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Effect of Fenfluramine on Sympathetic Firing Rate

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ARASE, K., T. SAKAGUCHI AND G. A. BRAY. *Effect of fenfluramine on sympathetic firing rate.* PHARMACOL BIOCHEM BEHAV **29**(4) 675–680, 1988.—The effects of acute and chronic treatment with fenfluramine have been explored in two experiments. Three and twenty-four hours following the injection of fenfluramine 20 mg/kg the firing rate of sympathetic efferent nerves to brown adipose tissue was significantly increased compared to sham injected controls. Body weight loss following acute treatment with fenfluramine was significantly greater at three and twenty-four hours than in the vehicle-treated controls. In the chronic experiment animals were treated once daily for 12 days with 20 mg/kg of fenfluramine. There were two control groups. One control group ate ad lib and a second control group was pair fed to maintain body weight comparable to that of the fenfluramine-treated animals. By the twelfth day food intake in the fenfluramine-treated animals had returned to control levels. Sympathetic firing rate after three days of treatment with fenfluramine was significantly higher in the treated animals than in ad lib fed controls. The ad lib fed controls were likewise significantly higher than the vehicle-treated, pair-gained controls. After 12 days of treatment fenfluramine treated animals had sympathetic firing rates which were still slightly but significantly higher than those of the vehicle-treated controls whereas the vehicle-treated, pair-gained animals had a small but significantly reduced firing rate. These data support the hypothesis that fenfluramine can increase peripheral sympathetic activity.

Appetite suppressing drugs Food intake Weight loss Brown adipose tissue Neurophysiology

FENFLURAMINE is an appetite suppressing drug used for the treatment of obesity [4, 9, 16]. In animals [9] as well as man [4], this drug will significantly reduce food intake. Clinical studies demonstrate a reduction in body weight which can last up to 1 year [6]. Recent studies by Levitsky *et al.* [10] have suggested that fenfluramine may not be primarily an anorectic or appetite suppressing drug. They showed that when animals were deprived of food to lower their body weight prior to treatment with fenfluramine, the drug did not reduce food intake. Rather, food intake rose to normal levels when drug and food were given together. Body weight however remained below that of ad lib fed controls. These studies suggested that fenfluramine might have an effect on energy expenditure.

The possibility that fenfluramine might be a thermogenic drug has been supported by several studies [11–13, 22, 24]. Acute treatment with fenfluramine will significantly increase the thermogenic activity of brown adipose tissue as reflected in the increased binding of purine nucleotides (GDP) to mitochondria from this tissue [12,13]. Chronic treatment with fenfluramine will reduce food intake with a subsequent fall in body weight. Body weight remains low but food intake gradually returns almost to control levels during chronic treatment with fenfluramine [13]. Body weight remains depressed as long as treatment is continued. In animals treated with fenfluramine, the drug alone did not increase resting oxygen consumption, but it did potentiate post-prandial oxygen consumption [11].

Some of these effects of fenfluramine are reminiscent of the effects which follow lesions in the lateral hypothalamus of rats [7,8]. Lateral hypothalamic (LH) lesions acutely decrease food intake and body weight falls. There is a gradual recovery in food intake but body weight remains low [7,23]. This adaptation is often referred to as a change in the regulated set point since these LH-lesioned animals will "defend" this new body weight if it is disturbed by under- or over-feeding [8]. Fenfluramine-treated rats also defend their lower body weight [10]. Following LH-lesions there is an acute increase in the turnover of norepinephrine which persists for at least 3 weeks when compared to pair-fed control animals. LH-lesioned animals and those treated with fenfluramine also show higher GDP-binding in brown adipose tissue [13]. These studies suggest that both LH-lesions and treatment with fenfluramine activate the sympathetic nervous system.

Measurement of the electrical activity of the sympathetic nerves [17–20] provides a more direct approach to assessing the activity of the sympathetic nervous system. Direct measurements of sympathetic efferent activity have been reported from several laboratories [15, 17, 20, 26]. Ventrome-

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dial hypothalamic lesions have been shown to decrease firing rate of sympathetic nerves to brown adipose tissue [19]. Utilizing this technique we have explored the effects of acute and chronic treatment with high doses of fenfluramine on sympathetic firing rate.

METHOD

Animals

The 62 female Sprague-Dawley rats used in these experiments were purchased from Harlan Sprague-Dawley Laboratories (Madison, WI). They weighed about 225 to 250 g upon arrival in the laboratory. Animals for the acute experiments were housed in groups and those for the chronic experiments were individually housed in hanging wire bottom cages. Purina laboratory chow (Ralston Purina Co., St. Louis, MO) was available ad lib in the acute experiments. Ground chow was used for measurement of food intake in the chronic experiment where food was available from Wahman cups with a round hole in the middle to minimize spillage. The vivarium was illuminated from 0600 to 1800 hr each day and ambient temperature was maintained at 22±1°C.

Experimental Protocol

Acute experiment. Twenty rats were used in the acute experiment. A single injection of d,l-fenfluramine·HCl, 20 mg/kg in a volume of 0.25 ml/100 g body weight, was given to 8 rats and 12 rats received an equivalent of 0.15 M NaCl between 0900 and 1100 hr. Blood sampling and recording of nerve activity were carried out immediately after the injection (zero time) or at three and twenty-four hours after the injection. Animals had ad lib access to food until anesthetized for nerve recording.

Chronic experiment. Forty-two rats were used in the chronic experiment. Six rats were used for pre-treatment measurements of sympathetic firing rate. The other 36 rats were divided into three groups of 12 rats each. The experimental group received fenfluramine 20 mg/kg, IP each morning. The control group (N=12) and pair-fed group (N=12) received injections of 0.5 ml of 0.15 M NaCl IP. All animals were treated daily between 0900 and 0930 hr for three days (6 rats from each group) or for twelve days (6 rats from each group). Food consumption by the fenfluramine-treated rats was measured daily and the pair-fed group was given a comparable amount of food once in the morning. Daily food intake and body weight were measured at 0830 hr. At the beginning of day 4 and day 13, blood samples were obtained by cutting the tail under pentobarbital anesthesia, 35 mg/kg, IP. The measurement of electrical activity of sympathetic nerves was recorded next. Animals were sacrificed with an overdose of sodium pentobarbitol, and selected organ weights were recorded. Lee Index (LI) calculated as follows:

$$\text{Lee Index} = \frac{[\text{b.wt. (g)}]^{1/3}}{\text{naso-anal length (mm)}} \times 10^4.$$

Sympathetic Nerve Recording

Recording of sympathetic nerve activity was conducted under pentobarbital anesthesia (35 mg/kg) in animals allowed ad lib access to food until anesthetized. The sympathetic nerve bundles to the right lobes of the interscapular brown adipose tissue (IBAT) were carefully exposed by reflecting the IBAT through a dorsal incision. To record efferent nerve activity, one of the bundles to the middle of the IBAT was sectioned as close as possible to the IBAT and filaments were microdissected longitudinally. Recordings were made from two or three separate filaments placed on a pair of silver wire electrodes immersed in a mixture of liquid paraffin and petroleum jelly to prevent dehydration. The electrical activity was amplified by a differential amplifier and displayed on an oscilloscope. A window discriminator was used to eliminate background noise. The spikes from this fiber were cumulated in a rate meter with a reset time of 5 seconds. The output of this rate meter was used to drive a 1 millivolt strip recorder which recorded the spikes per five seconds. Sympathetic nerve activity was recorded from two or three different filaments in each rat and the data averaged. This averaged basal firing rate from each rat provided the data for statistical analysis [17–20].

Analytical Procedures

Plasma glucose was assayed with a glucokinase method (Sigma Chemicals, St. Louis, MO). The plasma insulin was assayed by a double antibody technique using iodinated porcine insulin and rat insulin standards [14].

Statistical Evaluation

Two-way analysis of variance with one repeat measure was carried out on the data for food intake and body weight values in the chronic experiment. Simple main effects of treatment on each day were evaluated by a one way ANOVA. Post-hoc comparisons between treatments were done using the Neuman-Keuls technique. For the acute experiment a two-way analysis of variance was carried out, and individual comparisons were conducted using the Q statistic in a two-by-two factorially designed experiment.

RESULTS

Acute Experiment

Rats for this experiment were allowed ad lib access to food and water throughout the experiment. As can be seen in Table 1, the fenfluramine-treated rats lost significantly more weight at three and twenty-four hours following the injection of fenfluramine than did the vehicle-treated animals. Neither plasma glucose nor insulin concentrations changed significantly by treatment with fenfluramine at either of these two time points. Sympathetic discharge rate at 3 and 24 hr following a single injection of fenfluramine is shown in Fig. 1, where each point represents the mean±SEM for the average of 2 to 3 filaments from each of 4 rats. The discharge rate was stable at approximately 39 spikes per five seconds for the four control rats. The fenfluramine-treated animals showed a significantly greater firing rate at three hours (63.6±5.0 vs. 38.5±0.5, $p<0.001$) and again at twenty-four hours ($p<0.01$) following the acute injection (N=4 rats per group).

Chronic Experiment

The time course of food intake and body weight in the animals treated for twelve days is shown in Fig. 2a and b. Following the initiation of treatment there was a significant reduction in food intake in the fenfluramine-treated animals (upper panel) which reached its nadir on the first day and gradually recovered to control levels by the 12th day, $F(1,10)=286.30$, $p<0.001$. The body weights of the fenfluramine-treated and pair-fed animals were significantly

TABLE 1

EFFECT OF FENFLURAMINE ON BODY WEIGHT, GLUCOSE AND INSULIN*

	Control			Fenfluramine	
	0 hr	3 hr	24 hr	3 hr	24 hr
Body Weight Change (g)		-0.3 ± 0.8^a	2.3 ± 2.1^b	-7.3 ± 0.8^a	-10.8 ± 1.7^b
Glucose (mg/dl)	150.0 ± 5.0	154.2 ± 1.6	149.1 ± 6.1	171.8 ± 14.8	150.0 ± 5.2
Insulin (ng/ml)	0.80 ± 0.13	0.54 ± 0.09	0.56 ± 0.14	0.46 ± 0.99	1.00 ± 0.28

*Data are mean ± SEM. Values with the same letter are significantly different.
$^a p<0.05$, $^b p<0.001$.

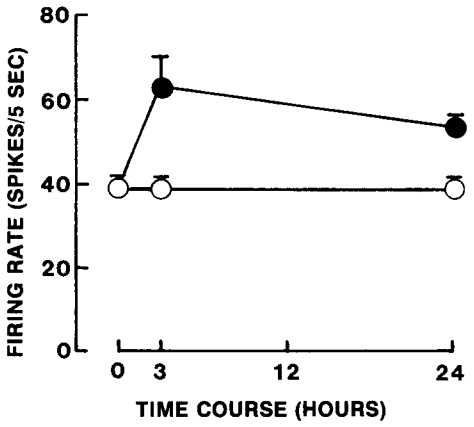

FIG. 1. Effect of fenfluramine on the sympathetic firing rate. Rats were treated with fenfluramine (solid circles) or vehicle (open circles), 20 mg/kg at 0 time and the firing rate of drug and vehicle-treated rats measured at 0, 3 and 24 hours following the injection. Firing rate is expressed as spikes/5 sec and each point is the mean±SEM for 4 rats.

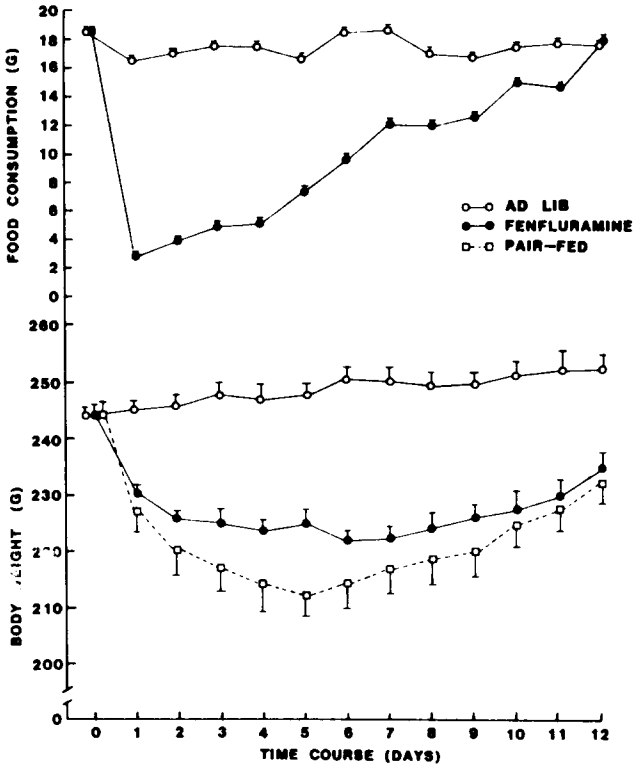

FIG. 2. Effect of chronic treatment with fenfluramine on food intake (top panel) and body weight (bottom panel). Two groups of 6 rats each were treated daily with an injection of vehicle (0.15 M NaCl) or fenfluramine (20 mg/kg) for 12 days. A third group of vehicle-treated rats were restricted to the quantity of food eaten by the fenfluramine-treated rats. Data are presented as mean±SEM.

lower throughout the experiment (Fig. 2b), $F(2,15)=45.65$, $p<0.001$. Weight gain between day 6 and 13 was greater for the pair-fed rats (17.8±2.3 g) than the fenfluramine-treated rats (12.8±1.8 g) but this difference was not statistically significant, $t(10)=1.75$.

The basal firing rate of sympathetic nerves during chronic treatment with fenfluramine is shown in Fig. 3, where each bar represents the mean±SEM for 6 rats. There was a significant difference between treatments, $F(2,30)=93.19$, $p<0.001$. At the beginning of the 4th day the pair-fed animals had significantly lower sympathetic firing rates than did the control animals ($p<0.01$). On the other hand, the fenfluramine-treated animals which consumed the same amount of food as the pair-fed animals had significantly higher basal rates than either the pair-fed or ad lib fed groups ($p<0.01$). When basal sympathetic firing rate was measured on the 13th day, the firing rate of the sympathetic nerves in the fenfluramine-treated animals (N=6) was reduced from 55.7 to 40.9 spikes/5 seconds (right-hand set of bars in Fig. 3). However, this value was still slightly but significantly higher ($p<0.01$) than in the control animals (N=6) and also significantly higher ($p<0.01$) than that of the pair-fed rats (N=6), whose body weights were comparable to those of the fenfluramine-treated animals. When the food intake and the sympathetic activity of the fenfluramine-treated and pair-fed animals were examined (Fig. 4), a highly significant negative correlation was found between food intake and nerve activity in the fenfluramine-treated rats ($r=-0.771$, $p<0.01$). At day 4, however, the relationship of sympathetic activity and food intake was positively correlated, y (firing rate)=2.6 X (food intake) + 39.2, $r=0.755$, $p=0.05$. There was also a positive correlation in the pair-fed animals between food intake and sympathetic activity ($r=0.878$, $p<0.01$).

Chronic treatment with fenfluramine did not affect the plasma glucose or insulin values. The Lee Index (Table 2), as a measure of body fatness, was significantly lower in the pair-fed and fenfluramine-treated rats than in the vehicle-

TABLE 2

EFFECT OF FENFLURAMINE AND PAIR-FEEDING ON LEE INDEX, ORGAN WEIGHTS, GLUCOSE, AND INSULIN

	Control			Fenfluramine		Pair-Fed	
	Day 0	Day 3	Day 12	Day 3	Day 12	Day 3	Day 12
Lee Index	308.0 ± 0.8	309.6 ± 1.1	310.8 ± 1.0	303.5 ± 1.6†	305.9 ± 0.5†	300.6 ± 1.3†	304.1 ± 1.6†
Liver Weight (g)	8.38 ± 0.19	7.68 ± 0.39	8.71 ± 0.41	8.12 ± 0.48	9.59 ± 0.30	5.52 ± 0.15†	6.92 ± 0.31*
Retroperitoneal Fat Pad (mg)	1528 ± 98	1864 ± 186	2304 ± 435	998 ± 251*	1446 ± 75	1093 ± 206*	1342 ± 470
Interscapular Brown Adipose Tissue (mg)	264.8 ± 9.1	258.7 ± 5.5	283.0 ± 9.9	196.2 ± 9.6†	271.7 ± 9.3	191.8 ± 10.7†	277.5 ± 23.7
Glucose (mg/dl)	152.6 ± 6.4	154.1 ± 8.6	145.9 ± 4.8	150.3 ± 6.3	147.6 ± 3.3	118.2 ± 5.5†‡	154.1 ± 9.3
Insulin (ng/ml)	1.06 ± 0.19	0.63 ± 0.26	1.03 ± 0.21	1.14 ± 0.37	1.16 ± 0.12	0.12 ± 0.04 (undetectable)	1.02 ± 0.57

Data are mean ± SEM.

*$p \leq 0.05$, †$p \leq 0.01$ when compared to appropriate days for the control group; ‡$p \leq 0.01$ when compared to day 3 of fenfluramine treatment.

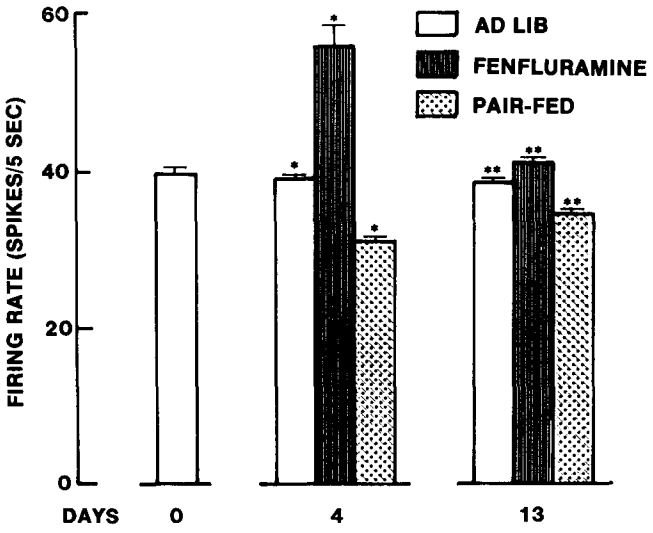

FIG. 3. Basal firing rate of sympathetic nerves during chronic treatment with fenfluramine. Data are presented for the basal sympathetic firing rate at day 4 and day 13 after beginning treatment. The last injection was 24 hours before study. Each bar represents the mean±SEM for basal sympathetic firing rate on each of 6 rats.

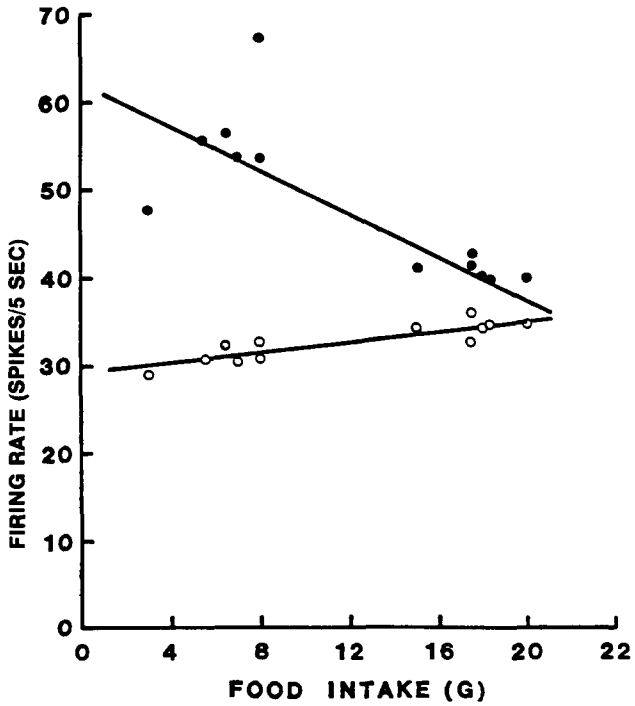

FIG. 4. Relationship of basal firing rate to food intake. The relation of basal firing rate to food intake for fenfluramine-treated and pair-fed controls is shown using data from day 4 and day 13. There was a highly significant negative correlation between the firing rate and food intake for the fenfluramine-treated rats ($y=67.1 - 1.1\ X$, $r=-0.771$, $p<0.01$) and a positive but significant one for the pair-fed controls ($y=29.2 + 0.3\ X$, $r=0.878$, $p<0.01$).

treated, ad lib fed animals. The weight of the liver was significantly decreased only in the pair-fed controls. The weight of retroperitoneal adipose tissue was significantly different between treatments, but there were no significant differences when individual comparisons were carried out.

DISCUSSION

The present experiments have demonstrated that acute treatment with fenfluramine significantly increases sympathetic firing rate. Chronic treatment with fenfluramine also substantially increased firing rate at the time of maximal suppression of food intake. When food intake had returned to normal, firing rate of sympathetic nerves of fenfluramine-treated rats was still slightly but significantly higher when compared with pair-fed animals of comparable body weight.

The present data measuring sympathetic firing rate are similar to previous data from this laboratory in which purine nucleotide binding to mitochondria from brown adipose tissue was estimated [12,13] as an index of sympathetic activity. In both sets of experiments, acute treatment with fenfluramine significantly increased the activity of the sympathetic nervous system whether measured by direct firing rate as in the present study or by the indirect measurement of purine nucleotide (GDP-binding to mitochondria from this

tissue [12,13]. In both studies a high pharmacologic dose of fenfluramine was chosen for two reasons. First, Levitsky *et al.* [10] in their initial studies had used this dose, and continuing with a similar dose would provide comparable data. Second, in dose-response studies using activity of GDP-binding as the end point, a lower dose (6 mg of fenfluramine/kg body weight) did not have a significant effect [12].

The effect of fenfluramine on the activity of the sympathetic nervous system is similar in some ways to the effect of a lesion in the lateral hypothalamus [13]. In both cases there is an acute activation of the sympathetic nervous system, whether measured as GDP-binding to mitochondria from IBAT [13], as sympathetic firing rate (this experiment) [1] or as norepinephrine turnover [23]. Our studies with LH-lesioned rats have demonstrated that, during the transition following an LH-lesion, increased sympathetic firing rate [1,25] and enhanced GDP-binding to brown adipose tissue [13] are both associated with, and may be the mechanism for, an adaptation to a reduced body weight. This suggests that the lateral hypothalamus may modulate the activity of the sympathetic nervous system in an inhibitory fashion. Removal of this inhibition by an electrolytic lesion allows the sympathetic system to be more active in response to the internal nutrient milieu in which it functions, thus driving body weight to a lower regulated level.

Fenfluramine appears to mimic the effects of an LH-lesion [13], and may provide some additional insight into the mechanism. Fenfluramine is a phenethylamine derivative which differs from most of the other appetite-suppressing drugs by the mechanism through which it acts [5, 9, 16]. Fenfluramine appears to enhance the release of serotonin and may also block its reuptake into the nerve endings [5]. This would suggest that serotonin is acting to remove an inhibitory system which is tonically suppressing the firing rate of the sympathetic nervous system. In the present study, chronic treatment with fenfluramine produces a significantly elevated firing rate of the sympathetic nervous system in association with the chronically reduced body weight which is maintained with ad lib food intake. This contrasts with the reduced sympathetic activity found when food intake is restricted below the level preferentially regulated by the animal itself.

One prediction of this model is that an LH-lesion might prevent the effects of fenfluramine. To the contrary, Blundell and Lesham [2] have shown that an LH-lesion actually enhances the anorectic effects of this drug. The similarity of fenfluramine and LH-lesions might be explained by the reciprocal control over the sympathetic nervous system exerted by the VMH and LH. The VMH may be viewed as an activator and the LH as an inhibitor of this system [3]. Similar effects would be produced by removing the LH inhibitory system as by activating the excitatory one in the VMH. If fenfluramine acted on the VMH, the LH-lesion which removed the inhibitory effects of the lateral hypothalamus on the sympathetic nervous system might actually enhance the effects of agents acting on the VMH. The implications of this potential interaction are currently under investigation.

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