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Effect of Fenfluramine on GDP-Binding to Brown Adipose Tissue Mitochondria

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LUPIEN, J. R. AND G. A. BRAY. *Effect of fenfluramine on GDP-binding to brown adipose tissue mitochondria.* PHARMACOL BIOCHEM BEHAV **23**(4) 509–513, 1985.—These experiments have tested the effects of treatment with fenfluramine on the GDP-binding to mitochondria isolated from interscapular brown adipose tissue in vitro. In acute studies, the binding of GDP was significantly increased after 3, 24, and 48 hours of treatment with a single dose of 20 mg/kg of body weight. Addition of fenfluramine in vitro, however was without effect. In a dose-response study there was no significant increase with doses of 2 or 6 mg/kg but there was a significant increase at 20 mg/kg. During eleven days of treatment, food intake was initially depressed by fenfluramine (20 mg/kg) and body weight was significantly reduced. By the ninth day of treatment however, food intake had returned to control values but the body weight remained significantly lower than in the control group. The weight of interscapular brown adipose tissue was not significantly altered but binding of GDP to isolated mitochondria was increased by 55%. These studies suggest a thermogenic effect of fenfluramine on the brown adipose tissue of rats.

Fenfluramine Brown adipose tissue GDP-binding

FENFLURAMINE is one of the appetite suppressing drugs used in the treatment of obesity [3,4]. On the one hand, there is a clear dose-related depression of food intake by this drug. Moreover, with chronic treatment there is a chronic reduction in body weight. On the other hand, if the body weight of experimental animals is reduced by food restriction prior to treatment with fenfluramine there is no anorectic effect from treatment with fenfluramine. Indeed such weight-reduced animals begin to eat when food is available [12]. These studies are reminiscent of those reported by Powley and Keesey [15] who demonstrated that lateral hypothalamic lesions produced anorexia in rats at normal body weight but that food intake resumed immediately following lesions in animals that were weight reduced by food restriction prior to the lesioning procedure. In the studies with weight reduced animals treated with fenfluramine, Levitsky *et al.* [12] observed that the food intake was normal during treatment with fenfluramine but that body weight remained significantly lower [12]. This suggested an increase in thermogenesis as a result of treatment with fenfluramine.

One of the mechanisms for thermogenesis in rodents is through activation of brown adipose tissue (BAT) [9, 13, 17]. Heat production in BAT involves a unique mitochondrial protein, forming a proton conductance pathway which allows the cells to oxidize substrate without this oxidation being linked to the synthesis of ATP. This protein in the mitochondrial membrane binds guanosine diphosphate (GDP) and other purine nucleotides. The level of GDP binding to BAT mitochondria has been considered a reliable indicator of the activity of the proton conductance pathway, namely a measure of the thermogenic capacity of this tissue [7, 9, 13, 17]. The present study was designed to test the possibility that fenfluramine might increase thermogenesis by activating brown adipose tissue via mitochondrial GDP binding.

METHOD

Animals

Female Wistar rats weighing approximately 250 grams were purchased from Simonsen Laboratory, Gilroy, CA. The animals were maintained in hanging wire bottom cages with tap water ad lib. Food was available as Purina laboratory chow except for experiments where food deprivation was produced.

GDP Binding

Rats were killed by decapitation and the interscapular BAT was carefully removed and trimmed from adhering white fat and muscle. The tissues were homogenized in a glass, motor driven tissue homogenizer in a medium containing 0.25 M sucrose, 0.2 mM EDTA(potassium salt), and 1 mM HEPES, pH 7.2 at 0°C and mitochondria were isolated as described by Slinde and Pedersen [19]. The binding of guanosine-5'diphosphate (GDP) to brown adipose tissue mitochondria was performed by the method of Nicholls [14], as modified by Desautels and Himms-Hagen [7]. Isolated mitochondria (0.5 mg of protein/ml) were incubated for 10 minutes at room temperature (20°C) in a medium containing 10 μM of 3H-GDP (1.25 μ Ci/ml) ^{14}C-sucrose (0.1 μ Ci/ml), 100 mM sucrose, 20 mM K-TES (pH 7.1), 1 mM K-EDTA, 10 mM choline chloride and 5 μM rotenone.

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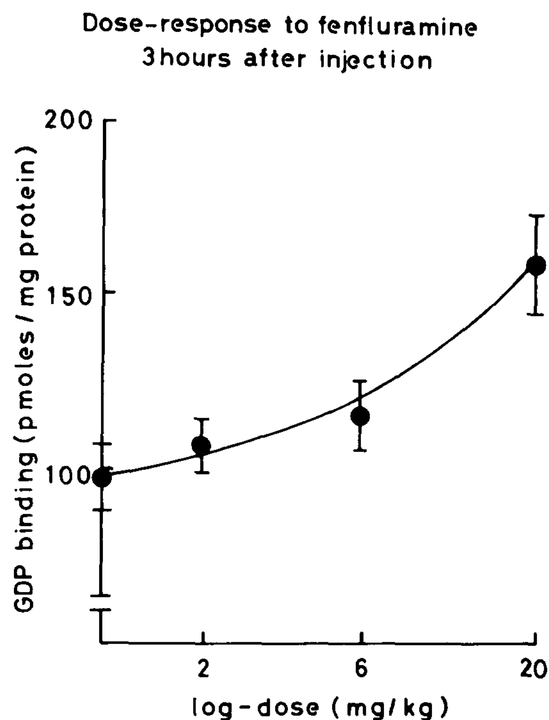

FIG. 1. Dose-response to fenfluramine 3 hours after injection. The animals received a single injection of vehicle or fenfluramine at doses of 2, 6 or 20 mg/kg and the GDP binding to isolated mitochondria was measured 3 hours later. All data are plotted as mean ± SEM for GDP Binding from five animals.

The mitochondria with the radioactive GDP bound are isolated by collecting the mitochondria on millipore filters and counting the radioactivity. A correction for the quantity of trapped medium was made from the ^{14}C-sucrose radioactivity on the filter. Protein was estimated by a modification of the Lowry method [18].

Experimental Procedures

Fenfluramine and saline (vehicle) were injected IP in a volume of 2.5 ml kg $-^{1}$ (0.5 ml/200 g body weight). Both the control groups and the experimental groups were injected in the morning between 0800–0900 hour. The food deprived groups were fasted during 3, 24 or 48 hours after a single dose of fenfluramine. The average half-life of fenfluramine and its metabolite, norfenfluramine, estimated from measurements of plasma concentrations was about 20 hours [1,4].

In Experiment 1, one-way analysis of variance was carried out on all data to test the effects of fenfluramine dose. In Experiment 2, a two-way analysis of variance was performed on all data including body weight and food intake values to examine the effects of time and treatment. In Experiment 3, a two-way analysis of variance procedure for repeated measures on the factor time was used to appraise statistical significance. On each analysis of variance, where necessary, the individual means were compared by Newman-Keuls technique [21]. The statistical significance of differences between groups in table 1 was assessed by Student's unpaired t-test.

TABLE 1

EFFECT OF FENFLURAMINE IN VITRO ON THE BINDING OF GUANOSINE 5′-DIPHOSPHATE (GDP) TO MITOCHONDRIA FROM BROWN ADIPOSE TISSUE*

	GDP Binding (p mole†/mg protein)
Additions in vitro	
None	99.2 ± 6.02 (6)
Fenfluramine (50 μM)	87.8 ± 11.9 (6)
Treatments in vivo	
Vehicle + Fenfluramine in vitro	161.7 ± 7.04 (3)
Fenfluramine (20 mg/kg)	166.9 ± 1.2 (3)

* Fenfluramine 20 mg/kg was given intraperitoneally 3 hr prior to study. The concentration of fenfluramine added in vitro was calculated to exceed the concentration achieved if fenfluramine were distributed in body water.

† Mean ± SEM (N = number of animals).

Chemicals

Fenfluramine was a gift of A. H. Robbins Company (Richmond, VA). Other chemicals were purchased from Sigma Chemicals Co. (St. Louis, MO). The radioactive ^{3}H-GDP and ^{14}C-sucrose were obtained from New England Nuclear Corporation (Boston, MA): (U-^{14}C-sucrose sp. radioactivity 360 mCi/mmol and 8-^{3}H-guanosine 5′-diphosphate, trisodium salt sp. radioactivity 7.8 Ci/mmol).

RESULTS

Experiment 1. Response to Fenfluramine In Vivo and In Vitro

The effect of fenfluramine at doses of 2, 6 and 20 mg/kg on GDP binding to mitochondria from interscapular brown adipose tissue 3 hours after drug administration is shown in Fig. 1. A dose response relationship is noted, but only the 20 mg/kg dose produced a significant increase in GDP binding, $F(3,16)=7.48$, $p<0.005$. Although a plateau was not obtained, the 20 mg/kg dose was used in subsequent experiments, because in vivo doses much greater than 20 mg/kg are lethal [11]. When fenfluramine was added in vitro to the incubation of mitochondria with GDP there was no change (Table 1). However the effect of 3 hours of pretreatment with fenfluramine is clearly seen (Table 1).

Experiment 2. Time Course of Response to Fenfluramine

The time course of response to fenfluramine in the presence and absence of food is shown in Fig. 2. The small decline in binding of GDP in control animals without food between 0 and 3 hr was not statistically significant. There was no further decline at 24 and 48 hours. In contrast, the stimulatory effect of fenfluramine was readily detected at 3 hours, $t(10)=6.32$, $p<0.01$, and GDP binding remained significantly elevated in animals which received food (open circles) as well as in animals that received no food (closed circles). GDP binding per mg of mitochondrial protein between the two fenfluramine-treated groups (with or without food) was not significantly different at either 24 hours or 48 hours after treatment.

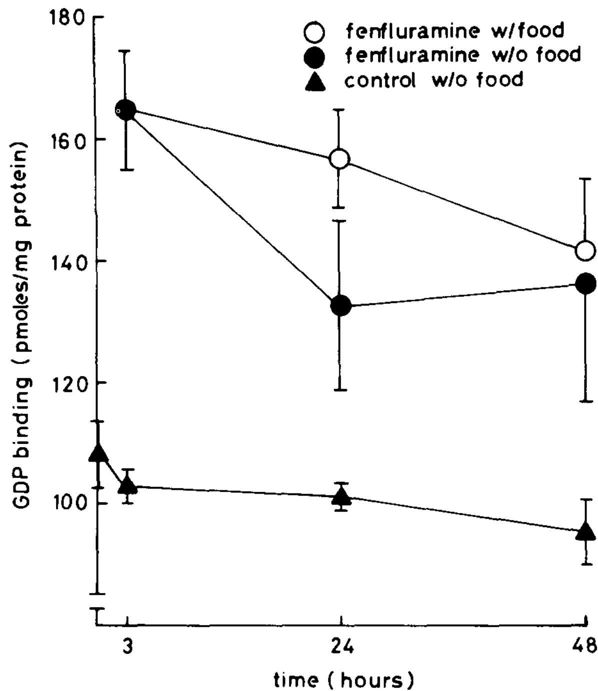

FIG. 2. Time course of response to fenfluramine in the presence or absence of food. Animals were divided into three groups: the two fenfluramine-treated groups received a single injection of fenfluramine (20 mg/kg, IP); half were food deprived and the other half ate ad-libitum. The control group was food-deprived and received a saline injection (IP). All data are plotted as mean ± SEM for GDP binding with four animals in each group. Open circles represent the fenfluramine-treated group with food, closed circles the fenfluramine-treated group without food and closed triangles control food-deprived group.

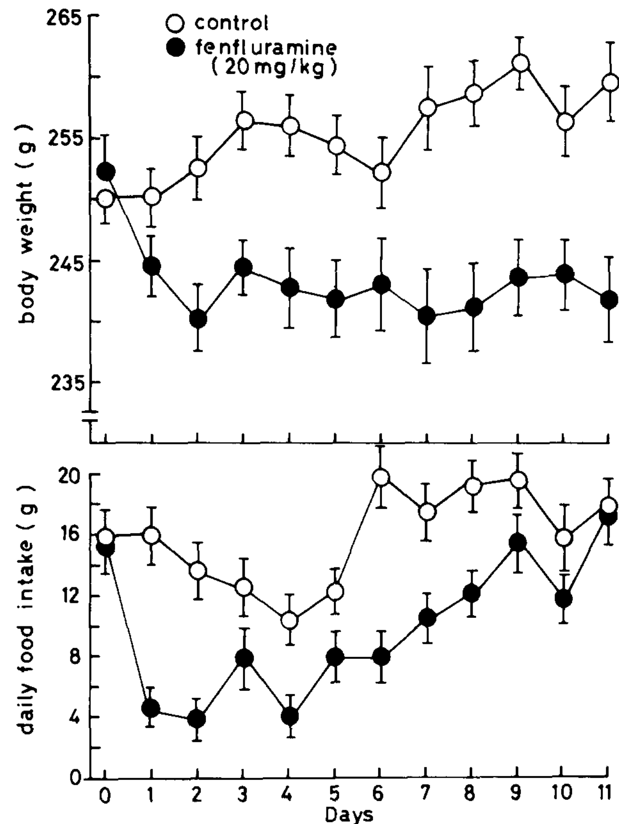

FIG. 3. Effects of chronic treatment with fenfluramine on food intake and body weight. Animals were divided into two groups of 8 animals each. The control group received an injection of saline injection (IP), and the experimental group received an injection of fenfluramine (20 mg/kg, IP). All animals were treated daily for 11 days. All data are plotted as mean ± SEM for body weight and daily food intake. Closed circles represent the fenfluramine-treated group, and the open circles the control group.

Experiment 3

The effects of chronic treatment with fenfluramine on food intake and body weight are shown in Fig. 3. For 11 days the animals were injected daily with fenfluramine (20 mg kg^{-1}, IP) and immediately replaced in their home cages. There was an initial drop in food intake and an associated decline in body weight from 252 to a low of 240 g. Thereafter the body weight of the fenfluramine-treated group remained essentially constant. The food intake after declining to 4 g/day gradually increased, and on the 9th, 10th and 11th days was not significantly different from the control values. The effects of this treatment on GDP binding to mitochondria from brown adipose tissue is shown in Table 2. The brown adipose tissue weight was not significantly different between the two groups either in absolute terms or on a body weight basis. The binding of GDP in pmole/mg protein was significantly higher in the fenfluramine-treated animals than in the vehicle-treated controls, $t(14)=3.82$, $p<0.01$.

DISCUSSION

The present studies have demonstrated a significant increase in GDP binding to mitochondria from rats treated with fenfluramine in vivo, but no effect when the drug is added in vitro. This in vivo effect of fenfluramine is dose dependent and detectable as early as 3 hours after drug administration and persists for at least 48 hours. After chronic treatment, when food intake has returned to normal, body weight remains significantly reduced, and the fenfluramine treated animals still have significantly elevated levels of GDP binding to mitochondria of brown adipose tissue.

Fenfluramine, a fluoromethyl substituted phenylethylamine differs in a number of respects from other anorectic drugs [4]. Amphetamine is thought to have its major effects through release of dopamine and/or norepinephrine from the neuroeffector junction [8]. Fenfluramine, on the other hand, appears to affect neurons involving serotonin [2,16]. Behaviorally, the effects of amphetamine are characterized by a long initial delay in the onset of eating followed by rapid eating in short infrequent bursts [6]. Fenfluramine, on the other hand, produces a marked slowing in the rate of eating with early onset of satiety [16]. Thus amphetamine appears to suppress hunger whereas fenfluramine enhances satiety.

The possibility that amphetamine might increase metabolic rate was clearly documented by Beyer [1] who found

TABLE 2

PURINE NUCLEOTIDE GDP BINDING TO INTRASCAPULAR BROWN ADIPOSE TISSUE MITOCHONDRIA FROM FENFLURAMINE AND CONTROL RATS

	Control	Fenfluramine
Animal body weights (g)	259.7 ± 2.87*	241.8 ± 4.47[a]
Intrascapular brown adipose tissue mass (mg)	260.1 ± 11.0	257.2 ± 30.1
Intrascapular brown adipose tissue/100 g body weight	100.4 ± 4.6	106.5 ± 12.6
Mitochondrial protein (mg)	3.92 ± 0.33	4.18 ± 0.19
GDP binding (pmol GDP/mg protein)	114.5 ± 7.45[a]	177.9 ± 15.11[a]
n	8	8

*Results are expressed as mean ± SEM. Groups with the same letter are significantly different $p < 0.01$.

an average 15% rise in metabolic rate after administration of amphetamine to normal subjects [1]. However, this mechanism for the action of this drug was largely ignored for more than 40 years. Rather the studies by Harris *et al.* [10] pointed to appetite suppression as the principal mechanism of action for this drug. They noted a dose-dependent reduction in food intake in amphetamine-treated dogs. Moreover there was no weight loss when human subjects who ate a constant diet were treated with amphetamine. Since these classic studies there has been a voluminous literature on the appetite suppressing effects of amphetamine and related derivatives [3,8].

The possibility that some of the appetite suppressant drugs might have important thermogenic effects has recently been resurrected. Sykas *et al.* [20] showed that both d-amphetamine and mazindol increased resting oxygen consumption in conscious rats, but that diethylpropion was without effect. In the anesthetized rat diethylpropion along with mazindol and amphetamine increased oxygen consumption, an effect which is thought be to mediated by peripheral mechanisms. Further evidence for an effect independent of food intake was published by Levitsky *et al.* [12] who demonstrated that fenfluramine was only anorectic in animals at normal body weight. When body weight was reduced 15% by food restriction prior to treatment with fenfluramine, the drug-treated animals ate amounts of food comparable to control animals during drug treatment, but nonetheless maintained a lower body weight. This effect was similar to the observation of Powley and Keesey [15] who showed that animals with lateral hypothalamic lesions which produced hypophagia in ad lib fed animals does not produce hypophagia if animals have been weight-reduced prior to introduction of these lesions. The recent demonstration that lateral hypothalamic lesions [22] are associated with an increased turnover of norephinephrine in brown adipose tissue suggested the possibility that the effects of fenfluramine observed by Levitsky *et al.* [12] might be the result of increased thermogenesis from this drug. The present experiments are consistent with that hypothesis. They have demonstrated an increase in the binding of GDP to the brown adipose tissue mitochondria. This index of brown adipose tissue activity is highly correlated with the thermogenic properties of this tissue [13]. It would thus appear that in addition to its influences on satiety, fenfluramine activates thermogenesis in brown adipose tissue.

Glick and his colleagues [9] have proposed that one mechanism for terminating eating may be related to the thermogenic effects of single meals. The present experiments are consistent with this mechanism of satiety. The enhanced thermogenesis in brown adipose tissue of fenfluramine-treated animals might produce a greater and/or earlier heat response to single meals. This in turn might trigger an earlier onset of satiety.

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