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Reduction of Fat and Protein Intakes But Not Carbohydrate Intake Following Acute and Chronic Fluoxetine in Female Rats

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HEISLER, L. K., R. B. KANAREK AND B. HOMOLESKI. *Reduction of fat and protein intakes, but not carbohydrate intake following acute and chronic fluoxetine in female rats.* PHARMACOL BIOCHEM BEHAV **63**(3) 377–385, 1999.—Fluoxetine hydrochloride, a selective serotonin reuptake inhibitor, leads to reductions in food intake and body weight and is under investigation as a possible treatment for obesity. Additionally, it has been suggested that fluoxetine administration could lead to a selective suppression in carbohydrate consumption. Because women more often than men seek weight reduction treatment, the present study examined the acute and chronic effects of fluoxetine on food intake, macronutrient selection, body weight, estrous cycle, and motor activity in female rats. Female Long–Evans rats were provided with separate sources of protein, fat and carbohydrate, and nutrient intakes were recorded following single (5.0, 10.0, and 20.0 mg/kg, IP) and chronic daily (10 mg/kg for 28 days) injections of fluoxetine. Acute and chronic administration of fluoxetine significantly reduced total caloric intake when compared to vehicle treatment. Moreover, fluoxetine significantly suppressed fat and protein intakes, but not carbohydrate intake following both acute and chronic drug administration. Animals chronically treated with fluoxetine gained significantly less weight than animals treated with vehicle. Chronic fluoxetine treatment did not significantly alter estrous cycle. However, in both fluoxetine- and vehicle-treated animals, total caloric intake, and carbohydrate and protein intakes were reduced and fat intake was increased when estrogen levels were high. Fluoxetine significantly reduced motor activity up to 4 h postinjection, and increased motor activity 24 h postinjection. © 1999 Elsevier Science Inc.

Fluoxetine Diet selection Carbohydrate Fat Protein Serotonin Food intake Body weight Reproductive cycle Estrus

IT has been proposed that an inverse relationship exists between central nervous system (CNS) serotonin (5-hydroxytryptamine) levels and energy intake [e.g., (6,8,16,17,24,29,39)]. In support of this proposal, drugs that increase serotonergic activity decrease energy intake, while drugs that block serotonergic activity increase energy consumption [e.g., (6,8,11,16,17, 28–30,38,39)]. Fluoxetine hydrochloride (Prozac), a drug that augments serotonergic activity by selectively inhibiting the reuptake of the neurotransmitter into the presynaptic nerve terminal (46,47), leads to reductions in food intake and body weight in both animal and human subjects, and thus, is being investigated for the treatment of obesity [e.g., (8,16,28,30,50)].

Past research on fluoxetine and other serotonergic drugs has addressed whether these agents produce a general or macronutrient-specific reduction in caloric intake. It has been hypothesized that serotonergic drugs selectively reduce carbohydrate intake due to a biological behavioral feedback mechanism [e.g., (12,25,33,50)]. This hypothesis is based on 1) research demonstrating that intake of pure carbohydrate increases CNS serotonin synthesis (12), and 2) studies reporting that increases in serotonergic activity lead to a selective decrease in carbohydrate consumption (25,29,30,33,48). Although some investigators have reported selective reductions in carbohydrate intake following administration of serotonin agonists, others have not (17,19,24,34,35,45). The differences among these studies indicate that other factors such as the mechanism of increasing central serotonin, diet composition, feeding schedule, and the hydration of the diets interact with the effects of these drugs on diet selection (7,21,22,26,27,32).

Another factor to consider when evaluating the effects of serotonergic drugs on caloric intake, macronutrient selection, and body weight is the sex of the animal. Most studies have

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assessed the actions of serotonergic drugs on food intake and body weight in male rats. However, research suggests that sex differences exist in the synthesis of serotonin (37), serotonin receptor binding (4, 13), and the effects of serotonergic drugs on behavior (10,11,43). The local hormonal environment during the reproductive cycle may also impact the effects of serotonergic drugs. For example, Uphouse and colleagues demonstrated that [^{3}H]5-HT binding was lowest during the morning of proestrus and highest during estrus and diestrus (44). Additionally, they reported that the response to a 5-HT_{1A} agonist on food intake was potentiated in diestrus compared to proestrus and estrus (43).

Energy intake and macronutrient selection vary across the female reproductive cycle [e.g., (1,5,14,23)]. More specifically, total energy intake of female rats decreases when circulating estradiol levels are high (in proestrus), and increases when estradiol levels are low (in diestrus) (5,41). Furthermore, ovariectomy, which eliminates gonadal steroids, produces increased food intake and body weight, whereas estradiol benzoate treatment reverses this effect (5,23,41). It has been suggested that the increase in food intake observed when levels of estrogen are reduced is the result of either increased consumption of all macronutrients (23) or a selective increase in fat intake (3).

Serotoninergic drugs not only affect feeding behavior, but also alter motor activity [e.g., (2,9,15,18,20,36,42)]. Whether activity is decreased or increased depends on the specific serotonergic drug administered, as well as the route of drug administration. Previous research has shown that peripheral administration of serotonin or precursors to serotonin reduce motor activity in experimental animals (36,42). The observation that serotonin can decrease locomotion is an important consideration in the analysis of fluoxetine anorexia because the desired effect, reduced food intake, could result from impaired movement. On the other hand, an increase in motor activity could accentuate body weight loss associated with fluoxetine administration.

The present study investigated the effects of acute and chronic fluoxetine administration on food intake, macronutrient selection, body weight, and locomotor activity in female rats. Additionally, by monitoring the reproductive cycle, potential interactions between hormonal status and fluoxetine treatment were assessed. Based on work with male rats, it was expected that fluoxetine would lead to hypophagia and reductions in body weight gain.

GENERAL METHOD

Animals

Adult female Long–Evans rats (CD outbred, Charles River Breeding Laboratories, Wilmington, MA) were used. Rats were housed individually in stainless steel cages in a temperature-controlled room (21 ± 1°C) maintained on a 12:12-h reversed light:dark cycle (lights off: 0800–2000 h).

Diets

Animals were allowed ad lib access to separate sources of protein, fat, and carbohydrate (Table 1). Protein and carbohydrate were provided in food cups (LC-306A, Wahmann, Timonium, MD), and fat was available in 50-ml glass jars. Protein and carbohydrate cups were alternated daily to avoid the development of position preferences. Food containers were secured to the cage to reduce spilling. The test diets were weighed to the nearest 0.1 g, and food cups refilled to the same level each day at the beginning of the dark portion of the 24-h cycle. Food spillage was observed for all rats each day; minor spills (<0.1 g) were not measured, but major spills (>0.1 g) were recorded as missing values. Water was continuously available.

Drug

Fluoxetine hydrochloride (Ly 110140) was supplied as a gift by Eli Lilly Co. Fluoxetine was dissolved in distilled water and administered IP in doses of 0.0, 5.0, 10.0, and 15.0 mg/kg in a volume of 1.0 ml/kg of body weight. Distilled water was the control vehicle. Doses of fluoxetine were chosen on the basis of previous studies examining food intake and diet selection in male rats (17,29,45,50).

Behavioral and Motor Assessment

The following behavioral and motor assessments were made after drug or vehicle injections.

Vocalizations. Multiple or loud vocalizations upon removal of the animal from its home cage were scored a 2, a single, low vocalization was assigned a 1, and no vocalizations were recorded as 0.

Hanging reflex. To evaluate this, animals were placed on a wire cage turned 90 degrees. Animals that successfully hung without slipping were assigned a 1, while those that slipped were assigned a 0.

Righting reflex. Animals able to right themselves when placed on their backs received a score of 1, and those that could not received a 0.

Activity. Activity was evaluated by small animal movement monitors (Colbourn Instruments). A cage similar to the rat's home cage was placed on a platform that electrically assessed small (e.g., grooming and sniffing) and large (e.g., walking and rearing) movements for 5 min.

All procedures were approved by the Tufts University Institutional Animal Care and Use Committee.

TABLE 1
DIETARY COMPONENTS

Protein Component (3.76 kcal/g)
960 g casein (ICN Pharmaceuticals, Cleveland, OH)
40 g AIN Mineral Mix (ICN Pharmaceuticals)
20 g Vitamin Diet Fortification Mix (ICN Pharmaceuticals)
Carbohydrate Component (3.76 kcal/g)
575 g corn starch (Teklad Test Diets, Madison, WI)
275 g dextrin (Teklad Test Diets)
100 g commercial-grade sucrose
10 g Solka-floc (BW-200, James River Corp., Berlin, NH)
40 g AIN Mineral Mix
20 g Vitamin Diet Fortification Mix
Fat Component (7.85 kcal/g)
912 g Crisco (Procter and Gamble, Cincinnati, OH)
48 g Safflower oil (Hollywood Health Foods, Los Angeles, CA)
90 g AIN Mineral Mix
50 g Vitamin Diet Fortification Mix

Vitamins and minerals were added to the components so that the three dietary rations contained equal amounts of these micronutrients on a per kilocalorie basis.

EXPERIMENT 1

Method

Animals. Sixteen female Long–Evans rats, weighing between 144–210 g at the start of the experiment, were used.

Procedure. To ensure stable patterns of nutrient intakes prior to drug administration, animals were given 2 weeks to acclimate to dietary conditions. During this period, nutrient intakes and body weights were recorded every other day at the beginning of the dark cycle. Two animals that failed to eat sufficient protein and to gain weight were eliminated from the study. At the end of this period, the remaining animals were given two pretest vehicle injections (distilled water) to familiarize them with all experimental procedure. These data were not included in the statistical analysis.

On each test day, food cups were removed at the beginning of the dark cycle (0830 h), weighed, and refilled. After a 2-h period without food (0830–1030 h), each rat was injected with fluoxetine (0.0, 5.0, 10.0, and 15.0 mg/kg, IP), and food was returned to the cage. Macronutrient intakes were measured 2, 4, 6, and 24 h after injections.

Fifteen minutes after drug injections and 15 min after each measurement of nutrient intake, rats were assessed for vocalization, hanging reflex, righting reflex, and locomotor activity. Rats had been habituated to the activity monitors and procedures on the two pretest vehicle injections days.

Each rat was tested with each dose of fluoxetine. Drug doses were given in a counterbalanced order to animals with a minimum of 5 days intervening between injections.

Data analysis. Nutrient intakes were analyzed at each measurement point using repeated-measures analysis of variance followed by comparisons between treatment groups using Tukey's HSD. All analyses that reached the $p < 0.05$ level of significance are reported.

Results

Fluoxetine produced significant dose-related decreases in total energy intake at all time points [hour 2: $F(3, 39) = 7.37$, $p < 0.001$; hour 4: $F(3, 39) = 5.33$, $p < 0.01$; hour 6: $F(3, 39) = 5.72$, $p < 0.01$; hour 24: $F(3, 39) = 8.99$, $p < 0.001$] (Fig. 1).

Examination of intake of individual macronutrients revealed that at all measurement points, fat intake was significantly reduced in a dose-related manner following fluoxetine injections [hour 2: $F(3, 39) = 6.64$, $p < 0.01$; hour 4: $F(3, 39) = 7.36$, $p < 0.01$; hour 6: $F(3, 39) = 10.96$, $p < 0.001$; hour 24: $F(3, 39) = 10.19$, $p < 0.001$] (Fig. 2, top). Additionally, fluoxetine administration was associated with a significant reduction in protein intake 24-h following injections, $F(3, 39) = 5.92$, $p < 0.01$ (Fig. 2, middle). Although carbohydrate intake was less following the administration of 5 mg/kg fluoxetine than following vehicle injections, neither this difference nor any other difference in carbohydrate intake as a function of fluoxetine was significant (Fig. 2, bottom).

Fluoxetine led to significant reductions in large movements 2 h, $F(3, 39) = 7.95$, $p < 0.001$, and 4 h, $F(3, 39) = 10.41$, $p < 0.001$, after injections (Fig. 3, bottom). In contrast, 24 h after drug injections, large movements were significantly, $F(3, 39) = 3.98$, $p < 0.05$, greater when rats were given 10.0 and 15.0 mg/kg fluoxetine than when they were injected with distilled water. Fluoxetine did not alter small movements (Fig. 3, top), or righting or hanging reflexes. However, when handled 15 min after injections, animals made significantly, $F(3, 39) = 5.20$, $p < 0.01$, more vocalizations when injected 15.0 mg/kg fluoxetine than when injected with distilled water.

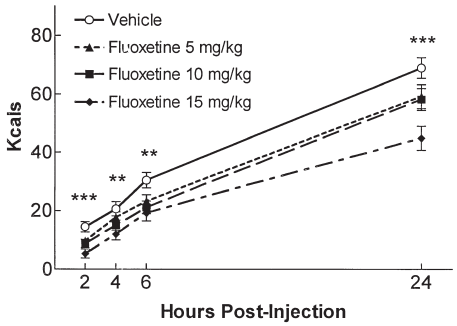

FIG. 1. The effect of acute fluoxetine administration on total cumulative 24-h caloric intake (mean ± SEM). Fluoxetine (5.0, 10.0, and 15.0 mg/kg) significantly suppressed food intake relative to saline at 2, 4, 6, and 24 h postinjections (***$p < 0.001$, **$p < 0.01$).

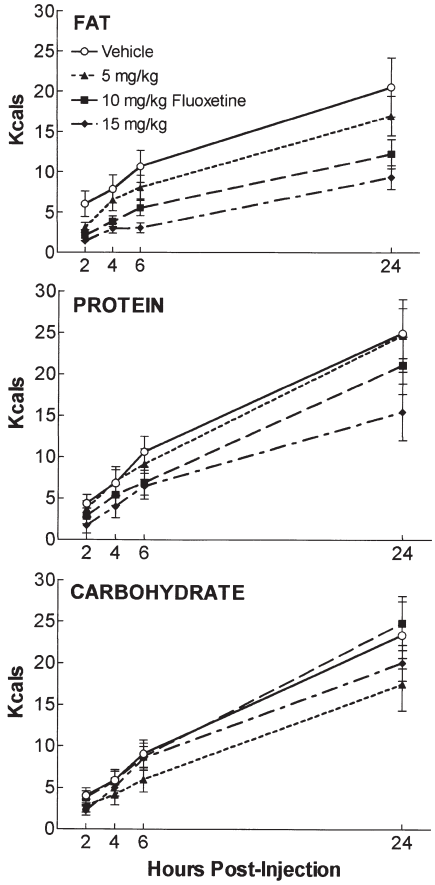

FIG. 2. The effect of acute fluoxetine on 24-h caloric intake (mean ± SEM) of fat (top), protein (middle), and carbohydrate (bottom). Fluoxetine (5.0, 10.0, and 15.0 mg/kg) significantly reduced fat at 2, 4, 6, and 24 h postinjection relative to vehicle [***$p < 0.001$ (except 5.0 mg/kg at hour 4)]. The higher doses of fluoxetine (10.0 and 15.0 mg/kg) were also significantly different than 5.0 mg/kg fluoxetine at hours 4, 6, and 24. Protein intake was significantly reduced by fluoxetine (10.0 and 15.0 mg/kg) 24 h postinjection (**$p < 0.01$). Carbohydrate intake did not vary as a function of fluoxetine treatment.

EXPERIMENT 2

Method

Animals. Twenty drug-naive, adult female Long–Evans rats, weighing between 173–233 g at the beginning of the experiment, were used.

Determination of the estrous cycle. Vaginal samples were collected daily between the third and fourth hour of the dark cycle (1030 to 1200 h) during the predrug, drug, and postdrug periods. Samples were placed on slides and dried at ambient temperature for at least 24 h. Samples were stained with cresyl violet and analyzed for cell distribution. Proestrus was recorded when the sample contained predominantly nucleated epithelial and cornified epithelial cells; estrus was recorded when the sample contained mainly cornified epithelial cells; metestrus was recorded when the sample contained cornified epithelial cells and leucocytes; and diestrus was recorded when the sample contained primarily leucocytes.

Procedure. To ensure stable patterns of nutrient intakes and body weights, animals were given 2 weeks to adapt to the dietary conditions. One rat, which failed to gain weight during this time, was removed from the experiment. Nutrient intakes and body weight continued to be recorded for a 2-week predrug period. Animals then were matched on the basis of patterns of macronutrient selection and body weight, and divided into two groups. During the drug period, one group ($n = 10$) received daily fluoxetine injections (10.0 mg/kg, IP), and the other group ($n = 9$), vehicle injections for 28 days. Drug injections were then stopped and nutrient intakes and body weight recorded for a 28-day postdrug period.

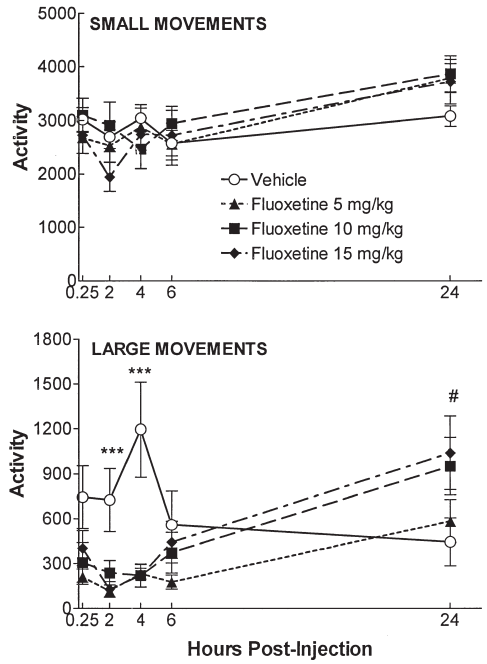

FIG. 3. The effect of acute fluoxetine administration on activity (mean ± SEM). Fluoxetine did not affect small movements (top). Large movements (bottom) were significantly reduced by fluoxetine (5.0, 10.0, and 15.0 mg/kg) relative to vehicle at 2 and 4 h postinjection (***$p < 0.001$). In contrast, 24 h after injections, the higher doses of fluoxetine (10.0 and 15.0 mg/kg) led to significantly more large movements than the low dose of fluoxetine (5.0 mg/kg) or vehicle (#$p < 0.05$).

Macronutrient intakes and body weights were recorded, and food cups refilled each day at the beginning of the dark cycle (0830–1030 h). During this 2-h measurement period, the rats were without food. During the drug phase, animals were injected with fluoxetine or vehicle immediately before nutrients were returned to the cages. Vaginal smears were collected each day at 1030 h.

Activity assessments were conducted every 7 days. Nutrients were removed at the beginning of the dark cycle (0830–1030 h) the rats were injected with either fluoxetine or vehicle and then returned to their cages. Fifteen minutes later, rats were assessed for vocalization, hanging reflex, and righting reflex. They then were placed in a cage on the platform of the small animal movement monitor for 5 min. In the pre- and postdrug periods, the rats were treated with vehicle, and during the drug phase, the rats were treated with either fluoxetine or vehicle.

Data analysis. Data on nutrient intakes and body weight gain were analyzed with repeated-measures analysis of variance followed by comparisons between treatment groups using Tukey's method. Macronutrient intakes were analyzed both on an absolute basis and as a percent of total energy intake (kcal intake for each macronutrient/total kcal intake). All analyses that reach the $p < 0.05$ level of significance have been reported.

Results

Fluoxetine significantly reduced total daily caloric intake compared to pre- and postdrug values and compared to vehicle during the treatment period [main effect of time: $F(2, 34) = 34.49$, $p < 0.001$; time × treatment interaction: $F(2, 34) = 38.67$, $p < 0.001$] (Fig. 4). Total daily caloric intake of drug-treated animals was maximally reduced on day 1 of treatment (33.82 kcal) and minimally reduced on day 28 of treatment (62.16 kcal). Despite this significant increase in caloric intake between the first and last days of treatment, $t(11) = -9.27$, $p < 0.001$], animals treated with fluoxetine consumed significantly fewer calories than vehicle-treated animals each day of treatment [smallest difference between groups on day 28 is significant, $t(17) = 3.10$, $p < 0.05$]. During the postdrug period, daily caloric intake of animals previously injected with fluoxetine was significantly greater than that of vehicle-treated animals. Intake was maximal the first week following fluoxetine withdrawal and deceased as a function of time (postdrug day 1: fluoxetine = 73.0 kcal, vehicle = 75.65

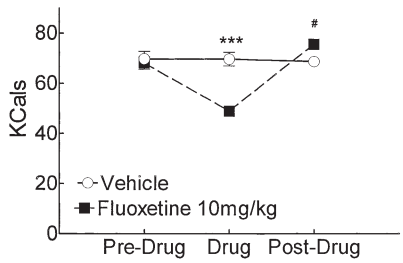

FIG. 4. The effect of chronic fluoxetine on total daily caloric intake averaged for each treatment period (mean ± SEM) in female rats. Fluoxetine-treated animals consumed significantly (***$p < 0.001$) less calories compared to vehicle-treated animals in the drug period, and significantly (#$p < 0.05$) more calories compared to vehicle-treated animals in the postdrug period.

kcal; day 6: fluoxetine = 95.61 kcal, vehicle = 61.58 kcal; day 28: fluoxetine = 74.02 kcal, vehicle = 69.37 kcal).

Chronic fluoxetine treatment significantly suppressed intakes of fat [main effect of time: $F(2, 34) = 24.18$, $p < 0.001$; and time × treatment interaction: $F(2, 34) = 10.60$, $p < 0.001$] and protein [main effect of time: $F(2, 34) = 15.21$, $p < 0.001$; and time × treatment interaction: $F(2, 34) = 18.62$, $p < 0.001$] compared to pre- and postdrug values and compared to vehicle during the treatment period (Fig. 5). Consumption of both fat and protein was consistently suppressed throughout the 28-day drug treatment period. In the postdrug period, intakes of both nutrients returned to predrug levels. In contrast to fat and protein intakes, carbohydrate intake slightly increased during both the fluoxetine treatment and the postdrug period. No differences between drug groups were observed in carbohydrate intakes in the predrug, drug, or postdrug periods.

The proportion of total caloric intake consumed from each macronutrient component was also assessed (Fig. 6). During the predrug period, rats consumed the majority of their calories as protein and fat. Although there was a trend for fat intake to increase with time, percent nutrient intakes of vehicle-treated animals did not vary across the experiment. However, during drug treatment, fluoxetine-treated rats consumed a significantly smaller proportion of their calories as fat [time × treatment interaction: $F(2, 34) = 6.07$, $p < 0.05$] and a significantly greater proportion of their calories as carbohydrate [time × treatment interaction: $F(2, 34) = 7.33$, $p < 0.05$] than during either the predrug or postdrug phases. The proportion of total energy intake consumed as fat and carbohydrate returned to approximately predrug levels when daily drug injections were stopped. The proportion of total energy intake consumed as protein did not vary as a function of fluoxetine administration.

To examine the effect of chronic fluoxetine treatment on body weight, body weight gain during each week of the drug and postdrug period was analyzed (Fig. 7). During the drug

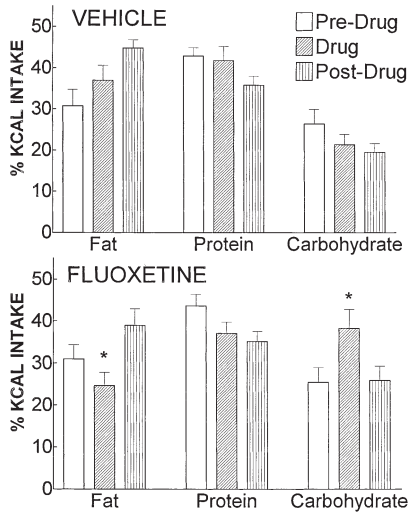

FIG. 6. The effect of chronic fluoxetine administration on percent of daily caloric intake consumed as each of the macronutrient averaged for each treatment period (mean ± SEM). Fluoxetine-treated rats (top) consumed a significantly smaller proportion of calories as fat ($*p < 0.05$) and a significantly greater proportion of calories as carbohydrate ($*p < 0.05$) during the drug phase compared to the pre- and postdrug phases. The proportion of calories consumed from each macronutrient did not vary across the experiment for vehicle-treated animals (bottom).

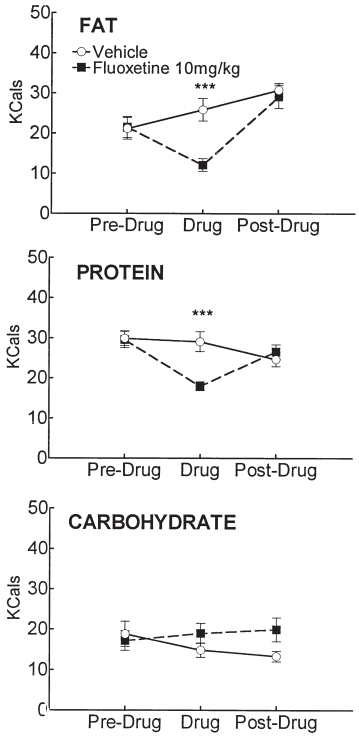

FIG. 5. The effect of chronic fluoxetine on daily caloric intake of fat (top), protein (middle), and carbohydrate (bottom) averaged for each treatment period (mean ± SEM). Fluoxetine-treated animals consumed significantly less fat ($***p < 0.001$) and protein ($***p < 0.001$) compared to vehicle-treated animals during the drug phase. Daily carbohydrate intake did not differ between fluoxetine and vehicle-treated animals, or across the treatment periods.

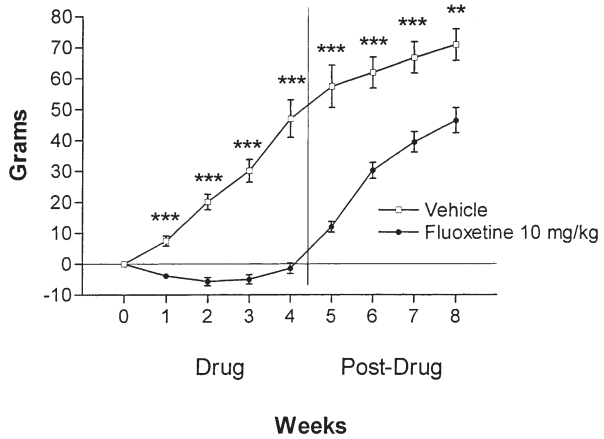

FIG. 7. The effect of chronic fluoxetine administration on cumulative body weight gain (values are mean change from baseline weight) in grams averaged for each week (mean ± SEM). Cumulative weight gain of fluoxetine-treated animals was significantly less than weight gain of vehicle-treated animals during drug treatment ($***p < 0.001$) and following drug withdrawal ($**p < 0.01$; $***p < 0.001$).

treatment period, rats injected with fluoxetine gained significantly less weight than vehicle-injected controls [main effect of time: $F(3, 51) = 56.11$, $p < 0.001$; main effect of treatment: $F(1, 17) = 117.44$, $p < 0.001$; and time × treatment interaction: $F(3, 51) = 43.13$, $p < 0.001$]. At the end of the drug phase, rats given fluoxetine had gained an average of 3.75 g, while rats given the vehicle gained an average of 55.42 g, $t(17) = -6.77$, $p < 0.001$.

During the postdrug period, female rats previously treated with fluoxetine gained weight at a faster rate than rats previously given the vehicle. However, at end of the 4-week postdrug period, rats previously treated with fluoxetine continued to weigh less (mean body weight = 288 g) than rats previously treated with vehicle (mean body weight = 315 g), although this difference was not significant.

To assess the effects of chronic fluoxetine on locomotor behavior, data were averaged across the weekly test sessions during the predrug, drug, and postdrug phase. Comparison were made between drug-treated and vehicle-treated animals and across phases of the experiment. Fluoxetine administration led to significant reductions in both large movements [time × treatment interaction: $F(2, 34) = 6.46$, $p < 0.05$] and small movements [time × treatment interaction: $F(2, 34) = 7.01$, $p < 0.05$] in comparison to vehicle-treated animals (Fig. 8). The animals appeared to adapt to the testing procedure as evidenced by significant increases in large [main effect of time: $F(2, 34) = 18.58$, $p < 0.001$] and small [main effect of time: $F(2, 34) = 18.45$, $p < 0.001$] movements by both fluoxetine and vehicle-treated animals in the postdrug period compared to the predrug and drug periods. No differences in vocalizations during handling 15 min postinjection, or the righting or hanging reflex were found as a function of drug treatment or phase of the experiment.

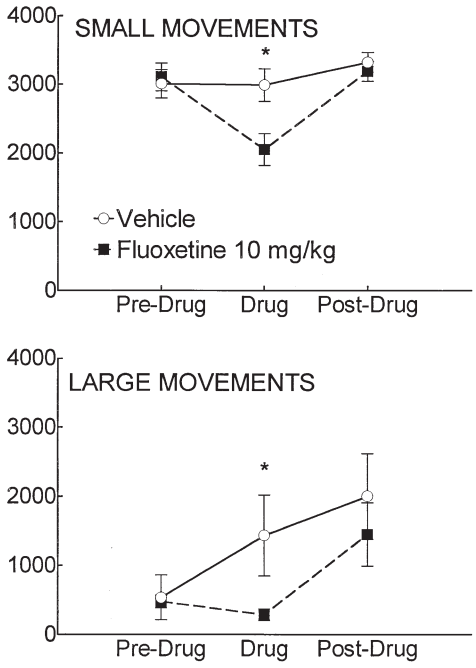

FIG. 8. The effect of chronic fluoxetine administration on activity 15 min postinjection and averaged for each treatment period (mean ± SEM) in female rats. Fluoxetine-treated animals made significantly fewer small (top) and large (bottom) movements compared to vehicle-treated animals (*$p < 0.05$). In addition, small movements of fluoxetine-treated animals were significantly ($p < 0.001$) lower during the drug phase compared to pre- and postdrug periods. Large movements for both fluoxetine- and vehicle-treated animals were significantly ($p < 0.001$) higher in the postdrug period compared to the predrug period.

Only rats that had a regular reproductive cycle (mean = 4.5 days; range = 3.7–5.0 days) throughout the predrug, drug, and postdrug periods were included in the analysis of estrous cycle ($n = 12$). Similar numbers of animals were eliminated from the fluoxetine and control groups. A repeated-measures analysis of variance was conducted for total caloric intake with cycle (proestrus, estrus, metestrus, diestrus) and time (predrug, drug, postdrug) as within-subjects variables, and treatment (fluoxetine and vehicle) as a between-subjects variable. Although fluoxetine treatment significantly reduced caloric intake, it did not affect the pattern of caloric intake across the estrous cycle. Total caloric intake was significantly reduced during proestrus compared to other stages of the reproductive cycle for both treatment groups throughout the predrug, drug, and postdrug periods [main effect of cycle: $F(3, 30) = 9.92$, $p < 0.001$] (Table 2).

Repeated-measures analysis of variance also were conducted to determine the effect of the reproductive cycle on macronutrient selection. Both protein, $F(3, 30) = 11.45$, $p < 0.001$, and carbohydrate, $F(3, 30) = 13.16$, $p < 0.001$, intakes were significantly reduced during proestrus compared to estrus, metestrus, and diestrus. This pattern was observed for both fluoxetine- and vehicle-treated rats throughout the predrug, drug, and postdrug periods. In contrast, fat intake significantly increased in estrus compared to metestrus, $F(3, 30) = 3.73$, $p < 0.05$.

GENERAL DISCUSSION

In concordance with previous research [e.g., (8,16,17,29)], acute and chronic fluoxetine administration significantly reduced total caloric intake in female rats. Because most of the past research had been conduced with male rats, it was not known if the findings would extend to females. Some differences were anticipated, because previous work has demonstrated that serotonin synthesis and receptor binding varies as a function of gender (13,37). Moreover, earlier studies reported sex differences in the effects of acute and chronic serotonin administration on food intake (11). Comparisons between this study and a previous one using the same experimental paradigm with male rats (17) revealed that acute administration of fluoxetine reduced total caloric intake to a greater degree in male rats than in females. In the previous study in male rats, total caloric intake was approximately 50% lower following acute injections of 10 mg/kg fluoxetine than after saline (17), while in the present study, caloric intake

TABLE 2

TOTAL CALORIC INTAKE ACROSS THE REPRODUCTIVE CYCLE

Group	Proestrus	Estrus	Metestrus	Diestrus
Fluoxetine (10.0 mg/kg)	55.88*	61.64	63.10	62.10
Vehicle	62.70*	69.15	69.16	68.91

*Total caloric intake during proestrus significantly ($p < 0.05$) less than during other stages of the reproductive cycle.

of females was about 30% lower after 10 mg/kg fluoxetine than after saline. These results suggest that fluoxetine has greater anorectic effects in males than in female rodents.

The results of the chronic phase of this study suggest that partial tolerance can develop to the anorectic effects of fluoxetine (Fig. 9). Although mean caloric intake of fluoxetine-treated rats was significantly less than that of vehicle-treated animals across the 28-day drug period, caloric intake of fluoxetine-treated animals increased as a function of time. In contrast to this finding, a number of researchers have reported that tolerance does not develop to fluoxetine's anorectic actions (8,17,30,38). However, in these studies, fluoxetine was administered for less time than in the present study. The one exception to this was our previous study with male rats, which used the same dose and time parameters as the present study, and which did not find tolerance to the anorectic effects of fluoxetine (17). These results suggest that gender differences may exist in the development of tolerance to fluoxetine's anorectic actions.

During drug withdrawal, rats previously treated with fluoxetine consumed significantly more calories than animals given the vehicle. Similar increases in caloric intake, relative to vehicle-treated controls, were observed in male rats when fluoxetine injections were terminated (17). The development of tolerance to the anorectic actions of fluoxetine in females, and the significant increase in caloric intake following the termination of treatment in both males and females are cause for caution in the use of the drug as a treatment for obesity.

The present studies provided no support for a biological behavioral feedback loop between central serotonin levels and carbohydrate intake. As mentioned, the results of previous experiments assessing the effects of serotonin agonists on nutrient selection are not entirely consistent. Some studies comparing intakes of high-carbohydrate/low-protein diets with low-carbohydrate/high-protein diets have reported selective decreases in intake of the high-carbohydrate/low-protein diet 1–3 h postinjection of serotonin agonists (29,31,32,48). However, other researchers, attempting to replicate these studies with L-tryptophan, the precursor to serotonin, have not observed selective reductions in carbohydrate intake (19,35). Most researchers who have reported significant reductions in carbohydrate intake following the administration of serotonin agonists have not used separate sources of the three macronutrients, making it impossible to determine the specific effect of these drugs on carbohydrate, protein, or fat intake (25,29–31,48). When separate macronutrients sources are provided, administration of serotonin agonists generally leads to decreases in fat and/or protein intakes, rather than in carbohydrate intake (17,24,34). Moreover, in both the present and previous experiments (17), fluoxetine treatment significantly reduced the proportion of calories consumed as fat and increased the proportion of calories consumed as carbohydrate. Results of other studies suggest that the effects of serotonin agonists on nutrient selection vary as a function of the time since injection (45) or drug dose (30). For example, Leibowitz and colleagues (45) reported a selective suppression of carbohydrate intake and no effect on fat or protein intake 1–2 h after fluoxetine injections. In contrast, fat and protein intakes, but not carbohydrate intake, were significantly suppressed 11–12 h after drug injections (45). One reason for the differences in these results, and those of the present study, may relate to baseline preferences for macronutrients. In the present study, the rats preferred fat, whereas in the previous study (45), the rats preferred carbohydrate. With respect to drug dose, it may be that lower doses of fluoxetine are more likely to lead to selective reductions in carbohydrate intake than higher doses (30). Indeed, in the present experiment, carbohydrate intake was reduced more following acute administration of 5 mg/kg fluoxetine than after injections of either 10 or 15 mg/kg. In comparison, fluoxetine led to dose-related reductions in both fat and protein intakes. These results indicate that drug dose also must be considered when assessing the effects of serotonergic agents on nutrient selection.

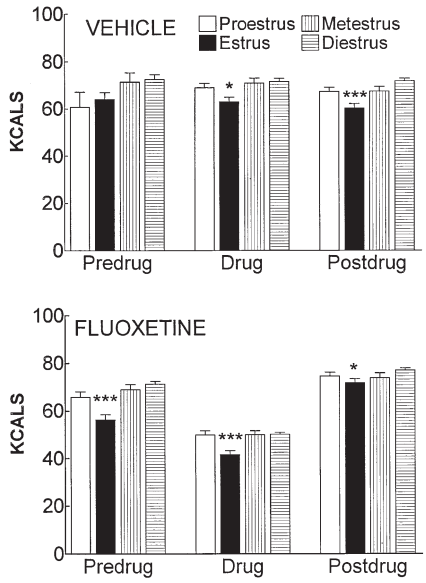

FIG. 9. Mean daily caloric intake across the estrous cycle during the predrug, drug and postdrug periods for rats chronically injected with vehicle (top) or fluoxetine (bottom). Mean daily caloric intake was significantly reduced during the estrous as compared to other stages in the cycle (***$p < 0.001$; *$p < 0.05$).

Female rats chronically injected with fluoxetine gained significantly less weight during the drug period than vehicle-treated animals. These results are consistent with past research with male rats (8,17,30,38). In the present study, the reduction in weight gain was relatively constant throughout the 28 days of fluoxetine treatment, suggesting that tolerance did not develop to the weight suppressing actions of the drug. These results are interesting in light of the effect of fluoxetine on total caloric intake. While female rats consumed more calories in the last 2 weeks of drug treatment compared to the first 2 weeks, their body weight changed very little. It is possible that fluoxetine causes both a decrease in food intake and metabolic efficiency, and while the anorectic properties become less effective, the metabolic properties become more effective over time.

As previously observed (5,41) total daily caloric intake was reduced when estrogen levels were high. The reduction in total caloric intake observed in proestrus was associated with significant reductions in protein and carbohydrate intakes, and a significant increase in fat intake relative to other stages of the cycle. These findings are similar to those reported by Bartness and Waldbillig (3), who found that estrogenic stimulation was associated with an increase in fat intake and a decrease in carbohydrate, and to a lesser extent protein, intakes in normally cycling and estradiol treated ovariectomized female rats. Wurtman and Baum (49) also found that carbohydrate intake was significantly reduced when estrogen levels were high in normally cycling and estradiol-treated ovariecto-

mized female rats. In contrast, Geiselman and colleagues (14) found the opposite pattern of macronutrient selection across the estrous in normally cycling female rats provided with pure sources of protein, fat, and carbohydrate. Differences in the composition of the nutrient source, or method of determining stages of the estrous cycle, could explain the discrepancies among these studies (22).

Both acute and chronic administration of fluoxetine led to reductions in motor activity. These findings are similar to those found following injections of other serotonin agonists [e.g., (9,18,20,40,42)]. These results are also consistent with past research assessing the effect of fluoxetine on the satiety sequence (8,17). More specifically, acute fluoxetine increased resting and decreased exploration within 1 h postinjection (8,17).

The results of the present studies provide support for the proposal that an inverse relationship exists between brain serotonin levels and food intake [e.g., (8,16,17,24,29,37,39)]. However, they do not support the idea that increases in central serotonin levels are associated with selective decreases in carbohydrate intake (12,25,29,30,33,50). In contrast, these results taken in conjunction with those of other experiments (17,21,24) suggest that carbohydrate intake is actually maintained while fat and protein intakes are reduced when animals are given serotonin agonists. The discrepancies among studies examining serotonergic influences on nutrient selection are evidence that factors such as diet composition, drug dose, baseline patterns of nutrient intake, and measurement times cannot be ignored in this type of research (21).

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