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Fenfluramine treatment in female rats accelerates the weight loss associated with activity-based anorexia

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Abstract

Serotonin plays an important role in controlling food intake and regulating body weight. Thus, altered serotonergic function may be involved in the etiology of anorexia nervosa. To investigate this hypothesis, we examined whether activation of the serotonin system increases the severity of activity-based anorexia, an animal model of anorexia nervosa in which food-restricted rats are housed with access to running wheels. This paradigm promotes symptoms of anorexia nervosa, including hypophagia, hyperactivity, and weight loss. Food-restricted rats received injections of a serotonin agonist, fenfluramine, or saline 1.5 h prior to their daily 2-h period of food access. A third saline-injected group was pair-fed to the fenfluramine group. Drug treatment and food restriction were terminated following a 25% weight loss. During food restriction, each group developed symptoms of activity-based anorexia. Although similar reductions in food intake were observed in fenfluramine-treated and pair-fed rats, only fenfluramine-treated rats displayed an accelerated rate of weight loss, relative to saline-treated rats. Thus, some other nonanorexic aspect of fenfluramine, perhaps its influence on metabolism, must underlie the accelerated rate of weight loss in this group. Our results suggest that increased activation of the serotonin system exacerbates the weight loss associated with activity-based anorexia.

Keywords: Anorexia nervosa; Serotonin; Food intake; Body weight regulation

1. Introduction

Anorexia nervosa is an eating disorder that predominantly affects young women. It is clinically diagnosed by a failure to maintain a healthy body weight, fear of becoming overweight, caloric restriction, and amenorrhea (American Psychiatric Association, 1994). While it is clear that environmental and sociocultural factors are involved in this disorder, recent advances in our understanding of the neural and endocrine systems controlling food intake have prompted renewed interest in investigating how biological factors may contribute to the etiology of anorexia nervosa.

While many anorexic women exhibit disturbances in neuropeptide, neuroendocrine, and neurotransmitter systems implicated in the normal control of food intake (reviewed in Bailer and Kaye, 2003), it remains to be determined whether such biological disturbances are a cause or a consequence of the weight loss associated with anorexia nervosa. Currently, there are two approaches to address this question because it is difficult, if not impossible, to conduct prospective studies of anorexia nervosa in humans. One approach involves studying whether the biological disturbances associated with anorexia nervosa persist in recovered anorexics that have achieved long-term maintenance of a healthy body weight. Clinical studies involving this retrospective approach indicate that most of the biological disturbances observed in underweight anorexic women are corrected by weight gain (reviewed in Barbarich et al., 2003). However, one biological disturbance that persists in weight-restored, recovered anorexics involves the serotonin system. Three lines of evidence demonstrate that serotonergic activity is

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chronically elevated in recovered anorexics. First, recovered anorexics have increased cerebrospinal fluid concentrations of the serotonin metabolite, 5-hydroxyindoleacetic acid (5-HIAA), compared to women of similar body weights who have never been diagnosed with an eating disorder (Kaye et al., 1991). Thus, serotonin turnover appears to be increased in recovered anorexics. Second, the ratio of tryptophan to large neutral amino acid concentration within plasma is greater in weight-restored anorexic women compared to control women of similar body weight. This suggests that recovered anorexics have greater tryptophan availability for transport across the blood brain barrier and thus a higher level of substrate for serotonin synthesis (Kaye et al., 2003). Third, chronic administration of pharmacological agents that function to deplete intracellular serotonin stores have been effective therapeutic agents to aid in the prevention of relapse in weight-restored, anorexic women (Kaye et al., 2001). Together, this evidence of elevated serotonin activity in weight-restored, recovered anorexics suggests that increased activation of the serotonin system is a pre-existing trait, rather than a physiological change produced by caloric restriction or the low body weight that accompanies anorexia nervosa. This hypothesis is of particular interest given the evidence, derived from both human and animal studies, that serotonin functions to inhibit meal size and increase metabolic activity (Foltin et al., 1996; Kaplan et al., 1997; Lupien and Bray, 1985; Rowland, 1986). Thus, it is possible that a primary defect in this neurotransmitter system, resulting in chronically elevated levels of serotonin activity, may contribute to the etiology of anorexia nervosa.

A second approach to studying the biological factors involved in anorexia nervosa involves an animal model. In this paradigm, rats are housed with the opportunity to exercise in running wheels and then placed on a restricted-feeding schedule consisting of 1–2 h access to food per day. Under these conditions, rats develop activity-based anorexia, a syndrome characterized by multiple symptoms of anorexia nervosa including weight loss, hyperactivity, and disruptions in the ovarian reproductive cycle (Dixon et al., 2003; Watanabe et al., 1992). This animal model is useful because it permits prospective investigation of biological factors involved in the development or maintenance of the symptoms of activity-based anorexia through direct manipulation of neural and endocrine systems involved in the control of food intake. Here, we used this model to investigate whether increased activation of the serotonin system exacerbates the symptoms of activity-based anorexia. Female rats were housed in cages that provided access to running wheels. Following adaptation to the novel housing conditions, rats were placed on a chronic food-restriction schedule and injected daily with fenfluramine, a serotonin agonist that functions to increase the release of serotonin into the synaptic cleft and prevent the reuptake of serotonin into presynaptic terminals (Rothman and Baumann, 2002). We hypothesized that if elevated serotonin activity plays a permissive role in the etiology of anorexia nervosa, then pharmacological manipulation of this system would increase the severity of activity-based anorexia in female rats.

2. Methods

2.1. Animals and housing

Twenty-four female Long–Evans rats (Charles River Breeding Laboratories, Raleigh, NC), weighing between 175 and 200 g at study onset, were housed individually in cages connected to Wahmann running wheels (35 cm in diameter). The running wheels were equipped with dipole magnets (DiLog Instruments, Tallahassee, FL) which signaled the occurrence of wheel revolutions. The testing room was maintained at 20±2 °C on a 12:12-h light–dark schedule (dark onset=1300 h). Powdered rat chow (Purina 5001) was presented in spill-resistant cups located in feeding niches that protruded from the cages. Removable blockers, located in the openings of the feeding niches, were used to block access to food during food restriction. Throughout the experiment, water was freely available. Rats were adapted to the novel housing conditions prior to data collection. Animal usage and all procedures were in compliance with the Florida State University Institutional Animal Care and Use Committee.

2.2. Behavioral measures, body weight, and estrous cycles

Food intake, wheel running, body weight, and the appearance of vaginal cytology samples were monitored daily. Between 1000 and 1100 h, food cups were weighed (±0.1 g), and any spillage was subtracted from the daily food intake measurement, wheel running was recorded (±0.5 rev), rats were weighed (±0.1 g), and vaginal cytology samples were collected. Stage of the estrous cycle (diestrus 1, diestrus 2, proestrus, or estrus) was then determined by examining the appearance and abundance of cells within each sample, as described previously (Eckel et al., 2000; Eckel and Geary, 1999). Using this strategy, proestrus included the light period peak in estradiol secretion, and estrus included the subsequent dark period when female rats ovulate and display increased sexual receptivity (Dixon et al., 2004). At study onset, all rats displayed regular, 4-day estrous cycles.

2.3. Procedure

Baseline measurements of food intake, running wheel activity, and body weight were obtained in free-fed rats across one estrous cycle, beginning on diestrus 1. At the start of the next cycle, rats were assigned to one of three groups. Two of the groups were placed on a 2 h restricted-feeding schedule (food available daily from 1400–1600 h).

Ninety minutes prior to food access, rats in these groups received intraperitoneal injections of 0.5 mg/kg d-fenfluramine (Sigma Chemical, Natick, MA; n=8) or 1 ml/kg physiological saline vehicle (n=8). Because this dose of fenfluramine decreases food intake in female rats (Rivera et al., 2003), we included a second control group (n=8) that received single, daily intraperitoneal injections of saline vehicle, 90 min prior to food access, and was then pair-fed to the fenfluramine-treated group. Starting at 1400 h, rats in the pair-fed group were given access to food until they had consumed the same amount as their partner in the fenfluramine-treated group. The average duration of food access in pair-fed rats, 1.9±0.2 h, did not differ from the 2-h feeding period of fenfluramine- and saline-treated treated rats, $F(2,21)=0.44$, n.s. Because rats exposed to the activity-based anorexia paradigm develop severe, often fatal, gastric lesions following a 30% weight loss (Doerries, 1991; Lambert and Kinsley, 1993; Tsuda et al., 1982), food restriction was terminated when individual rats either lost 25% of their baseline body weight or had been exposed to food restriction for 8 days, whichever occurred first. Restricted feeding was limited to 8 days based on our previous findings that 5–10% of rats exposed to this paradigm do not display rapid weight loss and are able to maintain a 10–15% weight loss indefinitely (Dixon et al., 2003). Upon reaching the 25% weight loss/8-day criterion, rats were given free access to food and recovery from activity-based anorexia was assessed by monitoring food intake, wheel running, body weight, and vaginal cytology samples until individual rats displayed one regular 4-day estrous cycle.

2.4. Data analysis

Data are presented as means±SEM. During the food-restriction phase, rats reached the 25% weight loss/8-day criterion at different times (range=3 to 8 days). To permit statistical analysis of this phase and to compare data obtained during this phase with data obtained during baseline and free-feeding phases, daily measures of food intake and wheel running from individual rats were averaged across days of each test phase as in previous studies (e.g., see Dixon et al., 2003). The severity of activity-based anorexia was determined by the mean latency (days) to reach the 25% weight loss/8-day criterion for termination of the restricted-feeding schedule and the percentage of rats displaying estrous cycle disruptions. Recovery from activity-based anorexia was determined by the mean latency (days) to body weight recovery and restoration of 4-day estrous cycles.

Initially, the effects of group and test phase on food intake and running wheel activity were analyzed using mixed-design ANOVAs. Group differences in food intake, wheel running, and body weight during each test phase were then analyzed using one-way ANOVAs. A Chi-square analysis was used to examine group differences in estrous cycle disruptions. Tukey's test was used to investigate differences between means following significant (p<0.05) main or interactive ANOVA effects.

3. Results

3.1. Baseline phase

Prior to food restriction, no significant differences in mean daily food intake, mean daily wheel running, or body weight were detected between groups (Fig. 1), and all rats displayed 4-day estrous cycles.

3.2. Induction of activity-based anorexia

Mean daily food intake and running wheel activity differed between the baseline and the restricted-feeding phases, $F(1,21)=414.57$ and 32.97, respectively, ps<0.01. In

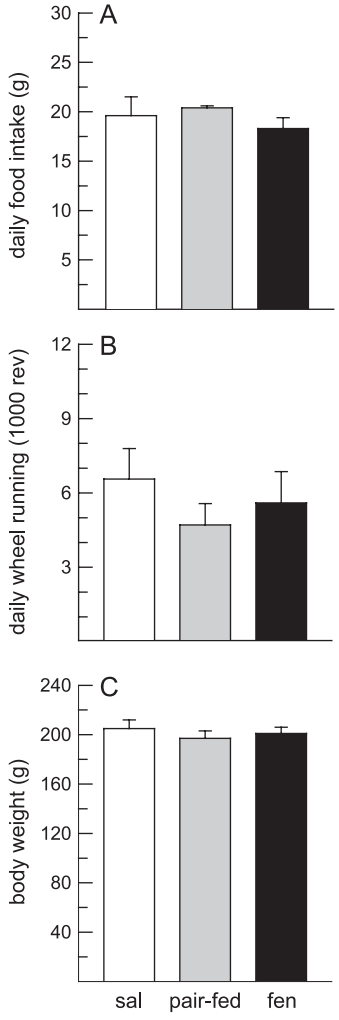

Fig. 1. Baseline measures of food intake, wheel running, and body weight. Prior to food restriction, no group differences in (A) food intake, (B) wheel running, and (C) body weight were detected. Abbreviations: sal—saline, fen—fenfluramine.

each group, introduction of the restricted-feeding schedule decreased daily food intake by 64±2% and increased daily wheel running by 105±22%, relative to that observed during baseline. Together, this decrease in energy intake and increase in energy expenditure promoted weight loss in all rats.

During restricted feeding, group differences in food intake were detected, $F(2,21)=4.64$, $p<0.05$ (Fig. 2A). Fenfluramine-treated and pair-fed rats consumed less food than saline-treated rats, $ps<0.05$. Food intake did not differ between fenfluramine-treated and pair-fed rats. No group differences in mean daily running wheel activity were detected during the restricted-feeding phase, $F(2,21)=0.85$, n.s. (Fig. 2B).

3.3. Severity of activity-based anorexia

During the restricted-feeding phase, all of the fenfluramine-treated rats lost 25% of their baseline body weight within 8 days, whereas only 7 of the 8 pair-fed rats and 6 of the 8 saline-treated rats met this weight loss criterion within 8 days. Following a 25% weight loss, or after 8 days, the restricted-feeding schedule was terminated, and rats were given free access to food. Group differences in the mean number of days to reach the 25% weight loss/8-day free-feeding criterion were detected, $F(2,21)=5.09$, $p<0.01$ (Fig. 3A). Fenfluramine-treated rats lost weight more rapidly than saline-treated or pair-fed rats ($ps<0.05$). Disruption of the

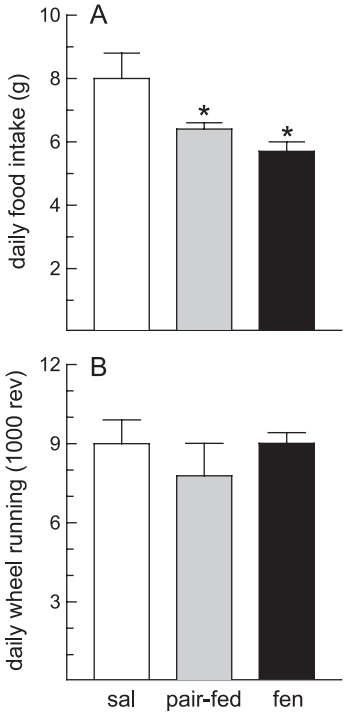

Fig. 2. Food intake and running wheel activity during restricted access to food. (A) Food intake was lower in fenfluramine-treated and pair-fed rats compared to saline-treated rats. (B) Wheel running did not differ between groups. *Less than saline-treated rats ($p<0.05$). Abbreviations: sal—saline, fen—fenfluramine.

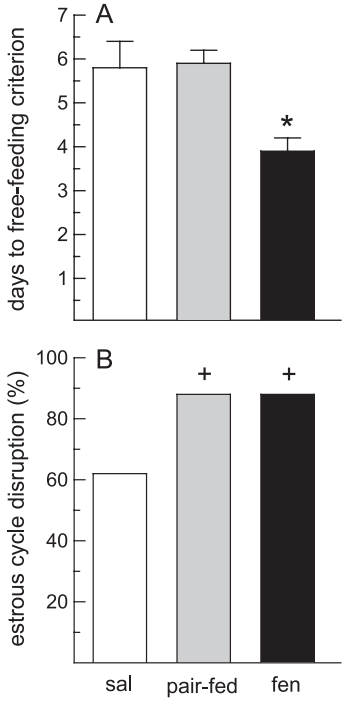

Fig. 3. Measures of activity-based anorexia symptoms in saline-treated, pair-fed, and fenfluramine-treated groups. (A) Fenfluramine-treated rats reached the 25% weight loss criterion faster than did saline-treated and pair-fed rats. (B) A greater percentage of fenfluramine-treated and pair-fed rats displayed estrous cycle disruptions compared to saline-treated rats. *Less than the saline-treated and pair-fed groups ($p<0.05$). $^{+}$Less than saline-treated rats ($p<0.05$). Abbreviations: sal—saline, fen—fenfluramine, rev—revolutions.

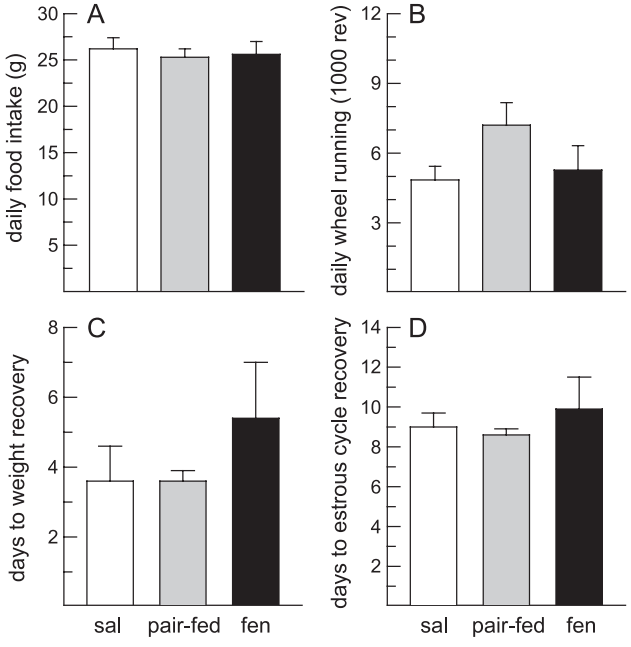

Fig. 4. Recovery from activity-based anorexia in saline-treated, pair-fed, and fenfluramine-treated groups. (A) Food intake during the free-feeding phase was similar in all groups. (B) There were no group differences in running wheel activity after restricted feeding. (C) Latency to regain body weight to baseline values was similar in all groups. (D) Latency to display one complete estrous cycle did not differ between groups. Abbreviations: sal—saline, fen—fenfluramine.

estrous cycle, characterized by a failure to go into estrus and perpetual vaginal cytology indicative of diestrus, differed between groups, χ^2=7.72, p<0.025 (Fig. 3B). Estrous cycle disruptions were apparent in 88% of fenfluramine-treated and pair-fed rats, whereas only 62% of the saline-treated rats displayed estrous cycle disruptions.

3.4. Recovery from activity-based anorexia

Mean daily food intake differed between the baseline and the free-feeding phases, F(1,21)=77.54, p<0.01. When free access to food was permitted following termination of the restricted-feeding schedule, each group displayed increased food intake relative to that observed during baseline by 37±7%, ps<0.01. Mean daily running wheel activity did not differ between the baseline and free-feeding phases, F(1,21)=0.06, n.s.

During free feeding, no group differences in daily food intake or running wheel activity were detected, F(2,21)=0.04 and 0.10, respectively, n.s. (Fig. 4A,B). No group differences in the number of days to body weight recovery and the restoration of 4-day estrous cycles were detected, F(2,21)=0.45 and 0.91, respectively, n.s. (Fig. 4C,D).

4. Discussion

The goal of the present study was to determine whether pharmacological manipulation of the serotonin system modulates the severity of activity-based anorexia in female rats. During the restricted-feeding phase, one group of rats received daily injections of fenfluramine, a serotonin agonist that increases the release of serotonin into the synaptic cleft while preventing the reuptake of serotonin into presynaptic terminals (Rothman and Baumann, 2002). A second group of rats received daily injections of saline vehicle. Because fenfluramine decreases food intake in free-fed, female rats (Rivera et al., 2003), we included a pair-fed control group to dissociate the effects of decreased food intake and drug treatment on the severity of activity-based anorexia. During the restricted-feeding phase, all rats displayed symptoms of activity-based anorexia. That is, all rats displayed decreased food intake, increased running wheel activity, and weight loss, relative to the baseline phase. As expected, fenfluramine-treated and pair-fed rats consumed less food than saline-treated rats during the restricted-feeding phase. However, only the fenfluramine-treated rats displayed a more rapid weight loss, relative to the saline-treated control rats. This suggests that it was the drug treatment, which presumably increased serotonergic activity, rather than the decrease in food intake, that increased the rate of weight loss in fenfluramine-treated rats with activity-based anorexia. If the biological processes involved in the development of anorexia nervosa and activity-based anorexia are similar, elevated serotonergic activity may increase the severity of symptoms associated with anorexia nervosa.

In the present study, fenfluramine treatment increased the rate of weight loss in female rats with activity-based anorexia. A similar finding was reported previously in male rats exposed to a similar paradigm (Rieg et al., 1994). In this study, chronic fenfluramine treatment decreased food intake during the restricted-feeding phase. This led the authors to conclude that the increased severity of activity-based anorexia, observed in their study involving male rats, was due to the anorectic effects of fenfluramine (Rieg et al., 1994). Because a pair-fed control group was not included, it is difficult to interpret the significance of this previous study. Here, the inclusion of such a group weakens the possibility that a reduction in food intake was the major factor that accelerated the weight loss associated with activity-based anorexia. While fenfluramine-treated and pair-fed rats consumed less food than saline-treated rats during the restricted-feeding phase, only the fenfluramine-treated rats displayed more severe symptoms of activity-based anorexia. Thus, our data suggest that some other action of fenfluramine was responsible for the increased rate of weight loss in female rats with activity-based anorexia. It is also interesting that, in the previous study, a 10-fold increase in the dose of fenfluramine was required to accelerate the development of activity-based anorexia in male rats, compared to the dose used in the present study. This finding is consistent with data in free-fed rats suggesting that sensitivity to fenfluramine is greater in females than in males (Rivera et al., 2003). Although the exact mechanism is not known, the greater sensitivity in female rats may be mediated by fluctuations in estradiol secretion across the estrous cycle. Recently, we reported that female rats are more sensitive to the anorectic effects of fenfluramine during estrus, immediately following the peak in estradiol secretion, compared to diestrus, when estradiol secretion is much lower (Rivera et al., 2003).

Our findings indicate that a decline in food intake does not account for the accelerated weight loss of fenfluramine-treated rats. Thus, some other mechanism must underlie fenfluramine's ability to increase the rate of weight loss associated with activity-based anorexia. Previous research indicates that fenfluramine increases metabolic energy expenditure. For example, fenfluramine treatment increases brown adipose tissue activation (Lupien and Bray, 1985) and lipid oxidation (Boschmann et al., 1996). Thus, it is possible that increased metabolic activity contributed to the accelerated weight loss of fenfluramine-treated rats, relative to pair-fed and saline-treated controls.

Another mechanism to account for the accelerated weight loss in fenfluramine-treated rats involves an alteration in release of hypothalamic neuropeptide Y (NPY). It is well established that NPY plays an important role in the regulation of energy balance, particularly during times of fasting. For example, food deprivation in sedentary rats increases arcuate NPY expression and NPY release in the paraventricular nucleus of the hypothalamus

(Kalra et al., 1991). Although not measured in the present study, it is likely that chronic food restriction increased release of hypothalamic NPY. This adaptive response would normally function to increase appetite and decrease metabolic energy expenditure. However, it has been shown that injection of a serotonin agonist reduces the concentration of hypothalamic NPY in food-deprived rats (Dube et al., 1992). It is possible, therefore, that the presumed increase in central serotonergic activity in fenfluramine-treated rats blunted the increased secretion of NPY associated with food restriction, relative to pair-fed and saline-treated rats. This could have functioned to elevate metabolic energy expenditure in the fenfluramine-treated group, relative to control groups.

The opportunity to exercise is critical for the development of activity-based anorexia. For example, when access to food is restricted to 2 h per day, sedentary rats display a slight decrease in body weight and, over time, either defend the lowered body weight or continue to gain weight (Dixon et al., 2003). Thus, treatments that suppress locomotor activity might be expected to decrease the rate of weight loss in rats with activity-based anorexia. Although fenfluramine decreases wheel running in free-fed rats (Aulakh et al., 1988; Rivera et al., 2003), it did not affect wheel running here. That is, no group differences in mean daily wheel running were observed during the restricted-feeding phase. Thus, the hypoactive effects of fenfluramine, observed in free-fed rats, may be suppressed by the increased drive to run while in the activity-based anorexia paradigm. Alternatively, the hypoactive effects of fenfluramine may have been minimal during the period when the wheel running associated with activity-based anorexia is maximal. In previous studies of activity-based anorexia, food-restricted rats displayed maximal locomotor activity during the 4-6 h period prior to food access, and preventing access to wheels during this time attenuated the development of activity-based anorexia (Beneke et al., 1995; Dwyer and Boakes, 1997). Here, fenfluramine was administered 90 min prior to food access. Because the suppressive effects of fenfluramine on wheel running are not apparent until 2 h after drug treatment (Rivera et al., 2003), it is possible that our daily injection of fenfluramine failed to suppress the anticipatory increase in running wheel activity that precedes food access in this paradigm.

In the present study, rats with activity-based anorexia displayed progressive weight loss that was often accompanied by estrous cycle disruptions. This is consistent with previous studies in which estrous cycle disruptions were reported in rats with sustained, low body adiposity (Dixon et al., 2003; Tropp and Markus, 2001). Reproductive functioning appears to be, at least in part, dependent upon plasma leptin level (Chehab et al., 1996; Ahima et al., 1997; Gruaz et al., 1998). Because leptin is secreted in proportion to body adiposity (Fredrich et al., 1997), the weight loss associated with activity-based anorexia undoubtedly results in decreased plasma leptin secretion. Thus, a reduction in leptin secretion may have been responsible for the estrous cycle disruptions observed here.

Estrous cycle disruptions during restricted feeding were more prevalent in fenfluramine-treated and pair-fed rats, compared to saline-treated rats. Because serotonin agonists, like fenfluramine, suppress hypothalamic NPY activity (Dube et al., 1992), and NPY plays an important role in regulating the luteinizing hormone surge during proestrus (Sahu et al., 1995; Xu et al., 2000), it is possible that decreased hypothalamic NPY activity contributed to estrous cycle disruptions in fenfluramine-treated rats. Of course, such a mechanism is unlikely to account for the estrous cycle disruptions in pair-fed rats.

Consistent with a previous study (Dixon et al., 2003), recovery from activity-based anorexia was associated with pronounced hyperphagia which resulted in rapid weight gain in all groups. While sustained hypoactivity was not observed during refeeding, a transient decrease in wheel running was observed on the first day of refeeding in each group. Thus, recovery of body weight was achieved primarily through a compensatory increase in food intake, rather than a compensatory decrease in wheel running. Overall, food intake, running wheel activity, recovery of body weight, and resumption of estrous cycles did not differ significantly between groups. Thus, fenfluramine treatment, concurrent with food restriction, does not appear to interfere with recovery from activity-based anorexia. This finding is consistent with the 2–4 h of pharmacological half-life of fenfluramine (Rowland and Carlton, 1986) and behavioral studies demonstrating that fenfluramine-induced hypophagia and hypoactivity persisted for ~6 h (Rivera et al., 2003).

The results of this study are consistent with the hypothesis that elevated serotonergic activity contributes to the development of anorexia nervosa. Here, we demonstrated that increased serotonergic activation, induced by chronic fenfluramine treatment, resulted in more rapid weight loss in female rats exposed to the activity-based anorexia paradigm. That pair-fed rats did not display accelerated weight loss suggests that it is the metabolic, rather than the anorectic, effects of fenfluramine that increased the weight loss associated with activity-based anorexia. Elucidation of how increased serotonergic activity increases the severity of activity-based anorexia represents a critical step towards understanding how this eating disorder develops. In future studies, it will be important to characterize how the serotonergic system changes as a result of exposure to this paradigm.

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