Preferential Growth Stimulation of Mammary Glands over Uterine Endometrium in Female Rats by a Naturally Occurring Estradiol-17β-fatty Acid Ester

Laura H. Mills, Anthony J. Lee, A. F. Parlow, and Bao Ting Zhu

Department of Basic Pharmaceutical Sciences, College of Pharmacy, University of South Carolina, Columbia, South Carolina 29208 (L. H. M., A. J. L., B. T. Z.), and National Hormone and Pituitary Program, Harbor-University of California Los Angeles Medical Center, Torrance, California 90509 (A. F. P.)

ABSTRACT

We hypothesize that the endogenously present lipoidal estrogen fatty acid esters may have a stronger mitogenic action in the fat-rich mammary tissues than in the uterus. To test this hypothesis, we compared the activity of estradiol-17β-stearate (E₂-17β-S) with that of estradiol-17β (E₂) in stimulating the growth of mammary glandular cells versus the growth of uterine endometrial cells in ovariecimated female Sprague Dawley rats. Experimentally, an estimated 0.5 or 5 nmol of E₂-17β-S or E₂ was released daily to ovariectomized female rats through an Alzet pump implanted under the back skin of the animal for 10 or 23 days. The growth-stimulatory effect of E₂-17β-S and E₂ on mammary glandular cells was determined according to 5-bromo-2′-deoxyuridine labeling indices, and their effect on the uterus was determined by measuring both the 5-bromo-2′-deoxyuridine labeling index and the uterine wet weight. Our results showed that chronic treatment of ovariectomized female rats with 0.5 or 5 nmol/day E₂-17β-S for 10 or 23 days had a stronger stimulatory effect on mammary glandular cell proliferation than treatment with equimolar doses of E₂. In the uterus, however, E₂ was more active in stimulating the proliferation of uterine endometrial cells than E₂-17β-S at equimolar doses. Our results demonstrated, for the first time, that a naturally occurring estradiol-17β-fatty acid ester has a differential, strong mitogenic effect in the fat-rich mammary tissues, and this effect was not observed with E₂. It is tempting to suggest that the fatty acid esters of the endogenous estrogens and their bioactive metabolites (e.g., 4-hydroxyestradiol and 16α-hydroxyestrone) may be of unique importance for stimulating cell growth and possibly also for inducing tumor formation in the fat-rich mammary tissues as compared with the uterus. More studies are warranted to test these ideas.

INTRODUCTION

It has been known for decades that chronic administration of an estrogen induces tumor formation in the target organs of a number of laboratory animals (1–7). In humans, there is strong evidence associating chronic estrogen administration with an increased risk of postmenopausal uterine endometrial cancer (8–12). Although there is also mounting evidence associating elevated human breast cancer risk with an increased total lifetime exposure to estrogens (13–16), this relationship generally is not as strong as the link between estrogen and human endometrial cancer. The mechanism underlying the difference is not fully understood at present. Zhu and Conney (17) recently pointed out that measurement of the circulating unmetabolized parent estrogens, such as E₂ (18) and estrone, might be too crude a parameter to be used successfully for associating the exposure of target tissues or cells to estrogens with the risk of hormonal cancers. It is possible that some of the biologically active estrogen derivatives (such as the fatty acid esters of endogenous E₂, 4-hydroxyestradiol, and 16α-hydroxyestrone) may be of unique importance in the fat-rich mammary tissues when compared with other estrogen target organs like the uterus.

Previous studies by Dr. Richard B. Hochberg and others have shown that the endogenous estrogen fatty acid esters have potent, long-lasting hormonal activity in certain target organs, which likely results from the slow enzymatic release of the parent estrogens in situ (18). Because of their extremely high lipophiliicity and slow metabolic disposition, estrogen fatty acid esters have very long half-lives in animals and humans (19–23). High concentrations of estrogen fatty acid esters are present in fat (24), whereas their blood concentrations are very low or undetectable (24). In addition, studies have suggested that large amounts of E₂ fatty acid esters are present in human follicular fluid, at concentrations comparable to those of follicular E₂ (25–28). This finding may suggest that the estrogen fatty acid esters account for a significant fraction of the endogenous ovarian estrogen pool.

The endogenous estrogen fatty acid esters are synthesized by estrogen acyltransferase using various fatty acyl-CoAs as cofactors (23, 29–35). This enzyme was found to be present in liver as well as in many extrahapatic tissues (such as the brain, fat, breast, ovary, uterus, and placenta) of rats (23, 29–35). Surprisingly, the brain and various fat tissues (including the fat-rich mammary tissue) of female rats contained higher or comparable levels of this enzyme activity when compared with the liver. The widespread presence of estrogen acyltransferase activity makes it possible that large quantities of these endogenous estrogen-fatty acid esters may be formed in the body, particularly when excess amounts of estrogens are present.

However, the physiological and pathophysiological importance of the endogenously formed estrogen fatty acid esters is mostly unknown at present. We hypothesize that the lipoidal estrogen fatty acid esters may be an especially important group of endogenous estrogens for stimulating cell growth and probably also for inducing tumor formation in the fat-rich mammary tissues when compared with the uterus. The estrogen fatty acid esters may serve as a reservoir for the sustained release of bioactive estrogens to mammary glandular cells because these cells are surrounded by large amounts of adipocytes that serve as a storage site for the lipoidal estrogen esters. In partial support of this interesting hypothesis, Xu et al. (34, 35) recently showed that marked induction of estrogen fatty acyltransferase activity in the liver of ovariectomized female rats by chronic administration of clofibrate resulted in selective enhancement of the mitogenic activity of E₂ in the breast but not in the uteri. In the present study, we compared the activity of E₂-17β-S (structure shown in Fig. 1), a representative naturally occurring E₂-17β-fatty acid ester, with E₂ for stimulating the growth of mammary glandular cells versus the growth of uterine endometrial cells in ovariecimated female Sprague Dawley rats. Our data demonstrated that chronic administration of E₂-17β-S had a differential, strong mitogenic effect in the fat-rich mammary tissues and that this effect was not observed with E₂.

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1 To whom requests for reprints should be addressed, at Department of Basic Pharmaceutical Sciences, College of Pharmacy, University of South Carolina, Coker Life Sciences Building, 700 Sumter Street, Columbia, SC 29208. Phone: (803) 777-4802; Fax: (803) 777-8356; E-mail: BTZhu@cop.sc.edu.
2 The abbreviations used are: E₂, estradiol-17β; E₂-17β-S, estradiol-17β-stearate; PRL, prolactin; LH, luteinizing hormone; FSH, follicle-stimulating hormone; RIA, radioimmunoassay; BrdUrd, 5-bromo-2′-deoxyuridine.


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**MATERIALS AND METHODS**

**Chemicals.** E₂-17β-S and E₂ were obtained from Steraloids (Newport, RI). Our high-performance liquid chromatography analysis of E₂-17β-S showed a purity of almost 100%. BrdUrd, 3,3-diaminobenzidine (as the peroxidase substrate, in a tablet set), acidic alcohol, and Scott’s tap water were purchased from the Sigma Chemical Co. (St. Louis, MO). Mouse anti-BrdUrd primary antibody was obtained from Novocastra Laboratory (New Castle upon Tyne, United Kingdom). The Vector ABC Elite kit, 1% normal horse serum, and biotinylated secondary antibody against mouse IgG were obtained from Vector Laboratories (Burlingame, CA).

**Animal Experiments.** Ovariectomized female Sprague Dawley rats (average age, 6.5 weeks old) were purchased from Harlan Sprague Dawley Laboratory (Houston, TX). After arrival, they were allowed to acclimate for a week before the experiment began. The animals were housed under controlled conditions of temperature and photoperiod (12-h light/12-h dark cycle) and had free access to food and water throughout the experiment period. For the first set of experiments, the animals were randomly divided into one control group and two treatment groups (one E₂-17β-S treatment group and one E₂ treatment group). Each animal in the E₂-17β-S or E₂ treatment group was surgically implanted under the back skin with an Alzet osmetic minipump (model 1002; release rate of 0.25 μl/h for up to 14 days) containing a 0.83 mM solution of estrogen, which was estimated to release 0.5 and 5 nmol/day E₂-17β-S or E₂. For this experiment, the animals were surgically implanted under the skin of each ovariectomized female rat under halothane anesthesia, a procedure approved by the Institutional Animal Care and Use Committee of the University of South Carolina.

For the second experiment, the same procedure was followed, except five groups of animals were used: one control group, two E₂-17β-S treatment groups, and two E₂ treatment groups. The Alzet minipumps were filled with a 0.083 or 0.83 mM solution of an estrogen, which was estimated to release a 0.5 and 5 nmol/day E₂-17β-S or E₂. In this animal experiment, a larger size of the Alzet minipump (model 2004) was used, and the pumps would continuously release the filled solution at a rate of 0.25 μl/h for up to 28 days. The procedure for preparation of the minipumps was the same as that described above for the first experiment. All of the animals used in this experiment were sacrificed 23 days after implantation of the Alzet minipumps.

**BrdUrd Labeling of Proliferating Cells in the Breast and Uterus of Female Rats.** The BrdUrd labeling of proliferating cells in the breast and uterus of female Sprague Dawley rats was carried out as described previously (36). Two h before sacrifice, the animals were injected i.p. with 50 μg BrdUrd/g body weight (dissolved in sterile saline). The animals were then euthanized in a small CO₂ chamber and immediately decapitated. Blood samples (average, 4–5 ml) were collected from each animal and placed in Vacutainer test tubes containing heparin sodium (purchased from Fisher Scientific, Suwanee, GA). The uteri were removed, trimmed of excess connective tissues, and weighed precisely to determine their wet weight. The mammary tissues were also removed. The mammary and uterine tissues were fixed in Carnoy’s fixative for 3 h and dehydrated by sequential transfer through a gradient of ethanol solutions with decreasing concentrations. Immediately after this step, the slides were placed in a solution of 3% H₂O₂ (in methanol) to quench endogenous peroxidase activity and then treated with 4.0 N HCl for 30 min to denature the cellular DNA. The sections were blocked using 1% normal horse serum and then incubated with anti-BrdUrd primary antibody (Novocastra Laboratory) at 1:100 dilution for 60 min, followed by incubation with a 1:100 dilution of a biotinylated secondary antibody (Vector Laboratories) for 45 min at room temperature. Detectors of BrdUrd-incorporated cells were done by using ABC Elite reagent (Vector Laboratories), and color was developed by incubation with 3,3′-diaminobenzidine for 5 min. Harris hematoxylin was used as a counterstain, and the slides were preserved with Cytoseal (Stephens Scientific, Kalamazoo, MI). Typically, the large nuclei of the BrdUrd-incorporated cells were selectively stained brown (some dark brown). The numbers of the stained and unstained mammary glandular cells or uterine endometrial cells for each tissue slide were determined as described in “Materials and Methods.” Each value was the mean ± SEM of five to six animals.
mined manually by counting ~10 representative fields under a light microscope (×40) in a double-blinded manner, and the BrdUrd labeling indices (the percentage of cells positively stained) were then calculated accordingly.

**RIA of the Plasma Levels of E₂, PRL, LH, and FSH.** The collected whole blood sample from each animal was kept in a Vacutainer test tube at 4°C for 2 h and then centrifuged for 15 min at 3000 rpm. Aliquots (200 μl) of the plasma samples were transferred precisely to another set of small vials with sealed caps and stored at −80°C. The plasma levels of E₂ were measured by using a double-antibody RIA kit provided by Diagnostic Products Corp. (Los Angeles, CA). Immediately before the assay, each plasma sample was diluted in the zero calibrator buffer provided by the vendor. Known standards, also provided by the vendor, and the rat plasma samples were incubated with 100 μl of rabbit anti-E₂ antibody for 2 h at room temperature followed by a 1-h incubation with 100 μl ¹²⁵I-labeled E₂. Aliquots of the ice-cold precipitating solution were then added to each tube, and all tubes were centrifuged for 15 min at 3000 × g. The supernatant was decanted, and the sediment in each tube was measured for radioactivity with a gamma counter. The concentration of E₂ in the unknown samples was calculated according to the standard curve generated. The assay sensitivity for E₂ was 5 pg/ml.

The plasma levels of rat LH, FSH, and PRL were determined by highly sensitive, specific, and quantitative RIAs, using the RIA reagent sets prepared and distributed by the National Hormone and Peptide Program of the NIDDK, directed by Dr. A. F. Parlow (Parlow@humc.edu). Results were expressed as ng/ml, in terms of rat LH (RP-3), FSH (RP-2), and PRL (RP-3).

**RESULTS**

**Effect of E₂-17β-S versus E₂ on Uterine and Mammary Cell Growth**

**Experiment I.** In this initial experiment, an estimated 5 nmol/day E₂-17β-S or E₂ was released to ovariectomized female Sprague Dawley rats for 10 days through an Alzet minipump implanted under the back skin of each animal at ~7.5 weeks of age. The growth-stimulatory effect of E₂-17β-S and E₂ on mammary glandular cells was determined by measuring the BrdUrd labeling index, and their effect on the uterus was determined by measuring both the uterine wet weight and the BrdUrd labeling index for uterine endometrium.

As shown in Fig. 2A, treatment of animals with 5 nmol/day E₂-17β-S or E₂ for 10 days significantly increased the uterine wet weight as compared with that of the controls, but the activities of these two estrogens were not significantly different. However, the rate of uterine endometrial cell proliferation (according to the BrdUrd labeling in-
In animals treated with 5 nmol/day E2-17β-S or E2, (0.5 and 5 nmol/day for 23 days) on uterine wet weight (A and A'), uterine endometrial cell proliferation (B and B'), and mammary glandular cell proliferation (C and C') in female rats. The ovariectomized female Sprague Dawley rats (6.5 weeks old) were randomly divided into one control group, two E2 treatment groups, and two E2-17β-S treatment groups. Each animal was surgically implanted under the back skin with an Alzet osmotic minipump (model 2004; release rate of 0.25 µl/h for up to 28 days) containing the desired concentration of E2, E2-17β-S, or vehicle only. Twenty-three days after estrogen treatment, the animals were sacrificed, and their uterine wet weights and BrdUrd labeling indices for uterine endometrial cells and mammary glandular cells were determined as described in "Materials and Methods." Each value was the mean ± SE of seven to eight animals.

Similar to the results from the first experiment, the uterine wet weight of each animal treated with either 0.5 or 5 nmol/day E2-17β-S or E2 for 23 days was significantly increased as compared with that of the controls (see Fig. 4, A and A'). At the lower dose level (0.5 nmol/day), the stimulatory effect of E2 on uterine wet weight gain was greater than that of E2-17β-S (P < 0.001, Fig. 4A), but their effects were not different at the higher dose (5 nmol/day; Fig. 4A'). Measurement of the BrdUrd labeling indices showed that 0.5 nmol/day E2 stimulated the proliferation of uterine endometrial cells ~5 times more than did E2-17β-S (P < 0.001; Fig. 4B). A 10× higher dose (5 nmol/day) of E2 had ~30% higher activity in stimulating the proliferation of endometrial cells than the lower dose; in contrast, the higher dose of E2-17β-S had ~300% higher activity than the lower dose (compare Fig. 4, B and B'). These data suggested that E2 had a higher potency than E2-17β-S in stimulating uterine endometrium growth.

According to the BrdUrd labeling index, the rate of mammary glandular cell growth in untreated ovariectomized female rats was 1.1 ± 0.1% (Fig. 4, C and C'). In animals treated with 0.5 nmol of E2-17β-S or E2 for 23 days, the rate of mammary cell growth was increased to 3.1 ± 0.2% (200% increase over the control; P < 0.0001) or 2.0 ± 0.2% (90% increase over the control; P < 0.01), respectively.
Effect of E2-17b-S on mammary glandular cell proliferation than did E2 at equimolar doses. In the uterus, however, E2 was much stronger stimulatory effect on mammary glandular cell proliferation into one control group, two E2 treatment groups, and two E2-17b-S treatment groups, with 7–8 animals/group. Each animal was surgically implanted under the back skin with an Alzet osmotic minipump (model 2004; release rate of 0.25 μl/h for up to 28 days) containing a desired concentration of E2, E2-17b-S, or vehicle only. Twenty-three days after estrogen treatment, the animals were decapitated and their blood samples were collected in Vacutainer test tubes containing heparin as described in “Materials and Methods.” The plasma levels of free E2 were determined by using the double-antibody RIA kit provided by Diagnostics Products Corp., and the plasma levels of LH, FSH, and PRL were analyzed at the National Hormone and Pituitary Program by using 125I-labeled RIAs. Each value is the mean ± SE of six to eight animals.  

**Table 1** Effect of chronic E2-17b-S or E2 administration (0.5 and 5 nmol/day for 23 days) on the plasma levels of free E2, LH, FSH, and PRL in ovariectomized female rats

<table>
<thead>
<tr>
<th>Treatment groups</th>
<th>E2 (pg/ml)</th>
<th>LH (ng/ml)</th>
<th>FSH (ng/ml)</th>
<th>PRL (ng/ml)</th>
</tr>
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<tr>
<td>Control</td>
<td>4.2 ± 1.6</td>
<td>18 ± 1.1</td>
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<td>5 ± 1.4</td>
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<td>E2-17b-S (0.5 nmol/day)</td>
<td>8.6 ± 0.5</td>
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<td>E2-17b-S (5 nmol/day)</td>
<td>8.2 ± 0.4</td>
<td>3 ± 0.7*</td>
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<td>E2 (0.5 nmol/day)</td>
<td>6.8 ± 1.3</td>
<td>10 ± 1.2*</td>
<td>48 ± 2.0</td>
<td>5 ± 0.7</td>
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<td>E2 (5 nmol/day)</td>
<td>10.6 ± 1.5</td>
<td>2 ± 0.7*</td>
<td>37 ± 2.8</td>
<td>12 ± 1.8*</td>
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* P < 0.01 as compared to the corresponding control.

**DISCUSSION**

In the present study, we compared the mitogenic effect of E2-17b-S with E2 in the breast and uterus of ovariectomized female rats. Our results showed that chronic administration of 0.5 or 5 nmol/day E2-17b-S to ovariectomized female rats for 10 or 23 days had a markedly stronger stimulatory effect on mammary glandular cell proliferation than did E2 at equimolar doses. In the uterus, however, E2 was much more active than E2-17b-S in stimulating the proliferation of endometrial cells.

**Effect of E2-17b-S versus E2 on Animal Body Weight and Plasma Levels of E2, PRL, FSH, and LH**

It is known that chronic estrogen administration decreases an animal’s body weight gain in a dose-dependent manner (37). Our results also showed that administration of 0.5 or 5 nmol/day E2-17b-S or E2 for 23 days decreased the animal’s body weight gain. Furthermore, the body weights of the animals treated with 0.5 or 5 nmol/day E2 were somewhat lower than those receiving equimolar doses of E2-17b-S (Fig. 5).

We also determined the plasma levels of free E2, PRL, FSH, and LH in ovariectomized female rats treated with 0.5 or 5 nmol/day E2-17b-S or E2 for 23 days. Analysis of plasma levels of free E2 by RIA showed that animals receiving 0.5 or 5 nmol/day E2-17b-S or E2 showed higher plasma concentrations of free E2 than control animals receiving no estrogen treatment, but the differences between E2-17b-S- and E2-treated animals were not statistically significant (Table 1). Our measurements of the plasma levels of free E2 concentrations in the control and E2-treated animals were in close agreement with an earlier report (37).  

Chronic administration of 0.5 nmol/day E2 for 23 days showed a stronger negative regulatory effect on the plasma LH levels than did administration of 0.5 nmol/day E2-17b-S (Table 1). However, both of the estrogens markedly decreased the plasma LH levels to a similar extent at a 10× higher dose (5 nmol/day; Table 1). The plasma levels of FSH in ovariectomized female rats were not significantly affected by treatment with 0.5 or 5 nmol/day E2-17b-S or E2 for 23 days (Table 1). These data suggested that the pituitary LH and FSH secretion had different sensitivities to estrogen feedback regulation. Analysis of the plasma levels of PRL indicated that chronic administration of 0.5 nmol/day E2-17b-S or E2 did not show detectable effects on the plasma levels of PRL. A 10× higher dose (5 nmol/day) of both estrogens markedly increased the plasma PRL levels, but their effects were not significantly different from each other (Table 1).  

In summary, administration of 0.5 or 5 nmol/day E2 for 23 days showed a somewhat stronger inhibitory effect on the animal’s body weight gain than did E2-17b-S. Chronic administration of 0.5 nmol/day E2 was significantly more repressive than an equimolar dose of E2-17b-S in lowering the postovariectomy plasma levels of LH. However, this difference was not evident either in relation to their plasma PRL-elevating activity or in relation to their virtual noneffect in repressing plasma FSH levels.

**Figure 5** Effect of chronic E2-17b-S or E2 administration (0.5 and 5 nmol/day) on the body weight of female rats. The treatment of the animals was the same as that described in the Fig. 3 legend. The animal’s body weight was measured immediately before and 6, 14, and 23 days after implantation of the Alzet minipumps. Each value was the mean ± SE of seven to eight animals.

**Table 1** Effect of chronic E2-17b-S or E2 administration (0.5 and 5 nmol/day for 23 days) on the plasma levels of free E2, LH, FSH, and PRL in ovariectomized female rats

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* P < 0.01 as compared to the corresponding control.

**Figure 4** When the animals received a 10× higher dose of E2-17b-S or E2 (5 nmol/day) for 23 days, the rate of mammary glandular cell growth was increased to 12.3 ± 1.2% (10.7-fold increase over the control; P < 0.001) and 5.6 ± 0.3% (4.3-fold increase over the control; P < 0.001), respectively (Fig. 4).

In summary, chronic administration of 0.5 or 5 nmol/day E2-17b-S to ovariectomized female rats for 10 or 23 days had a markedly stronger stimulatory effect on mammary glandular cell proliferation than did E2 at equimolar doses. In the uterus, however, E2 was much more active than E2-17b-S in stimulating the proliferation of endometrial cells.
enous estrogen-fatty acid esters were present in the blood, but much higher concentrations were present in the fat tissues (24). Because the mammary glandular cells are surrounded by large amounts of adipocytes when compared with uterine endometrial cells, it is possible that the large amounts of mammary adipocytes may serve as a storage site for the endogenously formed estrogen fatty acid esters, which may partially contribute to the stronger mitogenic activity of E₂-17β-S in mammary glands compared with the uterus.

It is known that the estrogen fatty acid esters themselves cannot activate the estrogen receptor. Their potent, long-lasting hormonal activity in the target organs results largely from the slow in situ release of the parent estrogens through esterase-mediated cleavage of the fatty acid chain (18). The differential mitogenic activity of E₂-17β-S in the mammary glands observed in this study may also be due to the presence of higher estrogen esterase activity in the breast tissues as compared with the uterus, which would release more bioactive estrogens from their respective estrogen fatty acid esters for stimulation of mammary cell growth. In support of this speculation, an earlier study showed the presence of estrogen esterase activity in human breast cancer cell lines (38). Our recent preliminary studies also showed that high estrogen esterase activity was present in rat mammary crude homogenates and microsomes, and this esterase activity was markedly higher than that in the uterus. More research is warranted to advance our knowledge on the tissue/cell distribution of estrogen esterase activity and its regulation by hormonal and environmental factors.

It should be noted that several earlier studies have compared the uterotrophic effect of a few endogenously present estrogen fatty acid esters with that of E₂ when given to female animals (18, 19, 21). In most of these studies, the immature female rats or mice usually received a single s.c. or i.v. injection of an estrogen-fatty acid ester or E₂ (in oil solutions), and the uterotrophic effect was determined by measuring uterine wet weight at various time points after estrogen injection (18, 19, 21). The results of these studies showed that the estrogen fatty acid esters had a longer-lasting and stronger stimulatory effect than E₂ on uterine wet weight increase (18, 19, 21). Although similar doses or the same doses (such as 5 nmol/day) of the estrogen fatty acid esters were also used in some of the earlier studies, the routes of administration were different; whereas we gave the estrogen by using minipump-controlled continuous release, most of the earlier studies gave the total amount of an estrogen with a single i.p. or i.v. injection. Comparing these two common methods of estrogen administration, we believe that the minipump-controlled continuous release may bear a greater resemblance to endogenous hormone secretion. This difference in the route of estrogen administration is likely a major contributing factor to the observed differences in the biological activity in the uterus.

Lastly, it is also of interest to note that our results indicated that the uterine wet weight gain in female rats treated chronically with an estrogen did not fully agree with the rate of uterine endometrial cell proliferation as determined by measuring the BrdUrd labeling indices. Assaying the uterotrophic activity in mice or rats by measuring uterine wet weight as determined by measuring the BrdUrd labeling indices. Assaying the uterotrophic activity in mice or rats by measuring uterine wet weight gain has been the most commonly used in vivo method for evaluating the hormonal activity (potency and efficacy) of an estrogen. Because of its convenience and readily reproducible dose-response curves, this bioassay has recently been recommended as a basic assay for evaluation of the estrogenic hormonal activity of a very large number of potential endocrine disruptors. It should be emphasized, in light of our observations, that the original assay conditions under which this assay was established preferred that the animals be treated with an estrogen for only a short period of time (6 h to 2 days). Measurement of uterine wet weight after chronic administration of an estrogen to immature or ovariectomized rats or mice may not provide a reliable estimation of the hormonal potency and efficacy of an estrogen.

In summary, we demonstrated that E₂-17β-S, a representative endogenous E₂-17β-fatty acid ester, has a differential, strong mitogenic effect in the fat-rich mammary tissues and that this effect was not observed with E₂. Based on this observation, it is tempting to speculate that the estrogen fatty acid esters may be a group of endogenous estrogens that are particularly important for stimulating cell growth and possibly for inducing tumor formation in the fat-rich mammary tissues as compared with some other estrogen target organs such the uterus. The estrogen fatty acid esters may serve as a reservoir for the sustained release of parent estrogens and their bioactive metabolites (such as 4-hydroxyestradiol and 16α-hydroxyestrone) in mammary glandular cells because they are surrounded by large amounts of adipocytes (which can be a storage site for the lipoidal estrogen esters) and also because they contain high levels of estrogen esterase activity (which catalyzes the release of bioactive estrogens). It is also worth suggesting that the levels of endogenous estrogen fatty acid esters and the activity of estrogen esterase present in the breast might be of more relevance as risk factors for human breast cancer than the circulating levels of unesterified estrogens that were usually measured in earlier epidemiological studies.

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