Estradiol increases glucagon’s satiating potency in ovariectomized rats

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Geary, Nori, and Lori Asarian. Estradiol increases glucagon’s satiating potency in ovariectomized rats. Am J Physiol Regulatory Integrative Comp Physiol 281: R1290–R1294, 2001.—Estradiol decreases meal size, food intake, and body weight in female rats. To investigate whether these effects of estradiol involve a change in the sensitivity of the signaling pathway through which pancreatic glucagon released during meals contributes to meal termination (satiation), glucagon or glucagon antibodies were infused via the hepatic portal vein in ovariectomized rats that were chronically treated with estradiol benzoate (2 μg/day sc) or vehicle alone (100 μl sesame oil). Infusions began at 1 h after dark onset, as rats were refed after 7 h of food deprivation. Glucagon (3 μg/min for 30 min) decreased feeding during the initial 45 min of food access in both groups of rats, but the inhibition was significantly greater in estradiol- than in oil-treated rats. Similarly, antagonism of endogenous glucagon by infusion of glucagon antibodies (a dose neutralizing 3 ng of glucagon in vitro during the first 3 min of refedding) increased feeding significantly more in estradiol- than in oil-treated rats. These data indicate that an increase in the activity of the endogenous glucagon satiation-signaling pathway may be part of the mechanism for estradiol’s inhibitory effect on feeding.

feeding; satiety; gender differences; meal size; ovarian hormones

There is accumulating support for the hypothesis that prandial secretion of the pancreatic peptide hormone glucagon plays a physiological role in the control of meal termination, or satiation. In rats, endogenous glucagon meets current operational criteria for a satiation signal (12–14). This includes demonstrations that acute antagonism of glucagon during meals is sufficient to increase meal size in male (20, 25, 27) and female (30) rats. Intravenous infusion of a physiological dose of glucagon also decreased meal size in men without physical or subjective side effects (19).

The possibility that glucagon’s satiating action may be sexually differentiated has received no attention. This is surprising, because it has long been known that ovariectomy elicits hyperphagia and weight gain in many species, that estradiol treatment reverses these effects, and that the inhibitory effect of estradiol on feeding is expressed as a decrease in meal size, without changes in meal frequency (2–4, 6, 7, 15, 24, 35, 37, 38). Furthermore, in intact cycling rats and mice, increased estradiol secretion during diestrus and proestrus decreases spontaneous meal size (and total food intake) during the subsequent period of estrus after the luteinizing hormone surge (2, 7, 8, 15, 32). Estradiol also increases sexual receptivity and locomotor activity during estrus, but each of these effects appears to be under separate neural control (4, 11, 33).

Comparisons of the feeding patterns of cycling female rats and untreated ovariectomized rats indicate that estradiol has phasic (or cyclic) and tonic inhibitory effects on feeding. The phasic inhibition refers to the reductions in meal size and food intake during the estrous phase of the ovarian cycle compared with the rest of the cycle in intact rats, and the tonic inhibition refers to the reductions in meal size and food intake throughout the ovarian cycle in intact rats compared with untreated ovariectomized rats (6, 7). Continuous estradiol treatment is sufficient to produce the tonic inhibition in ovariectomized rats (3), and cyclic estradiol treatment is sufficient to produce the tonic and the phasic inhibitions (15, 17). One mechanism of the cyclic inhibitory effect of estradiol appears to be an increase in the satiating potency of CCK released from the small intestine during meals (2, 8, 15). The mechanisms of the tonic inhibitory effect are entirely unknown.

Here we report that estradiol treatment increases the satiating potency of glucagon in ovariectomized rats. Thus an increase in the activity of the endogenous glucagon satiation-signaling pathway may be another part of the mechanism for estradiol’s cyclic or tonic inhibitory effect on feeding.

METHODS

Animals and housing. Female Sprague-Dawley rats (Charles River, Wilmington, MA; 175–225 g body wt) were housed in Plexiglas infusion cages (floor area ~475 cm², height 50 cm) with grated stainless steel floors and offered ad

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lubritum access to ground chow (no. 5001, Purina, St. Louis, MO), and tap water. The room was maintained at 20 ± 2°C with a 12:12-h light-dark cycle (lights off from 1500 to 0300). Six 34-W fluorescent ceiling lamps were lit during the bright phase, and two red 25-W incandescent bulbs provided dim illumination during the dark phase.

**Ovariectomy and estradiol treatment.** After 1 wk of adaptation, rats were food deprived overnight, anesthetized with intraperitoneal injections of a combination of 70 mg/kg ketamine (Ketaset, Park-Davis, Morris Plains, NJ) and 4.5 mg/kg xylazine (Sigma Chemical, St. Louis, MO), and bilaterally ovariectomized using an intra-abdominal approach. Three days later, rats were divided into two groups of similar body weight: one group received daily subcutaneous injections of 2 μg of estradiol benzoate (Sigma Chemical) in 100 μl of sesame oil (Sigma Chemical), and the other group received sesame oil alone. Injection sites were varied over the dorsum.

**Hepatic portal vein cannulation.** Beginning 10 days after ovariectomy, rats were deprived of food each day from 0900 to 1600 for 3–4 days. They were then reanesthetized and laparotomized 1 cm lateral to the initial incision, and chronic hepatic portal vein catheters were implanted via the ileocolic veins and exteriorized in the intrascapular area, as described previously (20, 27). Catheters were filled with heparinized (50 U/ml heparin sodium; Elkins-Sinn, Cherry Hill, NJ) bacteriostatic saline (0.9% benzyl alcohol and 0.9% NaCl; Abbott Laboratories, N. Chicago, IL) and flushed with 100 μl of heparinized bacteriostatic saline each 2–3 days. Gentamicin sulfate (0.1 ml; Steris, Phoenix, AZ) was injected into each thigh after surgery. Rats were offered food ad libitum for ≥7 days after surgery.

**Procedure.** Beginning 1 wk after surgery or when their food intake and body weights normalized, rats were returned to the 7-h daily food deprivation schedule. Between 1530 and 1545 each day, the catheter was flushed with 100 μl of bacteriostatic saline and attached to a syringe pump (model A99, Razel, Stamford, CT) by a length of polyethylene tubing (Intramedic PE-60, Clay Adams, Parsippany, NJ) that was filled with bacteriostatic saline and heat molded into an ~1-cm diameter coil to absorb the animal’s movements. At 1558, infusions of bacteriostatic saline (33 μl/min) were begun; at 1600, preweighed food cups were returned; at 1603, or 1630, infusions ended, as described below; and at 1645, the food cups were briefly removed and weighed and the catheters were detached and flushed. In male rats maintained under similar conditions, 45-min food intakes corresponded to the size of the first post-deprivation test meal (18). Tests began after 3 days of adaptation to the infusion procedure. At this time, oil-treated rats weighed 271 ± 6 g and estradiol-treated rats weighed 259 ± 4 g, a significant difference [t(25) = 4.79, P < 0.001]. This weight difference was maintained throughout testing.

Each rat underwent a series of independent crossover tests of the effects of glucagon or glucagon antibody vs. its vehicle. Test order was varied among rats. Infusates were loaded into the polyethylene tubing connected to the indwelling catheter. Because the dead space of the indwelling catheter was 65 μl, test infusates began to reach the rats just as food was returned at 1600.

Glucagon, containing 1 mg of glucagon hydrochloride and 49 mg of lactose (Glucagon for Injection, Eli Lilly, Indianapolis, IN), was diluted first in 1 ml of Lilly diluting fluid (1.6% glycerin and 0.2% phenol) and then in bacteriostatic saline to concentrations of 30 or 90 μg/ml glucagon, which yielded infusion rates of 1 or 3 μg/min glucagon. The vehicle was bacteriostatic saline alone. Infusions ended at 1630. Glucagon inhibited feeding under similar conditions in male rats (40).

Endogenous glucagon was antagonized with specific polyclonal antiglucagon antibodies raised in rabbits [Peninsula Laboratories, Belmont, CA; no cross-reactivity in vitro to human glucagon-like peptides-1 and -2, glucagon-(19–29), insulin, amylin, calcitonin gene-related peptide, or somatostatin]. Infusion duration was 3 min to ensure sufficient amounts of antagonist in the blood during the prandial increase in endogenous glucagon secretion, which begins during the first few minutes of meals in rats (5). Antibodies were diluted in Krebs-Henseleit buffer to concentrations of 0.28 μg/ml and infused between 1600 and 1603. According to the manufacturer’s specifications, these infusions yielded an antibody dose with an in vitro neutralizing capacity of 3 ng of glucagon. This dose of a similar glucagon antibody significantly increased the size of deprivation-induced and spontaneous meals early in the dark period in male rats (20, 25, 27). Control infusions were equal concentrations of nonimmunized rabbit IgG (Vector Laboratories, Burlingame, CA).

Because feeding baselines were less in estradiol- than in oil-treated rats, the capacity of the oil-treated rats to increase food intake was tested. After completion of the infusion tests, six oil-treated rats were maintained on the same feeding schedule, with chow presented at 1600. On 2 consecutive days, dilute sweetened condensed milk (Eagle Brand, Border, Columbus, OH; diluted 1:2 with water) was presented at 1645 for 30 min. Three days after the second adaptation trial, this unexpected milk “dessert” was offered for the third time, and 30-min milk intake was measured (±1 ml).

**Data analysis.** Cannula patency was verified after completion of the feeding tests by injecting 70 μl of the anesthesia mixture. Data were included only if the rat was anesthetized or heavily sedated within 1 min. Body weights and milk intakes were analyzed with t-tests. Chow intakes were analyzed with two-way ANOVAs (type III GLM Procedure, SAS Institute, Cary, NC), with estradiol treatment as a between-subjects factor and glucagon (or glucagon antibody) treatment as a within-subjects factor. Post hoc comparisons were done with Tukey’s honestly significant difference test. Differences were considered significant when P < 0.05. Values are means ± SE. The standard error of the difference (SED) is given as a measure of residual experiment-wide variability after ANOVA. Figure 1 was prepared using SigmaPlot (Jandel, San Rafael, CA).

**RESULTS**

Hepatic portal infusion of 3 μg/min glucagon decreased feeding significantly in oil- and estradiol-treated rats, but its effect was significantly larger in the estradiol-treated rats (Fig. 1A). That is, in estradiol-treated rats, glucagon infusion decreased 45-min food intake 1.3 ± 0.2 g, whereas in oil-treated rats, glucagon decreased food intake 0.7 ± 0.4 g, a significant difference (P < 0.05). These comparisons were based on a main effect of glucagon [F(1,16) = 24.91, P < 0.0001, SED = 0.3 g]; the main effect of estradiol was F(1,16) = 3.97, P < 0.07, and the estradiol-glucagon interaction effect was F(1,16) = 2.26, P > 0.15. Infusions of 1 μg/min glucagon significantly inhibited feeding only in the estradiol-treated rats, but the difference in glucagon’s effect between the two steroid hormone conditions was not significant [data not shown; main effect of glucagon, F(1,10) = 6.27, P < 0.05, SED = 0.3 g; other effects, not significant].
Fig. 1. Estradiol treatment increases the effect of glucagon or glucagon antagonism on feeding in rats. Data (means ± SE) are 45-min food intakes. A: hepatic portal vein infusion of glucagon (3 µg/min for the first 30 min of the test) decreased feeding more in ovariectomized rats that received daily subcutaneous injections of 2 µg of estradiol (right, n = 10) than in oil-treated rats (left, n = 8). **Increase in food intake compared with saline infusion [F(1,22) = 7.47, P < 0.02] and of estradiol [F(1,22) = 11.54, P < 0.003, SED = 0.3 g]; the interaction effect was F(1,22) = 2.63, P > 0.10. Baseline 45-min food intakes were significantly more in the oil- than in the estradiol-treated rats during this test (Fig. 1B). Nevertheless, when oil-treated rats were offered unscheduled access to sweetened condensed milk immediately after the scheduled chow feeding test, they ate 11.5 ± 1.0 ml ([t(5) = 8.00, P < 0.001]. This volume of milk contained 3.0 ± 0.3 g of solids.

DISCUSSION

We report here the first evidence that the satiating action of pancreatic glucagon is sexually differentiated. In ovariectomized rats, estradiol treatment increased the potency of prandial, hepatic portal vein infusions of glucagon to inhibit feeding and the potency of prandial, hepatic portal vein infusions of glucagon antibodies to stimulate feeding. Thus part of estradiol’s inhibitory action on feeding in normally cycling female rats may be mediated by modulation of the activity of the glucagon satiation-signaling pathway. Glucagon has previously been reported to decrease feeding in female rats (30), rabbits (36), ground squirrels (31), and dogs (23, 28). In these studies, however, estrous cycling was not monitored, the role of estradiol was not investigated, and the responses of male and female animals were not compared.

Hepatic portal infusion of glucagon decreased feeding more in estradiol- than in oil-treated rats, despite the fact that baseline feeding in estradiol-treated rats was slightly, although insignificantly, less than in oil-treated rats. During the tests of glucagon antagonism, however, baseline feeding was significantly less in estradiol- than in oil-treated rats. Thus it is possible that a ceiling effect may have obscured the influence of glucagon antibody infusion on feeding in the oil-treated rats. This is unlikely, however, because oil-treated rats consumed considerable amounts of an unscheduled milk dessert when it was offered immediately after the chow test period (indeed, their dessert intake expressed as solid material was more than twice as much as the increase in chow intake of estradiol-treated rats after glucagon antibody infusion). Thus the oil-treated rats certainly had the capacity to eat more when glucagon antibody was infused. Furthermore, in tests of estradiol’s effects on the satiating action of CCK, we observed similar results of CCK antagonism whether or not there were differences in baseline meal sizes (2, 8).

Estradiol’s inhibitory effects on feeding in cycling rodents are expressed as decreases in meal size (2, 3, 7, 24, 32). The release from this inhibition by ovariectomy produces chronic increases in meal size, total food intake, adiposity, and body weight (2, 3, 6, 15, 35, 37, 38). Only when adiposity increases 10–20% does meal frequency decrease so that total food intake approaches normal; the increase in meal size is permanent (3).
Here we treated ovariectomized rats with a dose of estradiol that produces steady plasma levels that are similar to the proestrous maximum estradiol concentration in cycling rats (15, 17). Previously, this treatment normalized spontaneous meal size and frequency, food intake, and body weight in ovariectomized rats (2, 15, 17). We now show that it also increased the satiating potency of glucagon, a physiological control of spontaneous meal size in male rats. Thus the control of glucagon satiation by estradiol may be part of the normal mechanism controlling feeding and body weight in female rats.

Estradiol's inhibitory effect on feeding in cycling rats includes a tonic component that reduces meal size and food intake throughout the ovarian cycle compared with untreated ovariectomized rats and a phasic, or cyclic, component that further reduces meal size and food intake during the estrous phase of the ovarian cycle (2, 6, 7, 15). Tests of CCK's satiating effect in intact cycling rats and in ovariectomized rats maintained on cyclic estradiol treatment indicate that estradiol phasically increases CCK's satiating action but does not tonically increase it (2, 8, 15). Because we used a quasi-continuous regimen of estradiol treatment, further tests are required to determine whether estradiol tonically or phasically increased glucagon's satiating action.

The sites and neural mechanisms of estradiol's actions on feeding are not known. The glucagon satiation-signaling pathway begins with a hepatic vagal afferent projection to the nucleus of the solitary tract (12–14, 36, 40), an area that contains estradiol receptors (22, 34). Furthermore, the neuronal activation in the nucleus of the solitary tract, as indexed by c-Fos protein expression, that is induced by food ingestion or CCK injection in ovariectomized animals is increased by estradiol treatment (9, 10). Thus estradiol may influence glucagon satiation in the initial central site of the glucagon satiation-signaling pathway.

Perspectives

It is increasingly apparent that gender differences are pervasive and important in physiology and medicine. There are prominent gender differences in the normal and pathophysiological controls of feeding in many animal species (15, 16). Some of these controls may have parallels in human eating behavior. For example, just as many animals decrease feeding during the estrous phase of the ovarian cycle, women also eat less during the periovulatory phase of the menstrual cycle (21, 29). Such parallels suggest that physiological factors may contribute to women's dramatically increased risks for anorexia nervosa, bulimia nervosa, binge eating disorder, and severe obesity (1, 26). This possibility underscores the importance of analyses of sexually differentiated controls of eating. It is clear that estradiol is a key mediator linking hypothalamic-pituitary-gonadal axis function to feeding. Yet, before the present report, only a single physiological control of feeding, the satiating action of CCK, has been shown to be estradiol dependent in normally cycling female animals (15). Our demonstration here that the satiating action of prandial hepatic portal glucagon infusion, as well as the desatiating action of prandial glucagon antagonism, is modulated by estradiol in ovariectomized rats strongly suggests that glucagon may be a second such control. Investigation of whether these or other estradiol-dependent controls of eating might contribute to the pathogenesis, course, or treatment of disordered eating in women is indicated.

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REFERENCES


