BRIEF COMMUNICATION

Early Exposure to Low Levels of Estradiol (E₂) Mitigates E₂-Induced Conditioned Taste Aversions in Prepubertally Ovariectomized Female Rats

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MERWIN, A. AND R. L. DOTY. Early exposure to low levels of estradiol (E₂) mitigates E₂-induced condition taste aversions in prepubertally ovariectomized female rats. PHYSIOL BEHAV 55(1) 185–187, 1994.—17β-Estradiol (E₂) can serve as a potent unconditioned stimulus in producing a conditioned taste aversion to saccharin in rats, with the effect being greater in males than in females. To determine whether the female’s lower susceptibility to such conditioning is due, in part, to early experience with estrogen, we performed the following experiment. First, we pretreated groups of prepubertally ovariectomized female rats with either sesame oil (control group) or low doses of E₂ (0.3, 0.75, or 1.50 μg). Second, in subsequent conditioning sessions, we presented a 0.2% saccharin solution to the rats prior to the injection of 100 μg of E₂ (the unconditioned stimulus). Third, we tested the magnitude of the conditioned aversion to the saccharin solution and its tendency to extinguish during the following week. The E₂-pretreated animals evidenced significantly weaker taste aversions and extinguished them more rapidly than did the oil-pretreated controls, even at the lowest E₂ pretreatment dose, suggesting that prior experience with low levels of estrogen can significantly mitigate, in female rats, the magnitude of a conditioned taste aversion produced by a high dose of estrogen.

METHOD

Subjects

Thirty-four female Sprague–Dawley rats (Charles River Breeding Laboratories, Kingston, NY) served as subjects. They arrived at the laboratory as weanlings (i.e., at about 22 days of age) and were housed through the duration of the study in 18 \( \frac{1}{2} \times 10 \times 7 \) in. polypropylene cages. The room was maintained at 64–76°F on a 12:12 light:dark schedule with lights on at 0600 h.

IT is well established that the estrogen 17β-estradiol (E₂), when administered exogenously at a nonphysiologic dose, can serve as the unconditioned stimulus in producing taste aversions to sweet-tasting solutions in both mice and rats (5,7). Male rats acquire this type of aversion more readily and extinguish it more slowly than do female rats (7), and prepubertally ovariectomized female mice extinguish the estradiol-induced taste aversion significantly more slowly than do sham-ovariectomized ones (5). Ovariectomy in adulthood fails to influence the acquisition of a conditioned saccharin taste aversion in female rats (7).

The observation that prepubertally ovariectomized female rats extinguish E₂-conditioned taste aversions more slowly than do sham-operates suggests the hypothesis that estrogens, which increase dramatically at the time of puberty, may serve to mitigate E₂-related conditioned taste aversions produced in adulthood. To test this hypothesis, we treated prepubertally ovariectomized female rats with low doses of E₂ (dissolved in sesame oil) and compared the magnitude of a conditioned taste aversion to saccharin induced by 100 μg of E₂ to that produced in control prepubertally ovariectomized female rats pretreated with sesame oil alone.

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Procedure

Each animal was assigned randomly to one of four groups: sesame oil vehicle (control; n = 8), 0.3 μg 17β-estradiol 3-benzoate (E2; Sigma Chemical Company, St. Louis, MO; n = 8), 0.75 μg E2 (n = 9), or 1.5 μg E2 (n = 9). These doses of E2 are believed to produce plasma levels of E2 that are within the physiological range (see the Discussion section). At approximately 25 days of age, all 34 rats were bilaterally ovariectomized under sodium pentobarbital anesthesia or a ketamine-xylazine anesthetic mixture in conjunction with atropine.

The experimental procedure was divided into three phases. During phase I (pretreatment phase), which began at approximately 44 days of age, the animals received daily SC injections for 8 days of the E2 dose to which they had been assigned. In addition, during the first 5 days of this phase, they were gradually acclimated to a water restriction routine (80 min of water access per day divided into two periods of 20 and 60 min separated by a 1-h period). On the last 3 days, intake of water for each rat during a 20-min access period was recorded to the nearest milliliter. The 3-day mean constituted the fluid consumption baseline for the animal, to which later saccharin solution consumption would be compared. In phase II (acquisition phase), the animals were conditioned by pairing a 0.2% (w/v in water) sodium saccharin solution (provided at the usual water time) with 0.1 mg E2 given 5–20 min after the end of the saccharin access period. This procedure occurred twice, with a recovery day in between, during which only water was offered. In phase III (extinction phase), the rats were tested every other day for their intake of saccharin solution. These consumption figures were transformed individually into a percentage of the water baseline figure, established during the pretreatment phase.

RESULTS

Saccharin consumption, as a percentage of baseline intake, is shown in Fig. 1 for each of the four conditions. It is clear from this figure that the rats pretreated with estradiol acquired weaker taste aversions (i.e., consumed more saccharin after the conditioning) than those pretreated with oil alone, regardless of E2 dose. A two-way analysis of variance (ANOVA) found significant group, F(3, 30) = 28.712, p < 0.0001, and day, F(3, 30) = 26.478, p < 0.0001, effects. Significant differences (p < 0.025) in the relative saccharin consumption were present between the control group and each of the three dose groups on the first 3 days of the experiment (Tukey HSD test). On the fourth day, only the high-dose group differed significantly from the control group (p = 0.012).

To establish whether the E2 pretreatment procedure altered baseline (i.e., preconditioning) fluid intake, we performed a subject group (E2 doses and oil control) by baseline day (days 1–3) ANOVA with repeated measures on the last factor. No meaningful group, baseline day, or group X baseline day interaction effects were found: group, F(3, 30) = 1.79, p = 0.17; baseline day, F(2, 60) = 0.08, p = 0.92; group X baseline day interaction, F(6, 60) = 1.39, p = 0.23. To determine whether, prior to aversive conditioning, saccharin solution intake differed among the groups, we performed an ANOVA on the saccharin intake data from the first conditioning day (i.e., prior to the administration of the conditioning dose of E2). No differences in saccharin intake among the four groups were found: group, F(3, 30) = 1.34, p = 0.28.

DISCUSSION

The present study demonstrates that prepubertally ovariectomized female rats, pretreated with E2, are protected to some degree from acquiring E2-induced saccharin taste aversions. Presumably, prepubertal ovariectomy minimized their early exposure to endogenous E2 and the E2 replacement regime provided their primary experience with this hormone. The lowest dose used in the pretreatment phase of the experiment (0.3 μg E2) was sufficient to attenuate the taste aversion; there was no significant dose-related effect. Thus, it appears that repeated exposures to low doses of E2 can protect females against the severity of E2’s aversion-inducing ability.

Several lines of evidence suggest that the E2 doses administered in the pretreatment phase of this study are at the minimal levels necessary to reverse various effects of ovariectomy and presumably reflect physiological levels of the drug. For example, Davidson et al. (2) reported that 1.6 μg of E2 was the minimum dose necessary to reverse ovariectomy-related changes in pituitary cytology, and that 0.8 μg distinctly reduced the number of pituitary basophils. Other researchers, studying topics ranging from food intake to sexual receptivity, have reported that daily E2 doses from 0.2 to 3.2 μg effectively reverse some or all of the consequences of ovariectomy (8,11).

Although one might argue that the results depicted in Fig. 1 reflect factors other than the aversion-inducing effects of E2 (e.g., metabolic influences of estrogen on saccharin preference or on fluid consumption), we feel this is unlikely for several reasons. First, Zucker (10) has shown that estradiol alone does not increase saccharin preferences in ovariectomized rats unless administered in combination with progesterone. Second, since E2 normally decreases fluid intake in rats, it would be expected that, after conditioning, the E2-pretreated rats would evidence lower, not higher, levels of fluid intake relative to their oil-treated counterparts. Third, the E2 treatments did not differentially alter the intake of saccharin solution prior to the conditioning event. Finally, there is a large literature that strongly implicates estrogen in aversive conditioning. For example, it is known that estrogen can cause nausea in humans when administered for therapeutic reasons (3,7), and nausea has been repeatedly shown to serve as the unconditioned stimulus for the induction of a wide variety of taste aversions (7).
in the brain (6), it may be the brain region responsible for producing the effects noted in this study. In regard to this hypothesis, it is of interest that a) Gustavson (4) has proposed that the decreased food intake that characterizes human anorexia nervosa (AN) may be due, in some cases, to abnormally low levels of estrogens at puberty and b) Young (9) suggests that the symptoms of AN may arise from a hypersensitivity of the hypothalamus to estrogen, as the hormone can induce the majority of the symptoms associated with the disorder (e.g., nausea, reduced body weight, abnormally low levels of LH, hyperactivity, insomnia), especially prior to puberty. Interestingly, AN patients respond to clomiphen, an antiestrogen drug, not with LH elevation, but with LH suppression, as occurs in children (1).

Regardless of the validity of the hypothesis that estrogen sensitivity may be related to the development of some cases of anorexia, the present findings suggest that prior experience with low levels of estrogen can significantly mitigate, in female rats, the magnitude of a conditioned taste aversion to saccharin subsequently produced by a high dose of estrogen. Whether pre-treatment of males with low E2 would similarly mitigate the magnitude of such a conditioned taste aversion requires further study.

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REFERENCES