brief anorexia of lean rats was then followed by compensatory feeding that was mediated by increases both in meal frequency and meal size. In contrast, obese rats recovered more slowly and showed no compensatory feeding. Thus, the greater overall effect of amphetamine on the intake of obese as opposed to lean rats may reflect either their greater initial susceptibility to the action of amphetamine, or a slower recovery from its effects. Our data are thus consistent with earlier reports that the Zucker obese rat is slower to adapt its food intake following chronic drug administration [14] or following substitution of poorly absorbed lipid [10] than the lean rat.

It is, of course, possible that the observed differences in the effects on the obese and lean rats are a direct consequence of differential drug doses. Based on mg/kg of body weight, the obese rats received approximately twice as much drug as did the lean rats. However, even the high dose of amphetamine failed to reduce the daily food intake of lean rats. Another possibility is that the kinetics of amphetamine action differ for obese and lean rats: since studies of drug disposition were not attempted, this explanation cannot be positively ruled out. These interpretations are unlikely given the equivalent effects of fenfluramine on obese and lean rats in both experiments.

GENERAL DISCUSSION

The present data document the temporal effects of two assumedly prototypic anorectic drugs, amphetamine and fenfluramine, on the food intake of obese and lean Zucker rats. Our data indicate that the two drugs had distinct behavioral profiles in the extent and the duration of their action on food intake, meal parameters and spontaneous activity. However, the data did not support the contention that amphetamine acts principally by suppressing appetite whereas fenfluramine acts by promoting satiety. Both drugs altered meal frequency and meal size by differing amounts. Furthermore, the effects of amphetamine on food consumption depended on genotype, drug dose, and the nature of the diet, while the action of fenfluramine was found to be consistent across diets and largely consistent across genotypes.

Differential responsiveness to anorectic agents depending upon nutritional status has been reported in mice and in rats following both peripheral and central administration [13,17]. Doses of amphetamine and fenfluramine necessary to suppress the food intake of freely feeding mice are double those required in deprived mice. Intra-hypothalamic administration of norepinephrine or amphetamine produces anorexia in starved rats, but elicits feeding in satiated rats [6,16]. Thus, the anorectic potency of a drug is directly dependent on the nutritional status and feeding history of the animal. The present data additionally suggest that rats of differing genotype may be differentially responsive to one drug and yet be equally responsive to another drug.

It has been suggested that the anorectic actions of amphetamine and fenfluramine are mediated by different central neurotransmitter systems; adrenergic for amphetamine and serotonergic for fenfluramine [3,15]. Thus, the effects of amphetamine are antagonized by lesions of the lateral hypothalamus whereas the effects of fenfluramine are antagonized by lesions of the raphe nuclei [22]. Agents such as methysergide diminish the effects of fenfluramine while having no effect on amphetamine action whereas agents such as haloperidol diminish amphetamine anorexia and have no effect on fenfluramine induced anorexia [9,19].

The present findings that fenfluramine has equivalent suppressive effects on feeding of obese and lean rats, whereas amphetamine is more effective in the obese rats, raise the possibility of differential mediation by brain monoamine systems. Differences in hypothalamic catecholamines may be related to the differential responsiveness of obese and lean rats to amphetamine administration. Obese Zucker rats have increased norepinephrine levels in the median eminence and decreased levels in the periventricular nucleus compared to lean rats [11,12]. Dopamine levels have also been reported to be decreased in the dorsomedial nucleus and C2 nucleus of the male obese Zucker rat [20]. Further investigation of responses to cen-

trally administered amphetamine and fenfluramine may reveal selective differences in neurotransmitter responses which may be related to the production and maintenance of obesity.

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