Pergamon

Physiology & Behavior, Vol. 57, No. 1, pp. 155–158, 1995

0031-9384/95 $9.50 + .00

0031-9384(94)00271-1

Estradiol Interacts With Gastric or Postgastric Food Stimuli to Decrease Sucrose Ingestion in Ovariectomized Rats

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Received 31 March 1994

GEARY, N., D. TRACE AND G. P. SMITH. *Estradiol interacts with gastric or postgastric food stimuli to decrease sucrose ingestion in ovariectomized rats.* PHYSIOL BEHAV **57**(1) 155–158, 1994.—The sham feeding preparation was used to determine whether systemic estradiol administration inhibits the intake of 0.8 M sucrose of ovariectomized rats by decreasing the potency of pregastric controls of ingestion. During real feeding, significant reductions in the sucrose intake of estradiol-treated rats appeared within 5–6 min. In contrast, estradiol had no effect on sham feeding at any time. The lack of effect of estradiol on sham feeding indicates that pregastric stimuli are not sufficient to mediate the inhibitory effect of estradiol on feeding in ovariectomized rats. Rather, because estradiol did inhibit real feeding, gastric and/or postgastric food stimuli are necessary for this inhibitory effect. The rapid onset of estradiol's inhibitory effect on real feeding suggests that these postingestive stimuli are selective for controls of the initial phase of the meal.

Food intake Sham feeding Estrus cycle Satiety Pregastric stimuli Palatability

OVARIECTOMY produces a dramatic increase in meal size in rats that is reversed by estradiol treatment (3,13,28). The physiological mechanism of this action of estradiol on meal size is unknown (29,30). Meal size is controlled in large part by afferent signals elicited by food stimuli that occur between the acceptance of food into the mouth until the absorption of the products of digestion (26). Therefore, one possibility is that estradiol affects meal size by influencing the central processing of one or more of these afferent signals. Pregastric afferent stimuli occurring during meals arise from olfactory, gustatory, chemoceptive, mechanoceptive, and proprioceptive receptors activated during ingestion. Pregastric afferent stimuli give rise to both positive feedback controls, which stimulate further ingestion and increase meal size, and negative feedback controls, which inhibit feeding and decrease meal size (26).

We took advantage of the sham feeding preparation to investigate whether estradiol decreases meal size by modulating the potency of pregastric signals, either decreasing positive feedback controls or increasing negative feedback controls. In our sham feeding preparation, ingested food drains from gastric cannulas and does not accumulate in the stomach or enter the intestines in appreciable amounts (32). This isolates the control of feeding from the influence of gastric and postgastric afferent stimuli.

METHOD

Subjects

Female Sprague–Dawley rats (n = 12, 175–225 g; Taconic Farms, Germantown, NY) were housed in solid-bottom Plexiglas cages (46 x 24 x 21 cm tall) with cob bedding (Bed-O-Cobs; Maumee, OH). Water and pelleted chow (#5001; Purina, St. Louis, MO) were provided ad lib. The room was maintained at 22 ± 3°C and brightly lit from 0130 to 1330 h.

After 3 wk in the laboratory, rats were food deprived overnight, anesthetized with a mixture of 80 mg/kg ketamine (Vetalar; Parke, Davis, Morristown, NJ) and 5 mg/kg xylazine (Rompun; Obay, Shawnee, KS), and bilaterally ovariectomized using an intraabdominal approach.

Cyclic ovarian hormone replacement began 1 wk postoperatively and continued throughout. Subcutaneous injections (0.1 ml) of 10 μg β-estradiol 3-benzoate in sesame oil (Sigma Chemical, St. Louis, MO) or the oil vehicle alone were done at 0930 h on Tuesday and Wednesday. Oil alone was injected Friday, and no injections were done on Thursday or Saturday-Monday. This cyclic regimen produces behavioral estrus and some of the neurochemical changes associated with it in ovariectomized rats tested on Friday afternoon (24,25). Further, the degree of sensitization of behavioral estrus remains stable through weeks of tests

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(24,25), in contrast to the progressive changes in ovarian hormone receptors or behavioral sensitivity to gonadal hormone administration that often accompany continuous ovarian hormone replacement (18,19,21).

Rats were food deprived by removing all but two pellets of chow (~5 g) at 1600 on Monday, Tuesday, Wednesday, and Thursday and offered 0.4 M sucrose from 1300 to 1400 on the following day before returning chow until 1600. They were maintained on this schedule during a 9-mo series of tests described elsewhere (ref. 10, Experiment 3, groups V-V and E-V).

The rats were then reoperated to install stainless steel gastric cannulas, as previously described (1,32). Chow was offered ad lib for 10 days postoperatively before reinstating the feeding schedule described above, with two changes. First, 0.8 M sucrose was offered instead of 0.4 M sucrose. Second, before presenting sucrose at 1330, the cap occluding the gastric cannula was unscrewed and the gastric contents were flushed out with 10 ml aliquots of warm 0.15 M NaCl until the drainage was free of food particles. For real feeding trials, the caps were replaced. For sham feeding trials, silastic drainage tubes were attached to the cannulas and hung beneath the cages. There were two criteria for successful sham feeding (14): First, the volume drained from the stomach had to be at least as much as the amount sham fed, and, second, gastric drainage had to be observed within 15 s of the onset of sham feeding. These criteria were met by each rat during each of the sham feeding trials described below.

Rats were adapted to both the real feeding and sham feeding procedure prior to the test trials. The sequence of both the adaptation and test trials, and their relation to estradiol replacement treatment, is shown in Table 1. Trial 12 was the sham feeding test trial. Note that this test then was done on Friday at 1330, 52 h after the second estradiol injection of the week. At this time, our cyclic estradiol replacement procedure produces behavioral estrus (24,25) and potentiates the satiating effect of exogenous cholecystokinin (10). The sham feeding test data were compared to real feeding data on trial 11, that is, the immediately preceding trial, and on trial 16, the subsequent trial that followed estradiol treatment by 52 h.

We analyzed two intake parameters. First, rates of ingestion early in the tests were estimated by converting intake measurements after 5 (trial 11) or 6 min (trials 12 and 16) intake to ml/min. This was done because rate of ingestion early in sucrose feeding bouts is thought to reflect the potency of positive pregastric controls of feeding. Rate data were analyzed by a repeated measures factorial ANOVA followed by pairwise comparisons with Tukey's HSD test. Second, 30-min cumulative sucrose intakes were analyzed. Intake during this interval in real feeding tests corresponded to the first sucrose meal. Estradiol's effects on 30-min sucrose intakes were analyzed with separate t-tests for trials 11, 12, and 16. An overall ANOVA was not used because, due to the increased variability of 30-min sham intakes in comparison to real intakes, the requirement of homogeneity of variance was violated, Cochran's $C(5,6) = 0.59$, $p < 0.01$. Because we expected estradiol to inhibit feeding, directional t-tests were used.

TABLE 1

SEQUENCE OF REAL AND SHAM FEEDING TRIALS AND THEIR RELATION TO ESTRADIOL REPLACEMENT TREATMENT

Trial No.	Day	Replacement Treatment	Feeding Condition
1	Tues	E	R
2	Wed	E	R
3	Thurs	—	R
4	Fri	—	R
5	Tues	E	R
6	Wed	E	R
7	Thurs	—	R
8	Fri	—	S
9	Tues	E	R
10	Wed	E	R
11*	Thurs	—	R*
12*	Fri	—	S*
13	Tues	E	R
14	Wed	E	R
15	Thurs	—	R
16*	Fri	—	R*

* Test trials. E indicates days on which rats in the experimental group received estradiol injections at 0930; rats in the control group received oil injections on these days. R indicates real feeding trials; S, sham feeding trials.

RESULTS

During both real feeding test trials, sucrose intake was reduced in estradiol-treated ovariectomized rats (Fig. 1a,c). It is interesting that this inhibitory effect of estradiol on real feeding occurred rapidly. Cumulative sucrose intake was significantly reduced within 5 min of the onset of sucrose ingestion in trial 11 and between 3 and 6 min after the onset of ingestion in trial 16 (3–6 min intake was 2.5 ± 0.5 ml in control rats vs. 1.2 ± 0.4 ml in estradiol-treated rats, $p < .05$). Further, after this early decrease in feeding in estradiol-treated, real feeding rats, no further inhibition was apparent. That is, although cumulative real intakes remained significantly decreased in estradiol-treated rats for >30 min in trial 11 and for 12 min in trial 16, none of the interval intakes after the first 5 or 6 min of sucrose ingestion were significantly affected by estradiol. This effect did not appear to be a statistical artifact due to the decreasing rate of real feeding in the control rats later in the meal. This is because the control rats' intake in the 5–15 min interval in trial 11 (4.5 ± 0.8 ml), in which estradiol did not inhibit feeding, was larger than the control rats' intake in the 3–6 min interval in trial 16 (2.5 ± 0.5 ml), during which estradiol did inhibit feeding.

In contrast to the significant inhibition of real feeding, estradiol had no effect on sham feeding at any time (Fig. 1b). Note especially that estradiol failed to inhibit the initial rate of sucrose intake only in sham feeding rats (Fig. 2). In the ANOVA of initial feeding rates, neither estradiol treatment nor feeding condition produced a simple main effect ($p > 0.25$), but the interaction effect of these two factors was significant, $F(2,20) = 4.39$, $p < 0.03$, standard error of the difference = 0.13 ml/min. Tukey's tests revealed that this interaction effect was due to an inhibitory effect of estradiol on the initial rate of real feeding of sucrose, with no effect on the initial rate of sham feeding.

DISCUSSION

When ovariectomized rats real fed 0.8 M sucrose, estradiol reduced the initial rate of ingestion and reduced meal size. Estradiol had neither effect when the same rats sham fed. These results have three implications. First, because sham feeding is a specific probe of the potency of pregastric food stimuli to control ingestion (7), the lack of an effect of estradiol on sham feeding indicates that these pregastric stimuli alone are not sufficient to mediate the inhibitory effect of systemic estradiol on feeding in ovariectomized rats under our experimental conditions. Second,

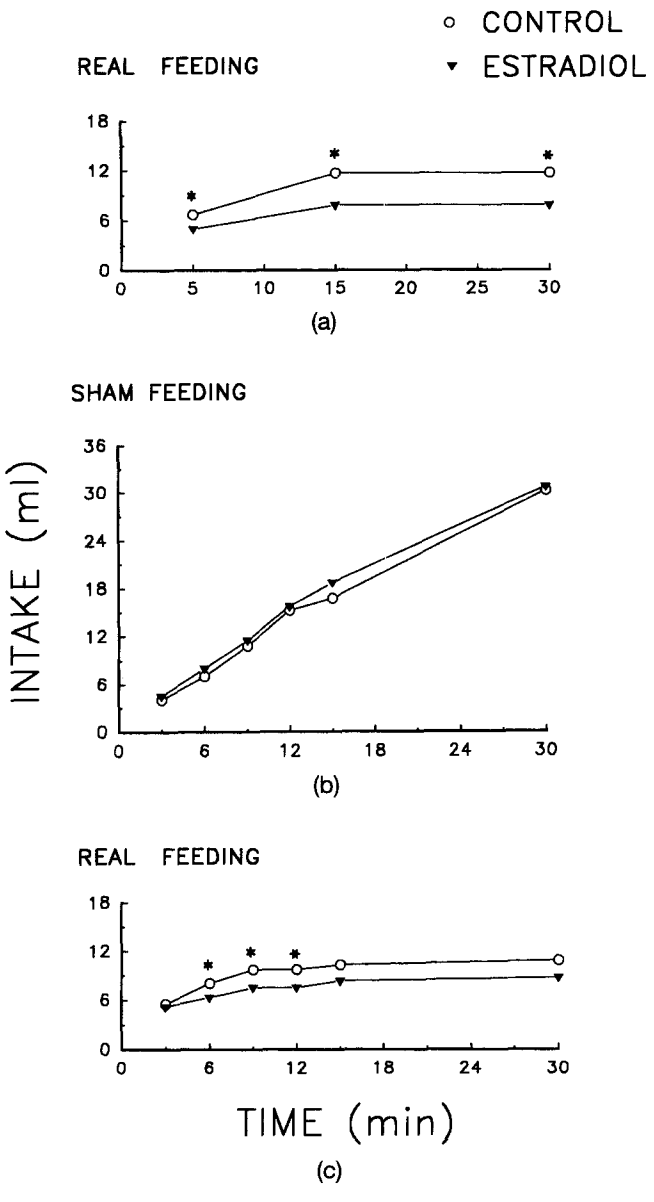

FIG. 1. Estradiol treatment reduced real intake of 0.8 M sucrose, but not sham intake of sucrose, in ovariectomized rats. (a) Mean cumulative real feeding intakes on Trial 11 (Thursday); (b) mean sham intakes on Trial 12 (Friday); and (c) mean real intakes on Trial 16 (Friday). *Different from value in estradiol-treated rats, t-test, $p < 0.05$.

because the estradiol treatments inhibited real feeding, gastric and/or postgastric food stimuli are necessary for this inhibitory effect. And, third, because estradiol's inhibitory effect influenced only the initial rate of real feeding, these postingestive stimuli appear selective for the controls of the initial phase of the meal.

Sham feeding isolates several pregastric food stimuli, which initiate conditioned and unconditioned excitatory and inhibitory controls of ingestion (6,7,14,26,31). For example, in 3–4 h food-deprived rats, the initial sham-fed meals are only modestly increased in size and end in the normal behavioral sequence of postprandial satiety, while subsequent sham-fed meals increase progressively in size as conditioned associations between pregastric food stimuli and postingestive satiety signals extinguish. In the present experiment, the rats' extensive experience real feeding 0.8 M sucrose prior to the sham feeding test provided ample opportunity for such conditioned pregastric satiety signals to increase in strength. Nevertheless, estradiol-treated and control ovariectomized rats sham fed identical amounts. Further, estradiol failed to inhibit sham feeding during the initial 6 min of the test, when the conditioned pregastric satiety signals that occur during sham feeding of 0.8 M sucrose are clearest (7). These results indicate that estradiol did not decrease real feeding solely by increasing the potency of pregastric satiety signals.

Our failure to identify any change in the potency of pregastric controls of food intake that might contribute to the inhibition of 0.8 M sucrose intake caused by estradiol in ovariectomized rats indicates that some gastric or postgastric food stimulus is involved in this inhibition. One hypothesis is that estradiol's feeding effects derive from its effects on energy metabolism in the postabsorptive compartment (29,30). Most tests of this idea, however, have produced negative results (12,17,20,22,23). Further, manipulations of the availability or utilization of metabolic fuels in the postabsorptive compartment generally lead to alterations in meal frequency rather than meal size (15), whereas both endogenous and exogenous estradiol selectively reduce meal size without affecting meal frequency (3,9,13,27). A more promising possibility is that estradiol may decrease meal size by potentiating the satiating effects of cholecystokinin (CCK) released from the small intestine by ingested food. The satiating effect of intraperitoneally injected CCK was increased in ovariectomized rats receiving either continuous (4,16) or cyclic (10) peripheral estradiol replacement. Sham feeding tests also revealed an interaction between pregastric food stimuli and exogenous CCK (2), although whether this interaction operates during the initial phase

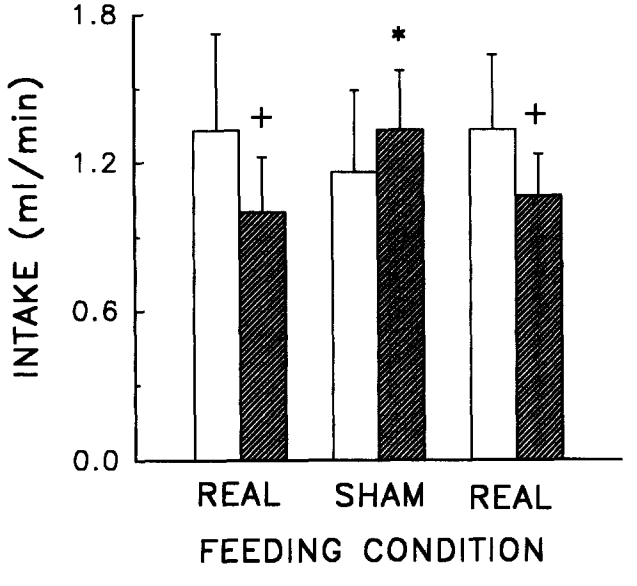

FIG. 2. Estradiol treatment reduced the initial rate of 0.8 M sucrose intake during real feeding, but not sham feeding, in ovariectomized rats. Open bars, control-treated rats; filled bars, estradiol-treated rats. Data are ml/min, M ± SEM, for the first 5 min for the first real feeding test (Trial 11) and 6 min for the sham feeding test (Trial 12) and the second real feeding test (Trial 16). +Different from control-treated rats during same trial, Tukey's HSD after significant ANOVA, $p < 0.05$. *Different from estradiol-treated rats during either real feeding trial, Tukey's HSD after significant ANOVA, $p < 0.05$.

of the meal and whether it is influenced by estradiol remain open questions.

Finally, the apparent functional localization of estradiol's inhibitory effect to the early phase of the meal suggests that future analyses of this phenomenon would profit from a microstructural analysis of feeding that can exactly characterize estradiol's effect on the behaviors (licking, biting, swallowing, etc.) that produce changes in meal size (5,8).

ACKNOWLEDGEMENTS

We thank Bruce McEwen, Ph.D., for help with the design of this experiment and Jane Magnetti for processing the manuscript. These data were reported at the Annual Meeting of the Society for Neuroscience, Washington, DC, November, 1993 (11). This research was supported by NIH research grants DK32448 and MH51135 (N.G.) and NIMH research grants MH40010 and MH00149 (G.P.S.).

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