The anorectic effect of fenfluramine is influenced by sex and stage of the estrous cycle in rats

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Eckel, Lisa A., Heidi M. Rivera, and Deann P. D. Atchley. The anorectic effect of fenfluramine is influenced by sex and stage of the estrous cycle in rats. Am J Physiol Regul Integr Comp Physiol 288: R1486–R1491, 2005. First published January 6, 2005; doi:10.1152/ajpregu.00779.2004.—The controls of food intake differ in male and female rats. Daily food intake is typically greater in male rats, relative to female rats, and a decrease in food intake, coincident with the estrous stage of the ovarian reproductive cycle, is well documented in female rats. This estrous-related decrease in food intake has been attributed to a transient increase in the female rat’s sensitivity to satiety signals generated during feeding bouts. Here, we investigated whether sex or stage of the estrous cycle modulate the satiety signal generated by fenfluramine, a potent serotonin (5-HT) releasing agent. To examine this hypothesis, food intake was monitored in male, diestrous female, and estrous female rats after intraperitoneal injections of 0, 0.25, and 1.0 mg/kg d-fenfluramine. The lower dose of fenfluramine decreased food intake only in diestrous and estrous females, suggesting that the minimally effective anorectic dose of fenfluramine is lower in female rats, relative to male rats. Although the larger dose of fenfluramine decreased food intake in both sexes, the duration of anorexia was greater in diestrous and estrous females, relative to male rats. Moreover, the magnitude of the anorectic effect of the larger dose of fenfluramine was greatest in estrous rats, intermediate in diestrous rats, and least in male rats. Thus our findings indicate that the anorectic effect of fenfluramine is modulated by gonadal hormone status.

Available data suggest that testosterone does not interact with the neural circuits implicated in the control of food intake (28). Rather, the orexigenic effect of testosterone appears to arise from its ability to stimulate the development of lean tissue mass, which functions to increase the male rat’s energy requirement (31). In contrast, considerable evidence suggests that estradiol decreases food intake by selectively affecting the neural controls of meal size. That is, the preovulatory increase in estradiol secretion decreases the size but not the number of meals consumed during estrus (reviewed in Ref. 14). This action of estradiol is mediated, in part, by its ability to increase the satiating effect of CCK, a gut peptide implicated in the physiological control of meal size (35). For example, the strength by which endogenous CCK inhibits meal size is increased by estradiol treatment in the ovariec-tomized rat (2), and during estrus in the cycling rat (15).

Like CCK, the serotonergic system has been implicated in the physiological control of meal size (34). Because an interaction between estradiol and CCK alone cannot account for the decrease in meal size in the estrous rat (14), our goal here was to investigate whether estradiol interacts with the serotonergic system to control meal size in the female rat. In particular, we were interested in determining whether the feeding response after an increase in serotonin (5-HT) neurotransmission was influenced by sex and/or stage of the estrous cycle. To investigate this hypothesis, food intake was monitored in male, diestrous female, and estrous female rats after acute administration of fenfluramine, a potent 5-HT releasing agent used extensively to investigate the contribution of endogenous 5-HT to food intake (34). Although the anorectic effect of fenfluramine is largely attributed to its ability to increase the strength of negative-feedback satiety signals during a meal, there is some evidence that fenfluramine may also decrease the strength of meal-related positive-feedback signals, or induce an aversive internal state that might promote early termination of a meal (reviewed in Ref. 34). As such, we also examined whether fenfluramine reduced the preference for a palatable sucrose solution in male and female rats.

METHODS

Animals. Twenty-four male and 24 female Long-Evans rats (Charles River Breeding Laboratories, Raleigh, NC; 45–55 days old at study onset), were housed in a room maintained at 20 ± 2°C under a 12:12-h light/dark cycle with food and water available ad libitum. Food and water intake were monitored daily from individual home cages. Food was not withheld prior to each test session.

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reverse 12:12-h light-dark cycle (dark phase = 1300–0100). Animal usage and all procedures were approved by the Florida State University Institutional Animal Care and Use Committee and the guidelines of the American Physiological Society (1).

**Estrous cycles.** Vaginal cytology samples were collected daily. Stages of the estrous cycle (diestrus 1, diestrus 2, proestrus, and estrus) were determined by examining the appearance and abundance of cells within samples, as described previously (15, 16). Using this strategy, proestrus included the light-phase peak in estradiol and luteinizing hormone secretion (19), and estrus included the subsequent dark phase when female rats ovulate and display increased sexual receptivity (6). At study onset, all rats displayed regular, four-day estrous cycles.

**Experiment 1: effect of fenfluramine on food intake.** Male and female rats (n = 8 per sex) were housed in cages equipped with feeding niches that provided access to spill-resistant food cups containing powdered chow (Purina 5001). Changes in food cup weights were monitored via custom-designed software (ESP 500; R. Henderson; Florida State University) connected, via a computer interface, to weight-sensitive load beams located below the food cups and infrared beams positioned above the food cups. Additional software (Meal Weight Analysis, R. Henderson, Florida State University) was used to monitor food intake at specific intervals.

The computerized system monitoring food intake was stopped daily at 0900 h. During the following 30 min, body weights were recorded, vaginal cytology samples were collected, food cups and water bottles were refilled, and the computer was then restarted. To prevent rats from consuming a meal before dark onset, food access was blocked during the last 4 h of the light phase (i.e., from 0900 to 1300). Rats were adapted to this restricted-feeding schedule before drug testing. In female rats, a within-subject design was used to examine the effects of varying doses of fenfluramine on food intake at different stages of the estrous cycle. During three consecutive cycles (i.e., 12 days), female rats received intraperitoneal injections of 0, 0.25, or 1.0 mg/kg fenfluramine (Sigma, St. Louis, MO), dissolved in physiological saline vehicle. Injections were administered 60 min before food access on diestrus 2, after a period of low estradiol secretion, and on estrus, after a period of high estradiol secretion (19). Injections were randomized with respect to drug dose and cycle phase. In male rats, the same schedule of injections was administered at 2-day intervals over the 12-day test period. Although daily variation in response to the same dose of fenfluramine was not expected in male rats, this protocol ensured that male and female rats received equal exposure to fenfluramine (i.e., two injections of each dose of fenfluramine).

**Experiment 2: Effect of fenfluramine on preference for a 5% sucrose solution.** Male and female rats (n = 16 per sex) were housed individually in custom-designed, polyethylene cages that provided access to chow (Purina 5001) and two drip-resistant drinking bottles. Rats were adapted to a 22-h water-deprivation schedule (daily water access from 1100 to 1300) over a 7-day period. On the eighth day of water deprivation, rats were given 2-h access to a novel 5% sucrose solution instead of water. Immediately after sucrose access, half of the male rats and half of the female rats received intraperitoneal injections of 1 mg/kg fenfluramine. The remaining rats received intraperitoneal injections of 1 ml/kg saline vehicle. In females, sucrose access and injections of fenfluramine or saline were administered when rats were either in diestrus 2 (n = 8) or estrus (n = 8). Preference for the sucrose solution was assessed on the following day. From 1100 to 1300, rats were given 2-h access to one bottle containing water and one bottle containing a 5% sucrose solution. To minimize the development of a drinking side preference, the position of the water and sucrose bottles was reversed during the second h of the test. The amount of 5% sucrose and water consumed was measured by weighing drinking bottles (±0.1 g) before and after the test.

**Data analysis.** In Experiment 1, the time course over which fenfluramine modulated feeding behavior was examined at three intervals: the first 2 h after drug treatment (i.e., from 1300 to 1500), the subsequent 4-h period (i.e., from 1500 to 1900), and the subsequent 14 h period (i.e., from 1900 to 0900). These intervals were chosen on the basis of the half-life of fenfluramine, which ranges from 2 to 4 h in the rat (10). The effects of injection day (diestrus and estrus in females; day 1 and day 2 in males) and drug dose (0, 0.25, and 1.0 mg/kg fenfluramine) on food intake during each of these intervals was analyzed using two-factor, repeated-measures ANOVAs in male and female rats. In males, these analyses failed to reveal any main or interactive effect of injection day (i.e., day 1 vs. day 2). Thus, to simplify the illustration of food intake in male rats, only data taken after the second injection of each drug dose are presented, and the effects of drug dose on food intake during each interval were analyzed using single-factor (drug dose), repeated-measures ANOVAs. The anorectic effect of fenfluramine was further characterized by calculating the decrease in food intake (±0.1 g) after the largest dose of fenfluramine, relative to saline vehicle, in male, diestrous female, and estrous female rats. One-way ANOVAs were then used to determine the influence of gonadal hormone status on the anorectic effect of fenfluramine. Tukey’s honestly significant difference test was used to investigate differences between means after significant main or interactive ANOVA effects.

In Experiment 2, preference for the sucrose solution was determined by the ratio of sucrose consumed to the total amount of fluid consumed by each rat during the two-bottle preference test. In the female group, an independent t-test was used to determine the influence of cycle stage on sucrose preference. Because this analysis revealed no significant effect of cycle stage, data were collapsed across cycle stage in female rats. In male and female rats, the effect of fenfluramine treatment on sucrose preference was analyzed using independent t-tests.

**RESULTS**

**Experiment 1.** In male rats, food intake was decreased only during the first 2-h period after drug treatment [F(2, 14) = 10.83, P < 0.005] (Fig. 1, left). During this interval, the largest dose of fenfluramine decreased food intake relative to the lower dose of fenfluramine and saline (P < 0.05). No influence of drug treatment was detected during the subsequent 4- or 14-h periods. In female rats, food intake during the first 2-h period and the subsequent 4-h period was decreased by estrous stage and drug treatment [F(2, 14) = 16.34 - 44.08, P < 0.005 - 0.0001] (Fig. 1, right). During these intervals, an estrous-related decrease in food intake was detected, regardless of drug treatment (P < 0.05). During the first 2-h period, both doses of fenfluramine decreased food intake in diestrous and estrous rats (P < 0.05). During the subsequent 4-h period, only the largest dose of fenfluramine decreased food intake in diestrous rats, whereas fenfluramine induced a dose-related decrease in food intake in estrous rats (P < 0.05). No influence of drug treatment was detected during the subsequent 14-h period in either diestrous or estrous rats.

Fig. 2 depicts the influence of gonadal hormone status on the magnitude of anorexia after treatment with the larger dose of fenfluramine. At 2 h after drug treatment, the anorectic effect of this dose of fenfluramine was similar across groups [F(2, 21) = 0.58, not significant (ns)] (Fig. 2A). That is, fenfluramine treatment produced about a 2-g reduction in food intake, relative to that consumed following saline vehicle treatment, in male, diestrous female, and estrous female rats. During the subsequent 4-h period, fenfluramine’s inhibitory effect on food intake was influenced by gonadal hormone status [F(2, 21) = 4.87, P < 0.01] (Fig. 2B). At this time, the
anorectic effect of fenfluramine was greater in diestrous and estrous female rats, relative to male rats (*P < 0.05). Among female rats, the anorectic effect of fenfluramine was greater during estrus, relative to diestrus (*P < 0.01).

Experiment 2. Before fenfluramine treatment, all rats avidly consumed the 5% sucrose solution on the conditioning day (mean intake = 24.7 ± 1.4 g/2 h). No estrous-related or sex differences in the amount of sucrose consumed during the 2-h access period were apparent. On the following day, all rats displayed a strong preference for the sucrose solution during the two-bottle sucrose preference test. Fenfluramine failed to suppress this preference for sucrose in either male or female rats [t(14) = 1.73 and 1.32, ns] (Fig. 3).

Fig. 2. Influence of gonadal hormone status on the anorectic effect of the larger (1 mg/kg) dose of fenfluramine during the first 2-h period and the subsequent 4-h period after drug treatment. Data are means ± SEs. A: anorectic effect of fenfluramine was similar in male, diestrous female, and estrous female rats during the first 2-h period. B: during the subsequent 4-h period, the anorectic effect of fenfluramine was greater in female rats, relative to male rats. Among female rats, the anorectic effect of fenfluramine was greater during estrus, relative to diestrus. *Greater than male rats, *P < 0.05. **Greater than diestrous and male rats, *P < 0.05. Fl: food intake.

Discussion

The major goal of the present study was to determine whether the anorexia resulting from increased serotonergic neurotransmission is influenced by sex or stage of the estrous cycle. To investigate this hypothesis, food intake was monitored in male, diestrous female, and estrous female rats treated with fenfluramine, a potent 5-HT agonist that increases extracellular concentration of 5-HT (32). In support of our hypothesis, the anorectic effect of fenfluramine was greater in female rats, relative to male rats. Among females, we also found that fenfluramine-induced anorexia was greater during estrus, relative to diestrus. It appears unlikely that the enhanced anorexia in female rats was secondary to any nonspecific, aversive effects of fenfluramine. Treatment with the larger dose of fenfluramine did not alter the female rat’s preference for a 5% sucrose solution.

Our data indicate that the anorectic effect of fenfluramine is sexually dimorphic. First, the lower dose of fenfluramine tested here decreased food intake in female rats, but failed to influence food intake in male rats. Thus the minimally effective
The anorectic dose of fenfluramine appears to be lower in the female rat. Second, the duration of the anorectic effect of the larger dose of fenfluramine was 6 h in diestrous and estrous female rats and 2 h in male rats. Third, the magnitude of the anorectic effect of the larger dose of fenfluramine was greater in diestrous and estrous female rats, relative to male rats. In males, the larger dose of fenfluramine reduced food intake by \( \sim 2 \) g. In females, the same dose of fenfluramine reduced food intake by \( \sim 3.5 \) g during diestrus and by \( \sim 6.5 \) g during estrus. Together, these results suggest that female rats are more sensitive than male rats to the anorectic effect of fenfluramine. Previous studies are consistent with our findings. Chronic administration of fenfluramine induced a larger decrease in daily food intake in female rats, relative to male rats (33), and acute administration of sibutramine, a 5-HT reuptake inhibitor, reduced consumption of a high-carbohydrate diet more in female rats, than in male rats (25). Although it was noted that the sex difference reported in the former study may have been related to the greater body weight of their female rats (33), such an explanation cannot account for the present findings. In our study, male rats weighed more than female rats, and female rats lost weight during estrus, relative to diestrus. In contrast to the results found here and in previous studies (25, 33), Dagnault and colleagues (13) reported that chronic fenfluramine treatment (6–12 mg·kg\(^{-1}\)·day\(^{-1}\)) produced similar reductions in food intake in male and female rats. The lack of a sexually dimorphic response in this study may be related to the fact that the very high, sustained doses of fenfluramine produced large decreases in food intake in both sexes such that a floor effect may have limited their ability to detect a sex difference in the anorectic effect of fenfluramine. Finally, our findings are consistent with other reports of sex differences in nonfeeding, behavioral responses induced by other treatments that modulate 5-HT neurotransmission. For example, the collection of responses that characterize the 5-HT behavioral syndrome (i.e., flattened body posture, resting tremor, forepaw treading, and head weaving), induced by paragline/tryptophan treatment, is more pronounced in female rats than in male rats (11, 18). Together, these studies suggest that females are more sensitive than males to the behavioral responses elicited by direct manipulation of the 5-HT system by several different compounds that increase 5-HT activity.

In the present study, the anorectic effect of fenfluramine was also influenced by stage of the estrous cycle. First, the duration of the anorectic response after administration of the lower dose of fenfluramine was greater during estrus, relative to diestrus. Second, the magnitude, but not the duration of the anorectic response after administration of the larger dose of fenfluramine was greater during estrus, relative to diestrus. We believe this to be the first demonstration of an estrous cycle-related change in the anorectic effect of any 5-HT agonist. There are, however, two previous reports of estrous cycle modulation of the hyperphagia induced by drugs that reduce 5-HT activity. In one study, posterior basolateral amygdala (pBLA) infusion of metergoline, a 5HT\(_{1A}\) receptor antagonist, induced a greater increase in food intake in estrous rats, relative to diestrous rats (30). Thus the estrous-related decrease in food intake may involve activation of postsynaptic 5-HT receptors within the pBLA. In another study, female rats treated with 8-OH-DPAT, a drug that reduces 5-HT neurotransmission via activation of presynaptic 5-HT\(_{1A}\) autoreceptors, displayed increased hyperphagia when the drug was administered during the diestrous stage of the cycle (37). However, vehicle-treated rats failed to display the typical, estrous-related decrease in food intake during multiple feeding tests, suggesting that the effect of 8-OH-DPAT on food intake may have been conducted at a time that was not optimal to detect an estrous-related change in the efficacy of the drug. The discrepant findings between these two studies may also be related to the fact that metergoline and 8-OH-DPAT reduce 5-HT activity via different mechanisms. Metergoline-induced hyperphagia is mediated by antagonism of postsynaptic 5-HT\(_{1/2/7}\) receptors, whereas 8-OH-DPAT-induced hyperphagia is mediated by agonism of presynaptic 5-HT\(_{1A}\) autoreceptors. Thus the hormonal changes that precede estrus may modulate the expression or binding affinity of postsynaptic, but not presynaptic, 5-HT receptors. Indeed, previous research indicates that 5-HT\(_{2A}\) receptor mRNA is increased during estrus, relative to nonestrous phases (36).

Differences in gonadal hormone status were associated with the changes in duration and magnitude of fenfluramine-induced anorexia observed here. Specifically, the anorectic effect of fenfluramine was greatest in estrous rats, intermediate in diestrous rats, and least in male rats when assessed 6 h after drug treatment. Our inability to detect a similar relationship within the first 2 h of drug treatment may be the result of a floor effect, as fenfluramine produced very large decreases in food intake, particularly in estrous rats, which consumed very little food (less than 0.5 g) at this time. Multiple mechanisms could account for the estrous cycle- and sex-related changes in the anorectic effect of fenfluramine. Because the duration of fenfluramine’s anorectic effect was greater in females, relative to males, it is possible that drug clearance occurs more slowly in the female rat. To test this hypothesis, hypothalamic concentrations of fenfluramine and its metabolite, norfenfluramine, were measured in male and female rats at 2, 6, and 20 h after acute injection of fenfluramine (29). At 2 h, hypothalamic concentrations of fenfluramine and norfenfluramine were similar in male and female rats. However, at 6 and 20 h, the concentration of fenfluramine was greater in female rats, whereas the concentration of norfenfluramine was greater in male rats. Importantly, the combined concentrations of fenfluramine and norfenfluramine, which are equipotent in their ability to act as 5-HT releasing agents when assessed in terms of brain concentration (9), were identical in male and female rats at both time points (29). Thus, the metabolism of fenfluramine to norfenfluramine may be increased in the female rat, the clearance of fenfluramine and norfenfluramine, assessed over a 20 h period, is similar in male and female rats, at least within the hypothalamus. Alternative mechanisms that could account for the estrous cycle and sex differences in the anorectic effect of fenfluramine reported here include a greater neuronal storage capacity for 5-HT, or reduced activity of inhibitory presynaptic 5-HT receptors, in the female rat. In support of these hypotheses, female rats were found to have greater storage capacity for neuronal 5-HT and higher levels of whole brain 5-HT than male rats (11). Additionally, the preovulatory rise in estradiol secretion in female rats was found to reduce the expression of somatodendritic 5-HT\(_{1A}\) receptors that function to decrease the firing rate of serotonergic neurons (26).

In addition to its anorectic effect, previous studies demonstrate that fenfluramine can induce an aversive internal state.
For example, acute injection of fenfluramine increased aversive taste reactivity responses during intraoral infusions of a 2% sucrose solution (5) and supported the development of a conditioned taste aversion to a novel tasting solution (12). Fenfluramine may also reduce the palatability of novel-tasting solutions. Fenfluramine treatment reduced the number of licks generated by rats consuming a sucrose solution (3, 4), and decreased sham intake of a sucrose solution in rats fitted with open gastric fistulas (27). Although our rats were fed a familiar chow diet that is usually resistant to conditioned taste aversion learning, we wanted to rule out the possibility that the large reductions in food intake observed in Experiment 1, particularly in female rats, were not partially mediated by the induction of an aversive internal state. Thus we examined whether the largest dose of fenfluramine used here was sufficient to induce a conditioned taste aversion to a novel, 5% sucrose solution using a sensitive, two-bottle preference test. Neither male nor female rats displayed any evidence of a conditioned taste aversion. Rather, all rats displayed a strong preference for the sucrose solution. Thus the fenfluramine-induced reductions in food intake observed here appear to be behaviorally specific and not secondary to an aversive effect of the largest dose of fenfluramine tested here.

In summary, the present study demonstrated that the anorectic effect of fenfluramine is modulated by sex and stage of the estrous cycle. Because fenfluramine’s anorectic effect is largely attributed to its ability to increase serotonergic neurotransmission (32), our findings suggest that 5-HT plays a role in the estrus-related decrease in food intake observed in female rats, as well as the lower daily food intake of female rats, relative to male rats. However, this interpretation is limited somewhat by the fact that fenfluramine can also affect the production or release of other neurotransmitters such as acetylcholine and GABA. Although it remains to be determined whether alterations in such transmitter systems are involved in fenfluramine’s ability to cause anorexia (21), and it has been shown that some of these alterations are secondary to fenfluramine’s action on serotonergic systems (20), additional research involving more selective 5-HT receptor agonists and antagonists is required to elucidate a definitive role for endogenous 5-HT in the differential control of food intake in male and female rats. Because peripherally administered fenfluramine readily crosses the blood-brain barrier, our results could have been mediated by a peripheral and/or central serotonergic mechanism. Additional research involving site-specific infusions of 5-HT receptor agonists/antagonists into brain regions implicated in the control of food intake, as well as peripheral administration of 5-HT agonists/antagonists that fail to cross the blood-brain barrier, will be useful in dissociating the relative roles of peripheral and central serotonergic mechanisms in the control of food intake in male and female rats.

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