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Individual effects of estradiol and progesterone on food intake and body weight in ovariectomized binge rats

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ABSTRACT

The individual roles of estradiol (E) and progesterone (P) in the control of food intake and body weight in ovariectomized (OVX) rats were investigated. Six groups of OVX Sprague–Dawley rats (n = 9/group) were assigned to one of three 4-day cyclic hormone treatments: two groups were treated with E benzoate; two groups were treated with P; two groups were treated with both (EP). All rats had continuous access to chow and water throughout this 4-week study. One group of rats within each hormone treatment condition was fed chow ad libitum, and the second was subjected to a binge schedule: chow ad libitum plus 1-h access to an optional fat source on Monday, Wednesday, and Friday. A seventh OVX group (n = 8) received the oil vehicle and chow. This group was included to monitor body weight and to verify hormone efficacy. The main findings were: (1) relative to rats receiving only P, E alone or EP attenuated 24-h chow intake tonically and cyclically, i.e. intake on Day 4, which models estrus, was lower in E and EP than in P, and also was lower than intake on Day 2, which models diestrus. In contrast, (2) neither E nor EP detectably affected optional fat intake during the 1-h fat access period relative to rats receiving only P when data were collapsed across the entire study. However, (3) E and EP had large effects on fat intake relative to P during the 1-h fat access period at the start of the study, but not at the end, when bingeing was fully established. (4) E and EP led to lower and apparently normal levels of body weight compared to rats receiving only the oil vehicle or only P. These results indicate that (1) administration of E alone has similar effects as co-administration of E and P on feeding and body weight in rats bingeing on fat, (2) with or without P, the inhibitory effects of E on meal size are compromised when bingeing on fat, and (3) the effects of E on binge size change dynamically as bingeing develops.

1. Introduction

Ovarian cycling affects food intake in both humans and animals, mainly via estrogens [1–3]. Estrogens have both tonic and cyclic inhibitory effects on eating [1,4,5]. Tonic inhibition is evidenced by the increases in food intake and adiposity that occur with ovariectomy (OVX) in animals and by the increase in adiposity after menopause in women [1–3,6–8]. Cyclic inhibition is evidenced by changes in food intake across the estrous cycle in rats and across the menstrual cycle in women. Female rats and mice eat less during the estrus phase, which occurs just after estrogens peak, and eat more during early diestrus, when estrogen levels are lower [1,2]. In women, food intake decreases in the peri-ovulatory phase, when estrogen levels are highest, and increases in the luteal phase, when both progestin and estrogen levels are high [9–11]. Treatment with cyclic regimens of estradiol (E) normalizes food intake and body weight in OVX rats [1–3,5]. Physiological levels of progesterone (P), on the other hand, do not affect eating in OVX rats; however, pharmacological P treatment can reverse the inhibitory effect of E on eating [3,9].

Ovarian hormones also modulate binge eating. In humans, the frequency of binge eating varies with the menstrual cycle, with higher binge frequency occurring during the luteal phase and menses [12–14]. A negative association between E level and binge frequency, and a positive association between P level and binge frequency, were reported in women with bulimia nervosa (BN) [15]. In addition, in a community sample, higher emotional eating scores (consistent with binge eating) on an eating behavior questionnaire were obtained when E was low and P was high [16]. Associations between E and binge size have not been reported.

In current animal models of binge-type eating, opportunities to binge are experimentally controlled, so that binge size rather than binge frequency is the outcome measure. We previously reported that binge size was tonically, but not cyclically, reduced in OVX rats treated with both E and P [17]. In the same rats, total daily food intake was reduced both tonically and cyclically, suggesting that the modulatory effects of E on food intake differ depending upon the conditions under

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which food is consumed. However, the individual contributions of E and P to binge eating were not ascertained in that study. Although E appears sufficient to explain the contribution of ovarian function to normal eating, in women with BN, cyclic changes in P levels have been independently associated with increased binge frequency [15] and emotional-eating scores [16]. Therefore, it is possible that P contributed to the loss of cyclic inhibitory effects of E on binge size in our previous report [17].

Here we sought to determine the individual and combined effects of E and P on food intake in bingeing rats. We hypothesized that: (1) binge size would be reduced tonically in E and EP rats relative to P rats, (2) binge size would not be cyclically reduced in any of the groups, (3) daily energy intake also would be reduced tonically in E and EP rats relative to P and control oil vehicle rats, and (4) that daily energy intake, in contrast to binge intake, would be cyclically reduced in E and EP, but not in P rats [17,18].

2. Materials and methods

2.1. Subjects

Female Sprague–Dawley rats (Harlan, Indianapolis, IN; 60 days of age) were individually housed in stainless-steel cages with ad libitum access to water and pelleted chow (Laboratory Rodent Diet 5001, PMI Feeds, Richmond, IN; macronutrient content (kcal/kg diet, percent of calories): protein (936, 28%), fat (405, 12%), carbohydrate (1960, 60%); 3.3 kcal/g). The vivarium was maintained at 22 ± 2 °C with a 12/12 h light–dark cycle (lights off at 1900 h). All procedures were approved by the Pennsylvania State University Institutional Animal Care and Use Committee.

2.2. Ovariectomy (OVX)

After one-week adaptation to the vivarium, rats were given a single period of overnight access to fat (Crisco® shortening [hydrogenated vegetable oil], J.M. Smucker Co., Orrville, OH; 9.17 kcal/g) in a bowl clipped to the front of the home cage. This was done to prevent neophobia during the rest of the study. In addition, the overnight fat intake data were used to group the rats after the surgery. Chow and water were available during the overnight fat access. Three days later, the rats were anesthetized (1 ml/kg body weight, IP) with a mixture of 70 mg/kg ketamine (Phoenix Science Inc., St. Joseph, MO) and 2 mg/kg xylazine (Phoenix Science), with 0.2 ml/kg supplements given as needed, and bilaterally OVX using a dorsal approach. Specifically, the surgical area was shaved and then cleaned by alternate iodine and 70% ethanol solutions for a total of 3 cycles. A midline dorsal incision (2.0–3.0 cm) was made through the skin about halfway between the shoulder blades and tail base. Parallel incisions (0.5–1.0 cm) were then made through the underlying abdominal musculature on both sides of the rat, about 2.0 cm lateral to the midline. The ovaries were pulled out through the incisions, the uterine horn and blood vessels were clamped, and a 4-knot ligature was placed around the blood vessels and uterine horn just distal to the clamp. The ovaries were then excised and the clamp was released. After checking for bleeding, the uterine horn was replaced into the abdominal cavity. Absorbable suture material (4-0 vicryl or a generic equivalent) was used to close the muscle layer. Surgical staples were used to close the skin; these were removed after 5–7 days.

2.3. Experimental design

Sixty-two OVX rats were used. After 5 days of postoperative recovery from OVX, rats were matched for body weight and fat intake during the single pre-surgical overnight exposure (above) and divided into seven groups. Two groups (n = 9/group) were maintained on the same 4-day hormone treatment cycle as EP groups in our previous study; that is, subcutaneous injection with 17-β-estradiol-benzoate (E, Sigma, 2 μg/100 μl sesame oil) on Day 2 followed by progesterone (P, Sigma, 500 μg/100 μl sesame oil) on Day 3 [17]; two groups (n = 9/group) had only E injections and another two groups (n = 9/group) had only P injections. These doses of E and P produce near-physiological levels of E [18] and P [19], and the cyclic regimen models the typical 4-day estrous cycle of intact rats; that is Day 1: Diestrus 1, Day 2: Diestrus 2, Day 3: Proestrus, and Day 4: Estrus [17]. Cyclic EP also maintains sexual receptivity (lordosis) in OVX rats [18], and normal body weight and food intake in OVX rats fed chow or allowed to binge on fat [17,18]. Hormone treatments were continued throughout the experiment. The seventh group (n = 8) had oil vehicle injections on Days 2 and 3. This group was maintained on chow throughout the study and was included to monitor body weight gain as an indicator of hormone treatment efficacy.

After four hormone treatment cycles, rats within each hormone treatment group were assigned to one of two feeding protocols: chow only (chow available ad libitum with no fat access) and our standard high-restriction "binge" protocol (chow available ad libitum with fat provided 1 h/day on Mon, Wed and Fri, 2 h prior to lights off). The experimental groups are summarized in Table 1. Chow intake was measured every 24 h, as well as during the 1-h fat access period on Mon, Wed, and Fri in all groups. Fat intake was measured during the 1-h fat access period on Mon, Wed and Fri in the H groups. Body weight was measured before OVX, daily during the 6 days post OVX and once a week thereafter.

2.4. Data analysis

Data were analyzed using SAS 9.1 for Windows (SAS Institute, Cary, NC). The outcomes analyzed were 1-h fat intake (kcal), 1-h chow intake (kcal), 1-h total energy intake (kcal), 24-h chow intake (kcal), 24-h total energy intake (kcal), and body weight (g). All data are presented as means ± SEM. Fat intake data were analyzed via 2-way ANOVA, since only the fat access groups consumed the fat (hormone treatment × cycle day); chow data and total energy intake data were analyzed via 3-way ANOVA, since these analyses included both the chow and the fat-access groups (diet group × hormone treatment × cycle day). Cycle Day 2 data were calculated by averaging all of the Day 2 data for each 4-day hormone treatment cycle across the 4-week study. Similarly, intake data on Day 4 were averaged across all 4 weeks. Cycle Day 2 data and Day 4 data were compared to examine the cyclic effects of E, EP and P because Day 2 models the diestrus phase, in which hormone effects are minimal, and Day 4 models the estrus phase, in which hormone effects are maximal. Significant differences among groups on each day were assessed using preplanned LS means comparison, corrected for the number of comparisons being made. For three comparisons, alpha was set at $0.05/(3-1)=0.025$; for six comparisons, alpha was set at $0.05/(6-1)=0.01$. T-tests were used to determine differences between Days 2 and 4 within each group. Since six t-tests were needed for some of the intake comparisons (for instance 24-h chow intake, which included all 6 groups), alpha was set at $0.05/(6-1)=0.01$ for all Day 2 vs. Day 4 comparisons.

Table 1
Experimental groups.

Group	Hormone treatment				Fat access
	Day 1	Day 2	Day 3	Day 4	
EPC		Estradiol	Progesterone		None
EPH		Estradiol	Progesterone		High-restriction
EC		Estradiol	Oil		None
EH		Estradiol	Oil		High-restriction
PC		Oil	Progesterone		None
PH		Oil	Progesterone		High-restriction