Estrogens Augment the Stimulation of Ovarian Aromatase Activity by Follicle-stimulating Hormone in Cultured Rat Granulosa Cells*

(Received for publication, November 13, 1981)

Eli Y. Adashi and Aaron J. W. Hsu$h
From the Department of Reproductive Medicine, University of California, San Diego, La Jolla, California 92039

The effects of estrogens on ovarian aromatase activity were investigated in vitro using granulosa cells from immature hypophysectomized estrogen-primed rats. The cells were cultured for 3 days in an androgen-free medium in the presence of follicle-stimulating hormone (FSH), with or without the specified estrogen. After washing, the cells were reincubated for 5 h with $10^{-7}$ M androstenedione, and the formation of estrogens was measured. Estrogen production by control and diethylstilbestrol-treated cells was negligible, while FSH stimulated aromatase activity. Furthermore, concomitant treatment with diethylstilbestrol led to dose-dependent increases in the FSH-induced aromatase activity with an $ED_{50}$ value of $4 \times 10^{-8}$ M and an apparent $V_{max}$ value 12- to 16-fold higher than those induced by FSH alone. The direct stimulatory effect of estrogens was time-dependent and was not accounted for by increases in cell protein. Various native and synthetic estrogens also augmented the FSH induction of aromatase activity in granulosa cells. The effect of estradiol-17$\beta$ was dose-dependent with an $ED_{50}$ value of $9 \times 10^{-8}$ M, which is within the physiological levels of follicular estradiol-17β. Although treatment with androgens also enhanced the FSH-induced aromatases, treatment with a progestin (R5020) or a mineralocorticoid (aldosterone) was without effect. Thus, estrogens directly augment the stimulation of granulosa cell aromatase activity by FSH. Follicular estrogens may activate intraovarian autoregulatory positive feedback mechanisms to enhance their own production, resulting in selective follicle maturation and the preovulatory estrogen surge.

It is now well accepted that follicle-stimulating hormone is the prime inducer of aromatase activity in rat (1-6) and human (7) ovaries based on in vitro experiments (1, 2, 4-7) and in vivo studies (3, 5) studies on granulosa cells. However, the possibility that this effect of FSH is subject to modulation by various steroid hormones has thus far received little attention. Although androgens have recently been shown to synergize with FSH in the induction of aromatase activity in rat granulosa cells (8), the possible involvement of estrogens has not been studied.

Estrogens have been implicated in several ways in the regulation of granulosa cell differentiation. Specifically, estrogen treatment of immature hypophysectomized female rats results in increased ovarian weight due to the proliferation of granulosa cells and growth of preovulatory follicles (9-23). Estrogens have also been reported to exert an antiatretic effect (13, 17, 18, 24), to increase granulosa cell estrogen receptor content (20), and to increase the coupling of granulosa cells through intercellular gap junctions (25). In addition, estrogens have been shown to synergize with FSH in the stimulation of follicular antrum formation (11, 16, 17, 19, 20, 22) and the induction of granulosa cell luteinizing hormone receptors (26, 27).

In view of the ability of estrogens to augment a broad range of FSH-dependent functions, it is of interest to study the interaction of estrogens with FSH in one of the key functions of ovarian follicles, i.e., the ability to synthesize estrogens through the activation of aromatases. Our present findings in cultured granulosa cells indicate that estrogens augment the FSH stimulation of aromatase activity thereby exerting a direct and positive autoregulatory feedback action on their own production.

EXPERIMENTAL PROCEDURES

Animals—Immature (23-25 days old) Sprague-Dawley female rats were hypophysectomized by Johnson Laboratories, Inc. (Bridgeview, IL) and delivered on the second postoperative day. Silastic capsules (10 mm) containing diethylstilbestrol were implanted subcutaneously at the time of hypophysectomy (5). The animals were housed in air-conditioned quarters and given physiological saline (0.9% NaCl) solution and a mixture of bread, milk, and tap water ad libitum. A 14:10 h light-dark cycle was maintained with the light cycle starting at 0600 h. The animals were sacrificed by cervical dislocation between 4-5 days after surgery. In some experiments, immature hypophysectomized Sprague-Dawley rats without diethylstilbestrol implants were also used between 4-6 days after surgery.

Reagents and Hormones—McCoy's 5a medium (modified, without serum), penicillin-streptomycin solution, 1-glutamine, and trypan blue stain (0.4%) were obtained from Grand Island Biological Co. (Grand Island, NY). Androstenedione (4-androstan-3,17-dione), diethylstilbestrol (3,4-bis-(p-hydroxyphenyl)-3-hexene), dihydrotestosterone (5α-androstan-3β,17β-diol-3-one), aldosterone (4-pregnen-18-al-11β,21-diol-3,20-dione), 17β-estradiol (E$_2$-17β; 1,3,5(10)-estratrien-3,17β-diol), 17α-estradiol (E$_2$-17α), 1,3,5(10)-estratrien-3,17α-diol, estrone (E$_1$, 1,3,5(10)-estratrien-3,17β-diol), estradiol (E$_2$, 1,3,5(10)-estratrien-3,17β-diol), estrone (E$_1$, 1,3,5(10)-estratrien-3,17α-diol), ethinyl estradiol (1,3,5(10)-estratrien-17α-ethyl-3,17β-diol), and mestranol (1,3,5(10)-estratrien-17α-ethyl-3,17β-diol-3-methylether) were from Sigma. Methyltrien-...
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Aromatase Activity—The accumulation of estrogen during the test interval was taken to reflect the level of aromatase activity acquired during the preincubation interval. Under the conditions of this study, aromatase activity was examined using a saturating substrate concentration (10⁻⁷ M). This substrate was not rate-limiting, and the rate of aromatase activity was calculated from the finding that ~30 ng/ml of 10⁻⁷ M androstenedione added per culture, only ~1 ng of estrogen was formed during the 5-h test interval.

Radioimmunoassay—The total estrogen content of the medium was determined by radioimmunoassay of unextracted medium using an antisera (5-104 s; 100 ng/ml) provided by Dr. W. W. L. Albrecht (Merrell Dow Pharmaceuticals, Inc., Cincinnati, OH).

Oviste FSH (oFSH, NIH-FSH-S13; FSH potency, 15 NIH-FSH-S1 units/mg; luteinizing hormone potency, 0.05 NIH-LH-S1 units/mg; prolactin activity, <0.1% by weight) was the generous gift of the National Pituitary Agency, Pituitary Hormone Distribution Program, National Institute of Arthritis, Metabolism, and Digestive Diseases. 

In Vitro Studies—Granulosa cells from hypophysecotomized diethylstilbestrol-treated rats were cultured as previously described (5). Granulosa cells (~1 x 10⁶ viable cells/culture unless indicated otherwise) were plated onto tissue culture dishes containing 1 ml of McCoy’s 5a medium (modified without serum) supplemented with l-glutamine (2 mM), penicillin (100 units/ml) and streptomycin sulfate (100 μg/ml). Cell cultures were maintained at 37 °C under a water-saturated atmosphere of 95% air and 5% CO₂. FSH was dissolved in sterile culture medium. Steroids and nonsteroidal estrogens were dissolved in ethanol followed by subsequent dilution in culture medium. Ethanol concentration in the cell cultures was less than 0.5%. All treatments were added in 50-μl aliquots. The cells were initially cultured for 3 days (unless indicated otherwise) in an androstenedione-free medium in the presence or absence of FSH with or without the specified experimental agents (the preincubation interval). At the conclusion of this period, media were collected, and the cells were washed twice with 2-ml portions of medium and reincubated for 5 additional hours in an androstenedione (10⁻⁷ M)-supplemented medium (the test interval). At the end of the experiment, the media were collected and stored at -20 °C until assayed for total estrogen content by radioimmunoassay. Following addition of 1.0 ml of distilled water, the dishes were also stored at -20 °C until assayed for their total cellular protein content. In certain control experiments, use was made of an androstenedione-free medium during the test interval. In some experiments, the cells were treated with diethylstilbestrol during the test interval. The presence or absence of diethylstilbestrol during the 5-h period did not affect aromatase activity (data not shown).

Determination of Cell Viability and Total Cellular Protein Content—Cell viability was estimated by determining the percentage of trypan blue excluding cells. In representative experiments, the percentages of viable cells were determined at the end of the 3-day treatment period by counting 2000 attached cells/dish and 6 dishes per treatment group. Treatment with diethylstilbestrol did not preferentially affect granulosa cell viability (FSH-treated cells: 51.0 ± 2.0%; FSH plus diethylstilbestrol-treated cells: 50.5 ± 1.5%). The total cellular protein content was determined after the 5-h incubation using the Coomassie brilliant blue G-250 protein assay of Bradford (28) (Bio-Rad protein assay kit). Following repeated (3 times) freezing and thawing of the frozen samples, the cells were scraped off the dish with a rubber policeman, and samples of each dish were taken for use in the assay. The results are expressed in terms of the bovine y-globulin standard.

RESULTS

Effect of Treatment with Diethylstilbestrol on Basal and FSH-Induced Aromatase Activity in Rat Granulosa Cells—To evaluate the effect of treatment with diethylstilbestrol (a nonsteroidal estrogen) on basal and FSH-induced aromatase activity, granulosa cells were cultured for 3 days in the presence or absence of FSH (10 ng/ml) with or without increasing concentrations (10⁻¹⁰-10⁻⁷ M) of diethylstilbestrol. Following this interval, the cells were washed twice and reincubated for a 5-h test interval in an androstenedione (10⁻⁷ M)-supplemented medium (Fig. 1). The estrogen production by control and diethylstilbestrol (10⁻⁷ M)-treated cells was negligible, whereas treatment with FSH brought about a substantial increase in aromatase activity (estrogen accumulation; 7.5 ± 1.0 ng/mg of protein/5 h). Furthermore, concomitant treatment with increasing concentrations (10⁻¹⁰-10⁻⁷ M) of diethylstilbestrol led to dose-dependent increases in the FSH-induced aromatase activity with an ED₅₀ value of 4.0 ± 0.5 × 10⁻⁸ M.

FIG. 1. Effect of treatment with diethylstilbestrol on the FSH-induced aromatase activity of rat granulosa cells. Granulosa cells were cultured for 3 days in the presence or absence of FSH (10 ng/ml) with or without increasing concentrations (10⁻¹⁰-10⁻⁷ M) of diethylstilbestrol. Three days later, the cells were washed with medium and reincubated for a 5-h test interval in an androstenedione (10⁻⁷ M)-supplemented medium. The accumulation of estrogen during this test period is taken as a measure of the level of aromatase activity. Media estrogen was measured by radioimmunoassay. The results represent the mean ± S.E. of triplicate determinations. C, controls; DES, diethylstilbestrol.
The maximal activity stimulated by $10^{-7}$ M diethylstilbestrol and FSH was 12-fold higher than that induced by FSH alone. These findings indicate that diethylstilbestrol augments the FSH stimulation of aromatase activity in a dose-dependent manner.

**Effect of Other Steroid Hormones on the FSH Induction of Aromatase Activity**—To examine the specificity of the stimulatory effect of diethylstilbestrol, granulosa cells were cultured for 3 days in the presence or absence of FSH (10 ng/ml) with or without $10^{-7}$ M of diethylstilbestrol, dihydrotestosterone (a nonaromatizable androgen), R1881 (a synthetic androgen), or aldosterone (a mineralocorticoid) (Fig. 2). Concomitant treatment with diethylstilbestrol, dihydrotestosterone, and R1881 brought about 14-, 9-, and 13-fold increases in the FSH-induced aromatase activity, respectively. In contrast, treatment with aldosterone was without effect. Furthermore, treatment with $10^{-7}$ M of R5020 (a synthetic progestin) did not affect the FSH induction of aromatases (estrogen production (ng/mg of protein/5 h): FSH-treated $= 8.4 \pm 1.4$; FSH plus R5020-treated, $10.3 \pm 1.0$). These findings indicate that the ability of diethylstilbestrol to augment the FSH induction of aromatase activity is shared by native and synthetic androgens but not by mineralocorticoids and progestins.

**Time Dependence of the Effect of Treatment with Diethylstilbestrol and FSH on Aromatase Activity**—To evaluate the time course of the effect of treatment with diethylstilbestrol and FSH on the aromatase activity, granulosa cells were cultured for increasing periods of time (1-3 days) in the presence or absence of FSH (10 ng/ml) with or without $10^{-7}$ M diethylstilbestrol (Fig. 3). Treatment with FSH by itself led to progressive time-dependent increases in aromatase activity, becoming significant at 2 days after incubation. Although concomitant treatment with diethylstilbestrol for 1 day was without effect on the FSH-induced aromatase activity, treatment for 2 and 3 days led to 7- and 12-fold increases in the FSH effect, respectively. These findings indicate that the stimulatory effect of diethylstilbestrol and FSH is time-dependent and that it becomes operative within 2 days of treatment.

**Effect of Treatment with Native Estrogens on the FSH Induction of Aromatase Activity**—To examine the effects of treatment with native estrogens on the FSH induction of aromatase activity, granulosa cells were cultured for 3 days in the presence or absence of FSH (10 ng/ml) with or without increasing concentrations ($10^{-10}$-$10^{-7}$ M) of E$_{2}$-17$\beta$, E$_{1}$, E$_{3}$, or E$_{2}$-17$\alpha$. Media estrogen was measured by radioimmunoassay. The results represent the mean ± S.E. of triplicate cultures. C, controls. DES, diethylstilbestrol.
dependent increases in the FSH-induced aromatase activity with an \( ED_{50} \) value of \( 9.2 \pm 0.7 \times 10^{-9} \) M and a maximal activity 8-fold higher than that induced by FSH alone. The projected minimal effective dose (defined as the dose leading to an increase of 2 standard deviations over FSH-induced aromatase activity) was \( 3.7 \pm 0.3 \times 10^{-9} \) M for both \( \text{E}_2-17\alpha \) and \( \text{E}_1 \). Although concomitant treatment with \( \text{E}_1 \) and \( \text{E}_2-17\alpha \) also led to dose-dependent increases in the FSH-induced aromatase activity, the maximal levels of aromatase in cells treated with FSH and \( 10^{-7} \) M of the estrogens were only 3- to 4-fold higher than those achieved by FSH alone. Since the effects of \( \text{E}_1 \) and \( \text{E}_2-17\alpha \) did not plateau at the highest dose used, these data did not permit accurate determination of \( ED_{50} \) values. However, estimations suggest that the \( ED_{50} \) values for these steroids are at least one order of magnitude higher than those for \( \text{E}_2-17\beta \) and \( \text{E}_1 \). These findings indicate that these native estrogens augment the FSH induction of aromatase activity (rank order of potency: \( \text{E}_2-17\beta = \text{E}_1 > \text{E}_2-17\alpha > \text{E}_0 \)).

**Effect of Treatment with Synthetic Estrogens on the FSH Induction of Aromatase Activity**—To evaluate the effect of treatment with synthetic estrogens on the FSH induction of aromatase activity, granulosa cells were cultured for 3 days in the presence or absence of FSH (10 ng/ml) with or without increasing concentrations \( (10^{-10} - 10^{-7}) \) M of various synthetic estrogens (Fig. 5, A and B). Concomitant treatment with increasing concentrations of hexestrol, moxestrol, or ethinyl estradiol led to dose-dependent increases in the FSH-induced aromatase activity with \( ED_{50} \) values of \( 2.0 \pm 0.4 \times 10^{-9} \), \( 9.3 \pm 0.6 \times 10^{-9} \), and \( 1.0 \pm 0.4 \times 10^{-8} \) M, respectively. However, concomitant treatments with increasing concentrations of chlorotrianisene or mestranol were virtually without effect. These findings indicate that these synthetic estrogens augment the FSH induction of aromatase activity (rank order of potency: hexestrol > moxestrol > ethinyl estradiol; 5.0:1.1:1.0).

**Effect of Treatment with Diethylstilbestrol and FSH on the Apparent Kinetic Parameters of the Aromatase Enzymes**—To determine the effect of treatment with diethylstilbestrol on the apparent \( K_m \) and \( V_{max} \) values of the FSH-induced aromatase enzymes, granulosa cells \( (3 \times 10^5 \text{ viable cells/culture}) \) were cultured for 3 days in the presence of FSH (10 ng/ml) with or without \( 10^{-7} \) M diethylstilbestrol. At the end of this incubation period, the media were removed and the cells were washed and reincubated for a 5-h interval in media containing increasing concentrations \( (1-1000 \text{ nm}) \) of androstenedione (Fig. 6A). As shown in Fig. 6A, provision of increasing concentrations of androstenedione led to dose-dependent increases in the accumulation of estrogen in cells treated with FSH or FSH plus diethylstilbestrol. Under the saturating dose \( (1000 \text{ nm}) \) of androstenedione, cells primed with both FSH and diethylstilbestrol accumulated about 16 times more estrogen as did cells treated with FSH alone. Lineweaver-Burk plot analysis of the data (Fig. 6B) revealed apparent \( V_{max} \) values of 4.9 and 79 ng/ng of protein/5 h for cells treated with FSH and FSH plus diethylstilbestrol, respectively. The corresponding apparent \( K_m \) values were 6.6 and \( 26.4 \times 10^{-9} \) M. These findings indicate that concomitant treatment with diethylstilbestrol brings about a 16-fold increase in the maximal activity of the FSH-induced aromatase enzymes.

**Effect of Treatment with Diethylstilbestrol on the FSH Induction of Aromatase Activity in Granulosa Cells from Hypophysectomized Rats without Diethylstilbestrol Implant**—To evaluate the effect of diethylstilbestrol priming in vivo on the in vitro action of the estrogens, granulosa cells were obtained from immature hypophysectomized female rats not bearing diethylstilbestrol capsules and cultured for 3 days in the presence or absence of FSH (10 ng/ml) with or without increasing concentrations \( (10^{-10} - 10^{-7}) \) M of diethylstilbestrol (Fig. 7). Concomitant treatment with increasing concentrations of diethylstilbestrol led to dose-dependent increases in the FSH-induced aromatase activity with an \( ED_{50} \) value of \( 7.5 \times 10^{-10} \) M and a maximal activity 4-fold higher than that induced by FSH alone. These findings indicate that diethylstilbestrol augments the FSH stimulation of aromatase activity in granulosa cells not primed with the estrogen in vivo.
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**Fig. 6.** Effect of treatment with diethylstilbestrol on apparent kinetic parameters of the FSH-induced aromatases. Granulosa cells were cultured as described in Fig. 1 in the presence or absence of FSH (10 ng/ml) with or without $10^{-7}$ M diethylstilbestrol. At the end of this induction interval, the medium was removed and the cells were washed with medium and reincubated for a 5-h test interval with increasing concentrations (1-1000 nM) of androstenedione. Media estrogen was measured by radioimmunoassay. A, enzyme saturation plots; B, Lineweaver-Burk plot analysis. The results represent the mean ± S.E. of triplicate cultures. DES, diethylstilbestrol.

**Fig. 7.** Effect of in vitro treatment with diethylstilbestrol on the FSH-induced aromatase activity of granulosa cells from rats without diethylstilbestrol capsules. Granulosa cells were obtained from immature hypophysectomized female rats not bearing diethylstilbestrol capsules and cultured as described in Fig. 1 in the presence or absence of FSH with or without $10^{-7}$ M diethylstilbestrol, E$_2$-17β, or E$_1$. At the end of the incubation, the cells were washed with medium and reincubated for 5 additional hours, and the total cellular protein content was measured (Table I). Treatment with FSH led to a 30% increase in the protein content of granulosa cells as compared with controls. However, concomitant treatment with diethylstilbestrol, E$_2$-17β, or E$_1$ did not lead to significant alterations in the protein content as compared with cells treated with FSH alone.

**Table I**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Cellular protein (μg/culture)</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>12.1 ± 0.4</td>
</tr>
<tr>
<td>Diethylstilbestrol</td>
<td>13.4 ± 1.0</td>
</tr>
<tr>
<td>E$_2$-17β</td>
<td>12.8 ± 0.7</td>
</tr>
<tr>
<td>E$_1$</td>
<td>11.5 ± 0.4</td>
</tr>
<tr>
<td>FSH</td>
<td>16.0 ± 0.5$^*$</td>
</tr>
<tr>
<td>FSH + diethylstilbestrol</td>
<td>15.8 ± 0.8$^*$</td>
</tr>
<tr>
<td>FSH + E$_2$-17β</td>
<td>14.7 ± 0.4$^*$</td>
</tr>
<tr>
<td>FSH + E$_1$</td>
<td>16.2 ± 0.4$^*$</td>
</tr>
</tbody>
</table>

$^*$Significantly ($p < 0.05$) different from untreated cells.

Effect of Treatment with FSH and Estrogens on the Cellular Protein Content—To determine the effects of treatment with FSH and estrogens on the cellular protein content, granulosa cells were cultured for 3 days in the presence or absence of FSH (10 ng/ml) with or without $10^{-7}$ M diethylstilbestrol, E$_2$-17β, or E$_1$. At the end of the incubation, the cells were washed with medium and reincubated for a 5-h test interval with increasing concentrations (1-1000 nM) of androstenedione. Media estrogen was measured by radioimmunoassay. The results represent the mean ± S.E. of triplicate cultures. DES, diethylstilbestrol.

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DISCUSSION

The modulatory role of estrogens in the induction of ovarian aromatase activity by FSH was investigated in vitro using granulosa cells from immature hypophysectomized female rats. Our findings indicate that various native and synthetic estrogens augment the FSH stimulation of aromatase activity. This direct and specific effect of estrogens is associated with
an increase in the apparent maximal velocity of the enzymes and is time- and dose-dependent.

The present findings constitute the first in vitro evidence to demonstrate the ability of estrogens to augment the FSH stimulation of aromatase activity in rat granulosa cells. Although the role of FSH in the induction of aromatases has been amply documented (1-6), possible estrogenic modulation of this process could not be evaluated as previous studies were conducted with media supplemented with aromatizable androgens. Since androgens may convert to estrogens during the induction period and may enhance the accumulation of progesterins (32-35), use of androgen-supplemented media precludes studies of the interaction between FSH and the various sex steroids. In fact, formerly reported “FSH-induced” aromatase activity probably represents the sum effect of FSH and some of the medium steroids. Given these considerations, this study employed an androgen-free induction period to investigate the effects of estrogens on the FSH-induced aromatase activity. Similar reasoning has recently prompted the use of an androgen-free induction period to study the interaction between androgens and FSH on aromatase activity (8, 36).

Our findings indicate that concomitant treatment with 10^{-7} M diethylstilbestrol results in a 12- to 16-fold increase in the apparent maximal velocity (V_m) of the FSH-induced aromatases along with a 4-fold decrease in the apparent affinity (K_a) of the enzymes for the substrate androstenedione. In view of the complexity of the system (i.e., androstenedione uptake and enzymatic aromatization by intact cells), conclusive demonstration of a change in the K_a of aromatases awaits future purification of these enzymes.

It is generally accepted that the actions of estrogens are mediated through specific receptors in target tissues (37) and that for a given estrogen, a good correlation exists between receptor binding affinity and biologic potency (38). That the ovary is endowed with estrogen receptors was suggested by the demonstration of specific ovarian uptake of [3H]estradiol-17β (39) and the localization of estrogen receptors in the granulosa cells (20, 40). The order of stimulatory potencies of estrogens used in the present study is E_2-17β > E_1 > E_3. This is in keeping with the fact that E_2-17β and E_1 are interconvertible in the granulosa cells by 17β-hydroxysteroid dehydrogenase and that E_1 binds with a lower affinity to the ovarian estrogen receptors (40). Furthermore, E_1-17α is also less effective than E_2-17β. The findings that mestrenol and E_2-17β are equipotent suggest that diethylstilbestrol are consistent with their relative binding affinities in mouse (41) and rat (42) uterine cytosols, respectively. Of the synthetic estrogens used in oral contraceptives, ethinyl estradiol proved to be far more potent than mestranol, consistent with their known binding affinities to estrogen receptors in the rat hypothalamus, anterior pituitary, and uterus (43). The relative binding affinity of chlorotrianisene to hamster uterine cytosol is known to be 0.1% of that of E_2-17β (44) and is, therefore, consistent with its low potency in the present system. It seems that both mestranol and chlorotrianisene require in vivo transformation to yield metabolites with higher estrogenicity. Taken together, these findings suggest that the ability of estrogens to augment the FSH stimulation of aromatases is mediated through granulosa cell estrogen receptors.

Although androgens also augment the FSH stimulation of aromatases (Fig. 2, Refs. 8 and 36), this effect has been shown to be blocked by synthetic antiandrogens (36). Furthermore, aldosterone (a mineralocorticoid) and R5020 (a synthetic progestin) were ineffective in the present system, thus reinforcing the hormonal specificity of the estrogen actions. However, possible interaction between estrogens and androgens in the present system awaits future studies.

Although the mechanism whereby estrogens exert their action on aromatase activity is not entirely clear, estrogens have been found to enhance the FSH-stimulated generation of cAMP (26) as well as the FSH-induced increase in cAMP-binding proteins (45, 46). We have also examined the possibility that the positive effect of estrogens on granulosa cell aromatase enzymes is due to their antiestrogenic properties. However, concomitant treatment with diethylstilbestrol did not result in significant alterations in the cellular protein content (Table I) or cell viability as compared with cells treated with FSH alone. It would, therefore, appear that the ability of estrogens to augment the FSH induction of aromatase activity represents a selective effect and is not due to an overall increase in granulosa cell viability and/or protein mass.

Most of the present experiments made use of granulosa cells obtained from immature hypophysectomized diethylstilbestrol-treated rats due to the availability of large numbers of granulosa cells from these estrogen-primed rats. Consequently, in vitro treatment of cultured granulosa cells with diethylstilbestrol was superimposed on prior in vivo priming with diethylstilbestrol implants. In view of the multiple actions of estrogens on granulosa cell functions (9-27), we examined the effect of in vitro diethylstilbestrol treatment on the aromatase activity of granulosa cells from rats not primed with diethylstilbestrol in vivo. Our findings (Fig. 7) indicate that, regardless of prior in vivo priming, diethylstilbestrol augments in vitro with FSH in the induction of aromatase activity. However, based on ED_50 values (compare Figs. 1 and 7), granulosa cells from rats not bearing diethylstilbestrol implants appear to be 5 times more sensitive to the action of diethylstilbestrol as compared with those cells from rats primed with the estrogen in vivo. It is conceivable that in vivo diethylstilbestrol priming reduces the sensitivity of cultured granulosa cells to estrogens in vitro.

Based on the present findings, one is led to propose that estrogens, in addition to their diverse roles in stimulating granulosa cell maturation (9-27), may also be important in the regulation of ovarian estrogen production and the maintenance of the dominant ovarian follicles. The projected minimal effective dose of E_2-17β capable of augmenting the aromatase activity is 3.7 × 10^{-10} M. This concentration is well within the reported range (up to 3 × 10^{-10} M) of E_2-17β measured in the follicular fluid of rat (47) and human (48) preovulatory follicles, suggesting that the findings reported herein are of physiologic importance. Estrogens may act as an end product amplifier of aromatase activity to further enhance the synergistic effect of thecal androgens (8, 36). Steroids within the microenvironment of the ovarian follicle may activate intraovarian autoregulatory positive feedback mechanisms to enhance the production of estrogens, leading to the preovulatory surge of estrogens.

It has been suggested that the selected follicle(s), by way of its estrogen production, reduces FSH levels thereby impeding the maturation of other follicles (49). However, it is not known how the selected follicle itself continues to mature in the face of declining FSH levels. The present finding suggests that intrafollicular estrogens can enhance the actions of FSH to stimulate aromatases. Once a “chosen” follicle(s) is producing a significant amount of estrogens, it has the capacity to produce more estrogens than neighboring follicles, and the selection of the dominant follicle(s) is secured. In contrast, follicles destined to undergo atresia may not be able to produce enough follicular estrogens to counteract the adverse effects of FSH deprivation. Future studies on the present
granulosa cell cultures should provide important clues on the role of estrogens in intraovarian regulations. The granulosa cell cultures may also be a valuable in vitro model for studying the mechanism of action of estrogens.

Acknowledgments—The interest and support of Dr. Samuel S. C. Yen are greatly appreciated. We also wish to thank C. Fabics and L. Tucker for expert technical assistance and Kayle Watts for typing the manuscript. Ovine FSH was provided by the National Pituitary Agency, Pituitary Hormone Distribution Program, National Institute of Arthritis, Metabolism, and Digestive Diseases. Methyltrienolone, R5020, and mestrol were the generous gifts of Dr. J. P. Raynaud (Centre de Recherches Roussel-UCLAF, Romainville, France). Chlorotrianisene was the generous gift of Dr. W. L. Albrecht (Merrel Dow Pharmaceuticals, Inc., Cincinnati, OH). We also thank Dr. David Rodbard (Biophysical Endocrinology Section, Endocrinology and Reproductive Research Branch, National Institute of Child Health and Human Development, National Institutes of Health) for providing the four parameter logistic curve fitting program.

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