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Chronic *d*-Fenfluramine Treatment Reduces Fat Intake Independent of Macronutrient Preference

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SMITH, B. K., D. A. YORK AND G. A. BRAY. *Chronic d-fenfluramine treatment reduces fat intake independent of macronutrient preference.* PHARMACOL BIOCHEM BEHAV **60**(1) 105–114, 1998.—We investigated the effect of chronic dexfenfluramine (DFEN) treatment on macronutrient selection in a three-choice diet paradigm using Sprague–Dawley rats. Baseline macronutrient intakes were measured for several days before the initiation of treatment. In Experiment 1, daily intraperitoneal injections of DFEN (1.5 mg/kg) or saline were administered 60 min before dark onset for 12 consecutive days and 24 h macronutrient intakes were measured. DFEN significantly reduced absolute fat intake (kcal) by 30% and relative fat intake (% of total energy) by 14% in animals that received dexfenfluramine treatment compared to controls over the 12-day period. Absolute carbohydrate intake was increased 24% compared to controls, but this difference was not significant. These changes in food intake resulted in a 10% lower total energy intake. Upon discontinuation of the drug, fat intake of the DFEN-treated rats rebounded to control levels within 24 h. In Experiment 2, rats were assigned to carbohydrate- or fat-preferring groups based on the ratio of their average daily carbohydrate to fat intake (kcal). All animals then received DFEN. During DFEN treatment, fat-preferring rats reduced their daily fat intake from 62 to 53% of total energy. The low baseline fat intake of carbohydrate-preferring rats was reduced even further by DFEN (from 24 to 15% of total energy). These corresponding effects of DFEN on macronutrient selection in both fat- and carbohydrate-preferring rats indicate that chronic DFEN treatment selectively suppressed fat intake independent of the preferred macronutrient diet. © 1998 Elsevier Science Inc.

Macronutrient selection Fat Carbohydrate Dexfenfluramine Food intake

DEXFENFLURAMINE, the dextro-stereoisomer of *d,l*-fenfluramine, is a selective serotonin agonist that enhances 5-hydroxytryptamine (5-HT) release and blocks its reuptake by presynaptic terminals. Systemic treatment with dexfenfluramine decreases caloric intake and induces weight loss in humans and laboratory animals by a mechanism(s) that remains controversial (13,30,33), although it is generally assumed that dexfenfluramine causes hypophagia by increasing synaptic 5-HT levels in the hypothalamus (37).

Over the last 2 decades, investigations of the effect of dexfenfluramine on food choice focused primarily on carbohydrate and protein selection. Most animal studies were not designed to evaluate the effect of dexfenfluramine on fat selection because the proportion of fat in the test diets was usually held constant (10,17,21,26,27,47). Using this experimental design, a reduction in the intake of a high-carbohydrate/low-protein diet was typically demonstrated. However, reductions in both fat and protein intake have also been reported after acute peripheral administration of fenfluramine when animals were given access to three separate macronutrient sources (31,39). Finally, recent evidence indicates that dexfenfluramine may also reduce human fat consumption, as measured by direct assessment of caloric intake, in obese (23) and in healthy lean subjects (15).

We and others have identified subpopulations of Sprague–Dawley rats with strong baseline preferences for fat or carbohydrate (32,38,40–41,43). Recent work has shown that these preexisting nutrient preferences significantly influence the animal's diet selection in response to various orexigenic stimuli (41,46). It is not known, however, whether macronutrient

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preferences such as these also influence feeding responses to anorexigenic stimuli. In the present study, we investigated the effect of long-term dexfenfluramine treatment on daily macronutrient intake of nondeprived rats in a choice paradigm where animals were allowed to select among three individual macronutrient sources. We hypothesized that, if the suppressive effect of dexfenfluramine on fat consumption is specific for this nutrient, the drug would reduce fat intake independent of the animal's baseline macronutrient preferences.

METHOD

Animals

Ten-week-old male Sprague–Dawley rats were purchased from Harlan–Sprague–Dawley (Indianapolis, IN) and housed individually in hanging wire-mesh cages (35 × 22 × 15 cm) with lights on from 0600 to 1800 h. The ambient room temperature was maintained at 21–23°C. The animals were fed rodent chow (Purina #5001) and tap water ad lib for several days after they arrived in the facility until they were placed on the experimental diets. For both experiments, the rats were approximately 14 weeks old when dexfenfluramine treatment began (average body weight: 362 ± 5 g in Experiment 1 and 347 ± 6 g in Experiment 2).

Experimental Diets

Animals were placed on the experimental diet at least 2 weeks before behavioral testing began. A three-choice macronutrient diet paradigm [e.g., (19,29)] was used in which rats self-select from three food cups, each containing a single macronutrient source supplemented with vitamins and minerals: carbohydrate (corn starch and powdered sugar), fat (vegetable shortening), and protein (casein) (see Table 1). The cups were secured with steel springs to the front of the cage and their positions alternated daily. The fat source was changed every 48 h in Experiment 1 and daily in Experiment 2.

Drugs

S(+)-fenfluramine hydrochloride (Research Biochemicals, Natick, MA) was prepared fresh daily for injection by dissolving in sterile 0.9% NaCl and administered as a 1.5 mg/ml solution. The 1.5 mg/kg/day dose was chosen because it has been shown to produce significant, yet moderate hypophagia (30–40% suppression of ad lib food intake) (35,36), and thus would allow us to observe the selectivity of dexfenfluramine on macronutrient selection.

TABLE 1
COMPOSITION OF MACRONUTRIENT DIETS*

	Carbohydrate	Fat	Protein
Corn starch	58.11	0.00	0.00
Powdered sugar	29.06	0.00	0.00
Casein	0.00	0.00	87.17
DL-Methionine	0.11	0.20	0.11
Vegetable shortening	0.00	75.12	0.00
AIN-76A vitamin mix†	0.77	1.49	0.77
AIN-76A mineral mix†	3.07	5.95	3.07
Choline chloride	0.18	0.34	0.18
Cellulose (Alphacel)	8.72	16.91	8.72
Energy density (kcal/g)	3.53	6.85	3.53

*Ingredients expressed as percent by weight.
†The vitamin and mineral mixes contain 97 and 12% sucrose, respectively.

Experimental Design

Two separate experiments were performed. In each experiment, baseline macronutrient preferences were determined by measuring 24 h intakes for several days before the initiation of dexfenfluramine treatment (1.5 mg/kg/day × 12 days). In Experiment 1, a between-subjects design was used in which groups of dexfenfluramine- or saline-injected rats were compared directly. Animals with a high baseline intake of fat were selected and the treatment groups were balanced for baseline fat intake by computing a ratio of the number of fat and carbohydrate calories consumed during the pretreatment period. Control animals received daily injections of sterile saline (SAL, 1 ml/kg IP). In Experiment 2, a within-subjects design was used in which the responses of animals to dexfenfluramine were compared across time periods. First, rats were assigned to carbohydrate- or fat-preferring groups based on the ratio of average carbohydrate to fat intake (kcal), i.e., carbohydrate-preferring rat's F:C ratio was less than 1. All animals then received DFEN. Control groups were not employed in this experiment because the saline comparison was previously performed in Experiment 1 using the same protocol.

Each day approximately 1 h before injection, the food jars were removed to be weighed and filled. Then 60–90 min before dark onset, rats were injected intraperitoneally with dexfenfluramine or sterile 0.9% NaCl. Preweighed jars of fresh food were placed in the cages 30 min after injection. The same balance was used throughout the study to weigh jars and spillage to the nearest 0.1 g. All spillage was collected on cardboard pads placed beneath the hanging wire bottom cages. Specifically, fat spillage (solid consistency) was minimal and was recovered manually; spillage from powdered diets was collected with a brush.

Data Analysis

Macronutrient intakes were converted to kilocalories and expressed as means ± SEM for each day of the study. In Experiment 1, a two-factor repeated measures (treatment × day) analysis of variance (ANOVA) was used to examine macronutrient intakes within diet and period (pre, treatment, and post). Secondary comparisons were made using the Tukey—Kramer adjustment. Body weight gains relative to the day before initiation of treatment were analyzed with repeated measures ANOVA with respect to day. In Experiment 2, one-way ANOVA was used to compare within preference groups (carbohydrate- and fat-preferring rats) the effect of dexfenfluramine on macronutrient intakes across three time periods (pretreatment, treatment, and posttreatment). If a main effect of time period was observed, then specific group comparisons were made using Tukey's Studentized Range (HSD) test.

RESULTS

Experiment 1: Effect of Chronic Dexfenfluramine on Macronutrient Selection

The effect of 12 days of dexfenfluramine (DFEN) treatment on macronutrient self-selection in rats with a high baseline intake of fat (DFEN, 62 ± 2% vs. SAL, 60 ± 4% of total energy) is presented in both Figs. 1 (daily time course) and 2 (averages for each time period). During the pretreatment period (days −3 to day 0), there were no differences in macro-

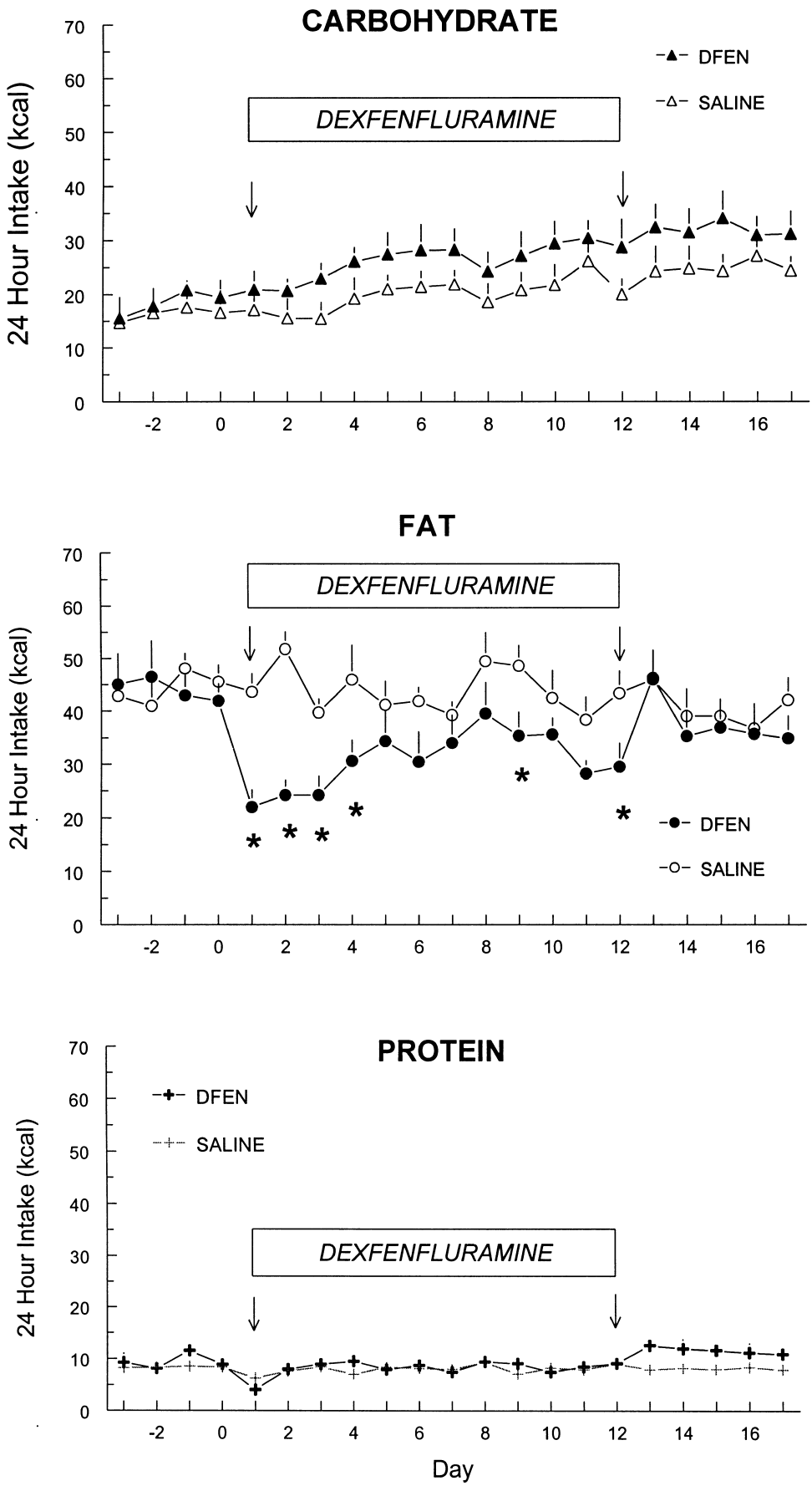

FIG. 1. Effect of 12 days of dexfenfluramine (DFEN, $n = 8$) or saline (SALINE, $n = 7$) injections on 24 h carbohydrate, fat, and protein intakes of Sprague–Dawley rats across the experimental period. Dexfenfluramine was injected daily approximately 90 min before dark onset on days 1 to 12. All values are mean ± SEM in this and subsequent figures. *Significantly different from saline, $p < 0.05$.

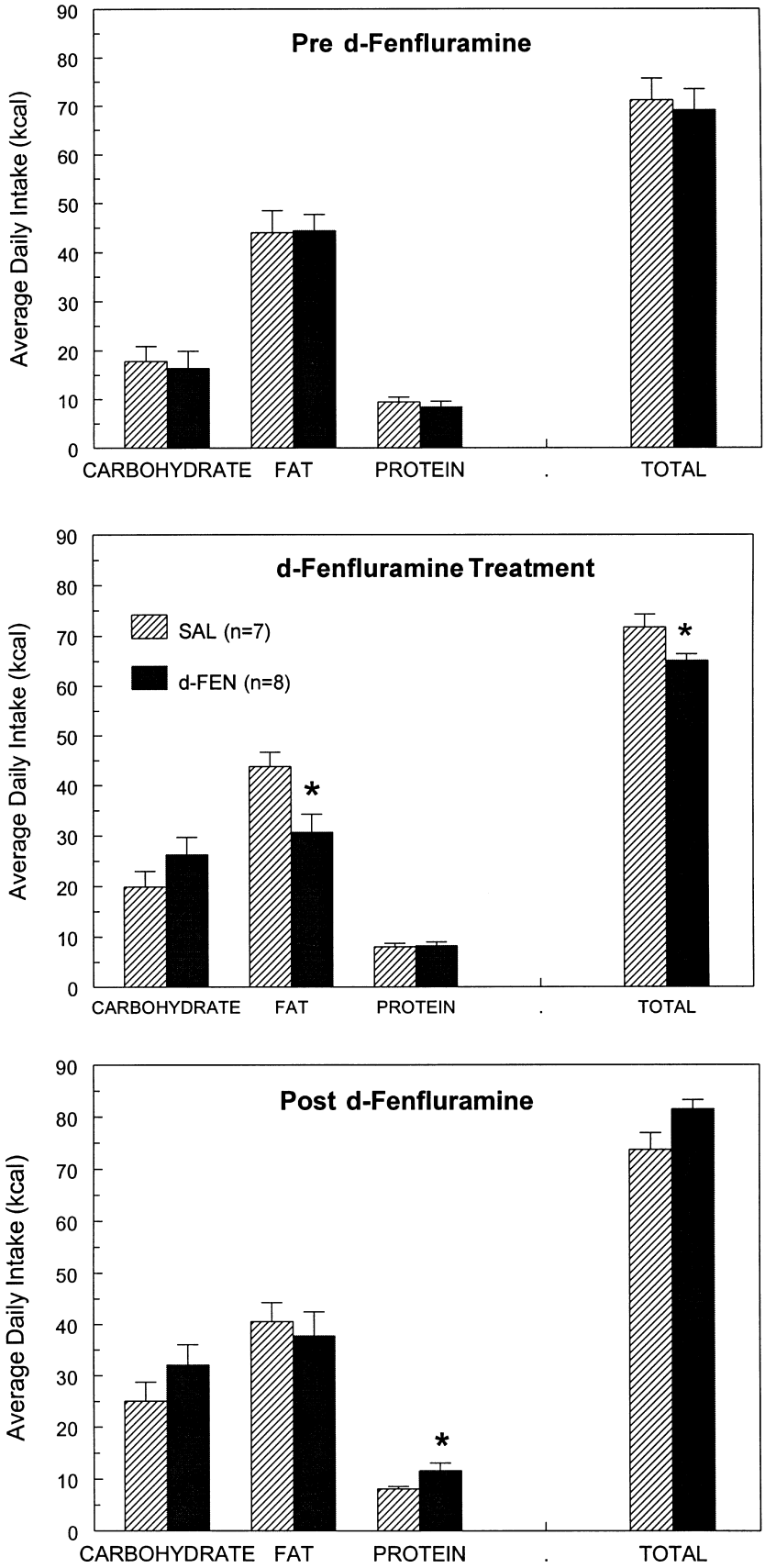

FIG. 2. Effect of 12 days of dexfenfluramine (DFEN, $n = 8$) or saline treatment (SAL, $n = 7$) on daily macronutrient intakes averaged across each time period: pretreatment, DFEN treatment, and posttreatment. *Significantly different from saline, $p < 0.05$.

nutrient intakes with respect to treatment or day. However, during dexfenfluramine administration, absolute fat intake was a significantly suppressed as a main effect of treatment, $F(1, 13) = 7.90$, $p < 0.05$, and of time, $F(11, 143) = 2.87$, $p < 0.005$, producing a significant interaction, $F(11, 143) = 2.25$, $p < 0.05$ (Fig. 1). Thus, the average daily fat intake of dexfenfluramine-treated rats during the treatment period was reduced by 30% compared to controls (DFEN, 31 ± 3 kcal vs. SAL, 44 ± 3 kcal, $p < 0.05$) (Fig. 2b).

Carbohydrate consumption did not change as a result of DFEN administration, $F(1, 13) = 1.82$, $p = 0.19$ but increased over time in both groups, $F(11, 143) = 6.03$, $p < 0.0001$ (Fig. 1). These changes amounted to a small but nonsignificant increase in average daily carbohydrate intake (23%) (DFEN, 26 ± 3 vs. SAL, 20 ± 3 kcal) (Fig. 2), while protein intake remained essentially unchanged. Thus, the rats that received dexfenfluramine reduced their average daily caloric intake by 9% ($p < 0.05$) (Fig. 2). After discontinuation of the drug, average daily fat intake returned to control level within 24 h, while protein intake remained significantly increased, $F(1, 13) = 5.04$, $p < 0.05$ (Fig. 2).

Total energy intake. Changes in total daily energy and body weight across the experimental period are shown in Fig. 3. There was no difference in energy intake between the two groups at baseline (DFEN, 71 ± 3 kcal vs. SAL, 69 ± 3 kcal) (Fig. 3a). Repeated-measures ANOVA revealed that although there was no main effect of DFEN treatment on daily energy intake, there was a significant treatment × time interaction, $F(21, 273) = 3.36$, $p < 0.001$. Specifically, dexfenfluramine decreased energy intake during the first 2 days of treatment ($p < 0.005$) and then intake gradually returned to control level for the remaining treatment days. After the last injection on day 12, energy intake of the DFEN-treated rats tended to remain slightly higher than that of controls during the posttreatment period.

Body weight gain. Changes in body weight gain across the experimental period are shown in Fig. 3. Initial body weight was not different between the two groups at the beginning of dexfenfluramine treatment (DFEN, 368 ± 5 g; SAL, 357 ± 9 g) (Fig. 3b). Only on treatment day 4 ($p < 0.05$) was the weight gain of the dexfenfluramine group significantly less than the control group. By the end of 12 days of treatment, the dexfenfluramine group had gained 22% less than the control group, and by day 20 weight gain of the DFEN group was essentially the same as that of control animals (Fig. 3b).

Experiment 2: Effects of Chronic Dexfenfluramine on Macronutrient Selection in Fat- and Carbohydrate-Preferring Rats

The effect of chronic dexfenfluramine treatment on macronutrient self-selection was examined separately in additional groups of fat- and carbohydrate-preferring Sprague–Dawley rats using a within-subjects design, and is illustrated in both Figs. 4 (daily time course) and 5 (averages for each time period). Figure 4 is presented for descriptive purposes, while results for the one-way ANOVAs on macronutrient intakes across the three time periods are presented in Fig. 5. During the pretreatment period (days −6 to 0), average daily fat intake of fat-preferring rats was 46 ± 2 kcals (see dotted line, Fig. 4a), while carbohydrate-preferring rats consumed an average of 18 ± 2 kcal (see dotted line, Fig. 4b). Thus, fat calories accounted for 62 and 24% of baseline energy intake for the two groups, respectively.

In fat-preferring rats, DFEN treatment resulted in significant changes across time periods for fat, $F(2, 36) = 7.34$, $p < 0.005$, and carbohydrate , $F(2, 36) = 8.53$, $p < 0.0001$, but not protein intake, $F(2, 36) = 1.25$, $p = 0.30$ (Fig. 5a). Thus, fat-preferring rats consumed an average of 56% less absolute fat during DFEN treatment (PRE-, 46 ± 2 vs. TRT, 20 ± 2 kcal), resulting in a 12% reduction in total energy, $F(2, 36) = 11.19$, $p < 0.001$). However, their intake of fat did not recover to baseline level after discontinuation of the drug; in fact, fat-preferring rats ate significantly more carbohydrate during the posttreatment period (POST-, 28 ± 2 vs. TRT, 20 ± 2 and PRE-, 16 ± 2 kcals, $p < 0.05$) (Fig. 5a).

In carbohydrate-preferring rats, DFEN treatment resulted in significant changes across time periods for fat, $F(2, 33) = 7.61$, $p < 0.005$, and protein, $F(2, 33) = 7.30$, $p < 0.005$, while carbohydrate intake, $F(2, 33) = 2.87$, $p = 0.07$, remained unchanged (Fig. 5b). Thus, DFEN treatment reduced absolute fat intake by 45% (PRE-, 18 ± 2 kcals vs. TRT, 10 ± 1 kcals) and protein intake by 20% (PRE-, 10 ± 1 kcals vs. TRT, 8 ± 0 kcals). These combined changes in macronutrient intakes account for an 11% decrease in total caloric intake observed during the treatment period, $F(2, 33) = 17.98$, $p < 0.001$). In the posttreatment period, the fat intake of carbohydrate-preferring rats returned to pretreatment level while protein consumption increased slightly (Fig. 5b). The increase in protein consumption, along with a nonsignificant increase in carbohydrate intake, produced a small rebound in the energy intake (9%) of carbohydrate-preferring rats during posttreatment.

DISCUSSION

To our knowledge, this report is the first to describe the effect of chronic dexfenfluramine treatment on diet selection using the three-choice macronutrient paradigm. The most important result of this investigation is that chronic systemic administration of dexfenfluramine significantly suppressed fat intake in freely feeding rats allowed a choice of macronutrient diets. In Experiment 1, we observed a 30% decrease in absolute fat intake (kcal) and a 14% reduction relative to total energy intake (% kcal) in animals that received dexfenfluramine treatment compared to controls over the 12-day period of drug administration. Absolute carbohydrate intake during treatment was 24% higher for dexfenfluramine-treated rats compared to controls, but this did not reach statistical significance. Total energy intake was significantly different, amounting to a 9% reduction in average daily calories for the duration of treatment. In Experiment 2, a significant suppression of fat intake was observed in both carbohydrate- and fat-preferring rats, indicating that dexfenfluramine does not simply decrease intake of the preferred macronutrient diet. Although in Experiment 2 the reduction in fat intake during dexfenfluramine treatment is greatest within the first 24 h, daily fat intake of both preference groups remained below the pretreatment average for the entire period of dexfenfluramine administration (see dotted lines, Fig. 4). Finally, the observation in Experiment 1 of a stable and persistent modification of macronutrient selection (i.e., reduced fat intake and slightly enhanced carbohydrate intake) after total energy intake had returned to baseline levels, is remarkable.

Consistent with previous studies of dexfenfluramine anorexia, the reduction in energy intake lasted for approximately 4–5 days (7,11,34), and then caloric intake gradually returned to baseline levels. The anorexia observed in the present study was attributed entirely to a reduction in the intake of dietary fat. The decrease in fat intake observed in these experiments agrees with the results of other studies examining the acute effects of serotonergic compounds on diet selection by laboratory animals (19,31). For example, a single low dose of dex-

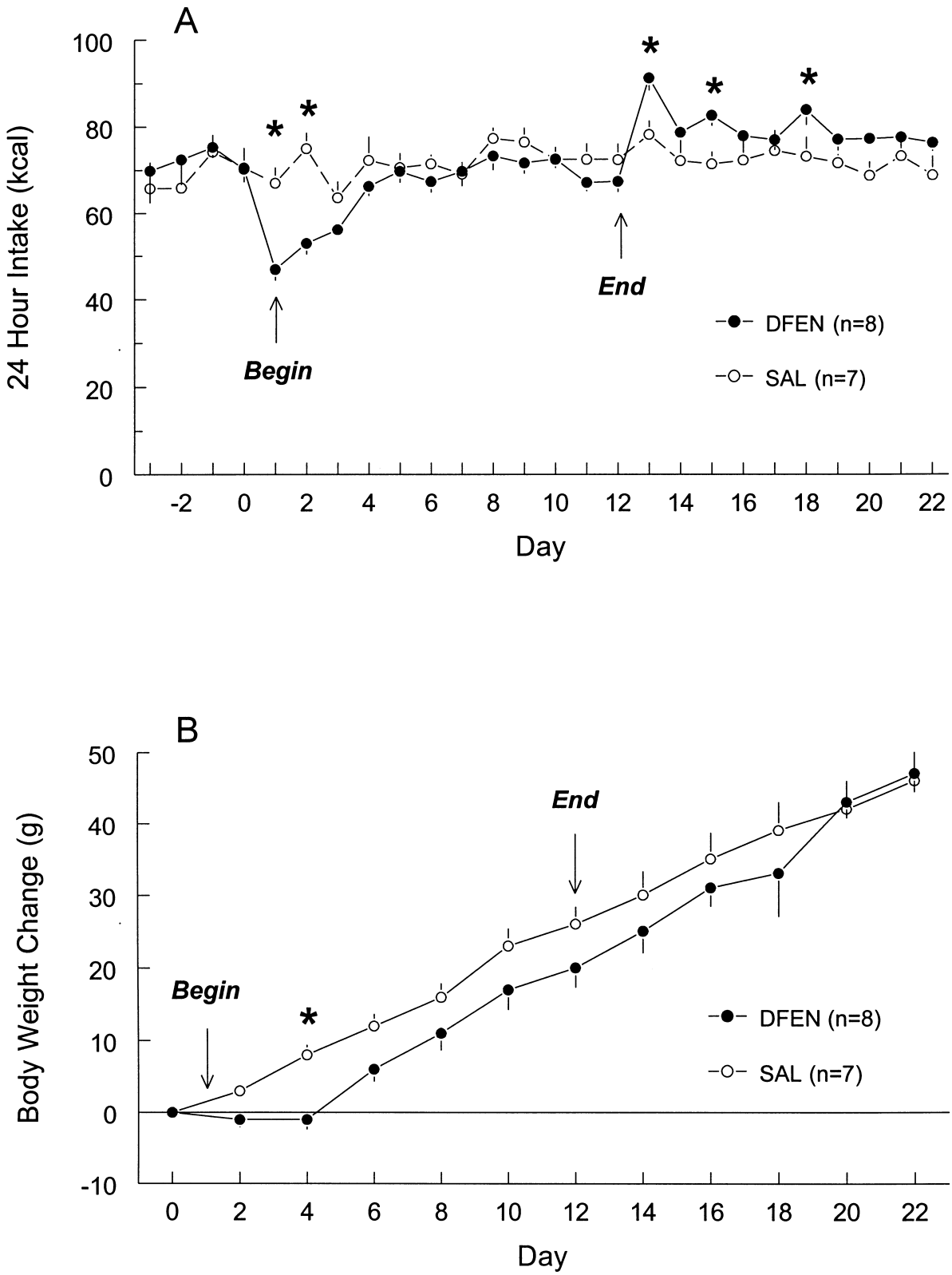

FIG. 3. Effect of 12 days of dexfenfluramine treatment on daily 24 h energy intake (A) and body weight gain (B) across the treatment period (days 1–12). *Significantly different from saline, $p < 0.05$.

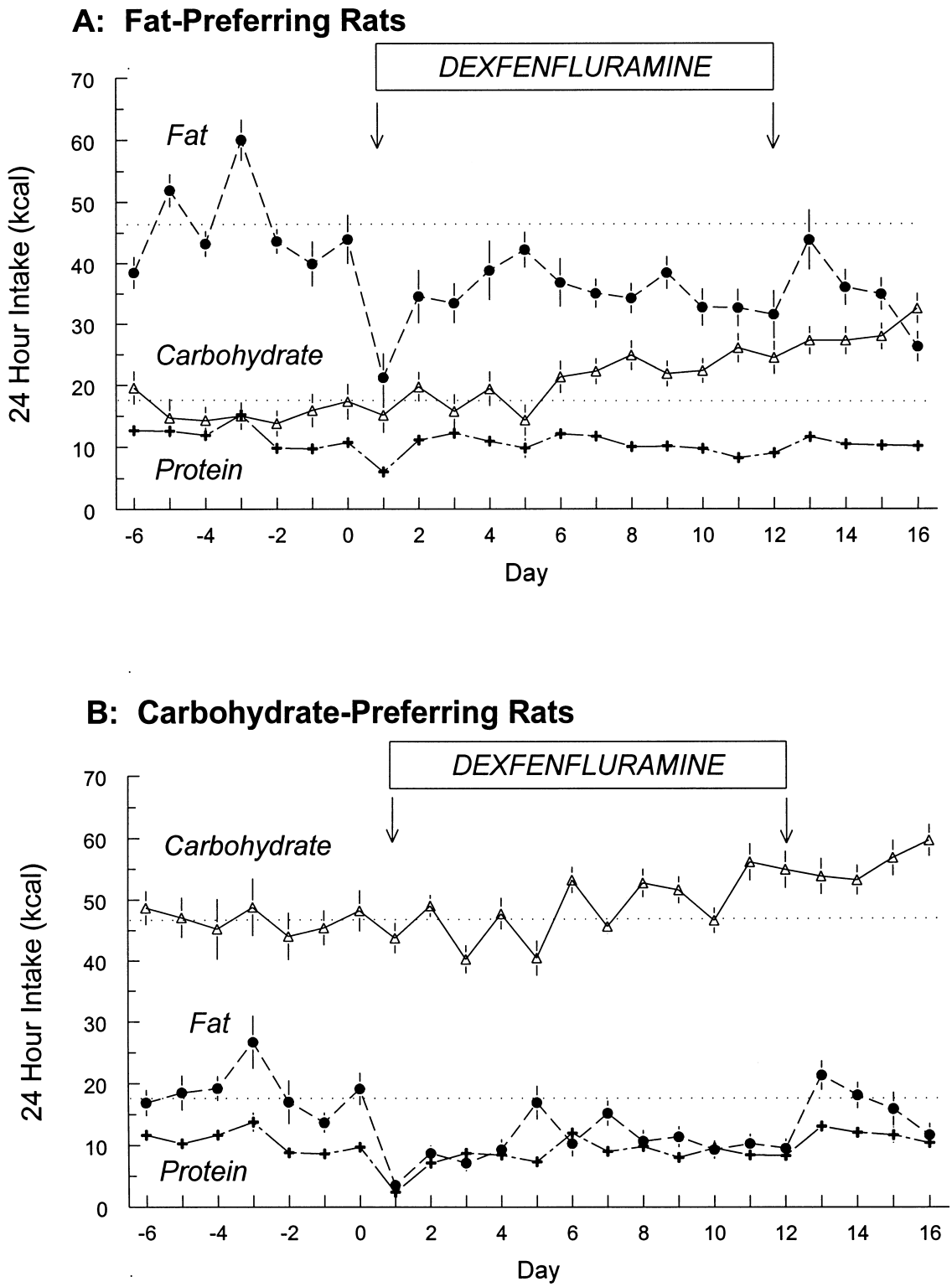

FIG. 4. Daily carbohydrate, fat, and protein intakes in kcal for fat- (upper panel) (n = 13) and carbohydrate-preferring (lower panel) (n = 12) rats across the experimental period. Dexfenfluramine was injected daily approximately 90 min before dark onset on days 1–12. The dotted lines represent the average baseline caloric intakes of fat and carbohydrate.

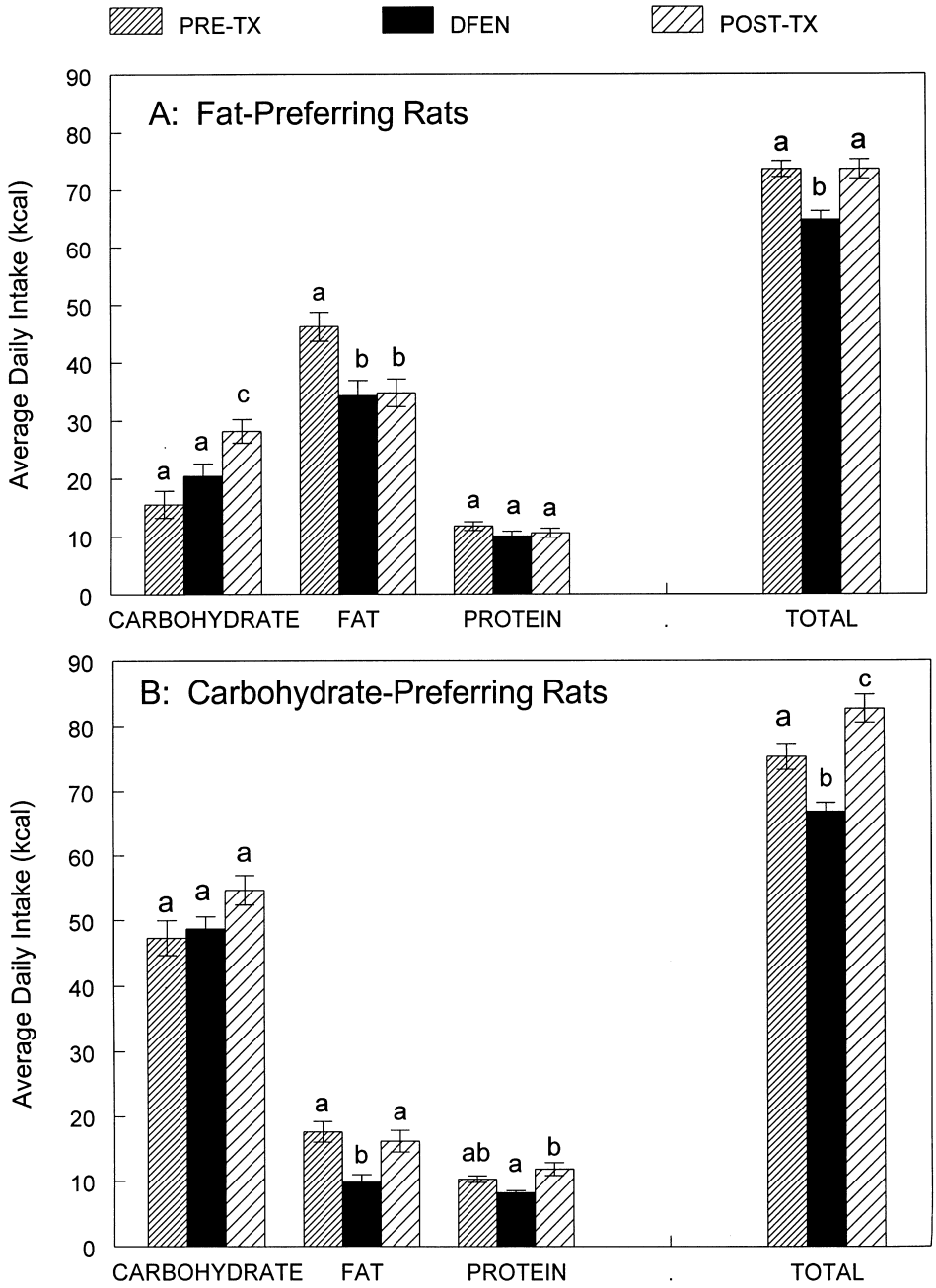

FIG. 5. Average daily macronutrient intakes of fat- (upper panel) and carbohydrate-preferring (lower panel) rats treated for 12 days with dexfenfluramine (DFEN). Within each macronutrient diet, means without common superscripts differ significantly between time periods, $^{*}p < 0.05$.

fenfluramine spared carbohydrate while suppressing both fat and protein intakes in schedule-fed rats (31). However, the present study constitutes the first report of a suppression of fat intake by with repeated daily administration of dexfenfluramine. Previous investigations of the chronic effect of dexfenfluramine on diet selection were limited to those employing a choice between two diets differing in protein and carbohydrate content (10,27), chow (34), chow plus supplement (2), a palatable dessert regimen (44), or a cafeteria diet (8). Yet in another study using single composite diets, the acute administration of a low dose of dexfenfluramine produced a greater reduction in food intake for rats fed the high-fat diet than for rats fed the high-carbohydrate diet (20).

For more than 2 decades, investigations of the effect of dexfenfluramine on diet selection have focused primarily on the intake of carbohydrate and protein. Most animal studies were not designed to evaluate the effect of dexfenfluramine on fat selection, i.e., the proportion of fat in the test diets was held constant. Using this type of experimental design, a reduction in the intake of a high-carbohydrate/low-protein diet was

typically demonstrated (17,21,26,27,47). Results from investigations of the effect of dexfenfluramine on food choice in humans were also influenced by methodologies (3), for example, the fat content of test foods was held constant, or not taken into consideration as in the case of high-carbohydrate snacks (48), thus precluding the possibility of detecting an effect of 5-HT manipulation on fat intake. More recently, however, a study of obese subjects showed that 3 months of dexfenfluramine treatment significantly reduced daily fat consumption, as measured by direct clinical assessment of caloric intake (23). In another study of long-term dexfenfluramine treatment in which macronutrient selection was measured by self-report, fat intake was not significantly different from the placebo group after 12 months of treatment (5). Within the drug and placebo groups, however, there appeared to be trend toward a reduction in energy intake from fat over the treatment period indicating a possible placebo effect (5). A role of 5-HT activation in fat selection is further corroborated by evidence that acute administration of dexfenfluramine can induce lean subjects to avoid fat when refed after an overnight fast (15).

The corresponding effects of dexfenfluramine on macronutrient selection in both fat- and carbohydrate-preferring rats demonstrate that dexfenfluramine specifically suppresses fat intake and does not simply modify intake of the preferred macronutrient. Thus, dexfenfluramine reduced the intake of fat when it was the preferred diet and when it was not. These results are in contrast with recent evidence that the effects of orexigenic peptides such as galanin and neuropeptide Y on diet selection are clearly dependent on baseline macronutrient preferences (41,43,46). Thus, it appears that the serotonin system exerts its effects on feeding in a manner that overrides the mechanism(s) responsible for dietary preference.

Systemically administered dexfenfluramine, along with its active metabolite *d*-norfenfluramine, readily cross the blood–brain barrier and decreases food intake by a mechanism involving 5-HT receptors. The anorectic effect of dexfenfluramine may be accounted for by its central action to increase serotonergic transmission in the hypothalamus (37), or by the direct agonist action of the metabolite *d*-norfenfluramine on postsynaptic 5-HT receptors (13,33). The local application of dexfenfluramine or *d*-norfenfluramine to the paraventricular nucleus of the hypothalamus suppresses food intake (28,42,45). The activation of postsynaptic 5-HT receptors by *d*-norfenfluramine is supported by evidence that 1) fluoxetine, a selective inhibitor of the 5-HT transporter, does not block the hypophagic activity of dexfenfluramine (33); and 2) the hypophagic effects of *d*-fenfluramine and *d*-norfenfluramine are not decreased by pretreatment with a 5-HT synthesis inhibitor (13). Despite the fact that direct application of 5-HT onto the PVN is sufficient to suppress feeding in nondeprived animals [unpublished data from our laboratory; (12)], some controversy remains as to whether increased synaptic 5-HT in the hypothalamus is necessary for hypophagia to occur following dexfenfluramine administration (30,33).

It is possible that the anorexic effect of dexfenfluramine is at least partially mediated by corticotropin-releasing hormone (CRH), a peptide whose ability to suppress food intake has been extensively described (14) and which may specifically inhibit fat intake (25). Acute administration of dexfenfluramine increases the concentration of CRH in the hypothalamus (18). Moreover, the anorexic effect of intracerebroventricular injection of fenfluramine is attenuated by pretreatment with a central CRH antibody (24). Recently it was reported that fenfluramine stimulated c-*fos* expression in CRH-containing neurons (16,22) and induced mRNA for the CRH_1 receptor in CRH cells of the paraventricular nucleus of the hypothalamus (22), providing further evidence for a mechanism related to the activity of the hypothalamic–pituitary–adrenal axis.

It is worth noting that the observed changes in macronutrient selection in the present study could also be related to alterations in peripheral energy metabolism induced by dexfenfluramine (4). Specifically, dexfenfluramine treatment decreases the synthesis of fatty acids and triacyglycerols and increases lactate production by adipose tissue in rats (1). In addition, it has been reported that fenfluramine increased lipid oxidation and decreased carbohydrate oxidation in rats with resulting increases in serum free fatty acids and triglycerides (4,6,9). Thus, it is possible that a drug-induced increase in fat mobilization could lower the drive for fat selection, as has been suggested previously to explain the reduction in fat intake of exercising rats (29).

The results of this study provide new information regarding the effects of dexfenfluramine on macronutrient selection. Repeated daily administration of low-dose dexfenfluramine significantly decreased fat intake and enhanced carbohydrate intake. Moreover, the prolonged modifications in macronutrient selection after total energy intake returned to control level indicate that dexfenfluramine may provide a pharmacological inhibition of fat appetite. Finally, we observed that dexfenfluramine selectively suppressed fat intake in animals with a low baseline intake of fat demonstrating that the drug's actions were independent of diet preference. These results provide evidence that the serotonergic system is an important component of the system controlling the appetite for dietary fat.

ACKNOWLEDGEMENTS

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