The modulation of ovarian steroidogenesis by epidermal growth factor (EGF) was investigated in cultured rat granulosa cells. Granulosa cells, obtained from ovaries of immature, hypophysectomized, estrogen-treated rats, were cultured for 2 days with EGF, follicle-stimulating hormone (FSH), or EGF plus FSH. Treatment with EGF did not affect estrogen production, but stimulated progesterin (i.e., progesterone and 20a-hydroxy pregn-4-en-3-one) production in a dose-dependent manner. Stimulation of progesterin production by EGF appears to be the result of an increase in pregnenolone biosynthesis as well as increases in the activities of 20a-hydroxysteroid dehydrogenase and 3β-hydroxysteroid dehydrogenase/isomerase. Treatment with FSH increased both estrogen and progesterin production by cultured granulosa cells, whereas progesterone production was further enhanced. The EGF enhancement of FSH-stimulated estrogen production was inhibited, while progesterin production was further enhanced. The EGF enhancement of FSH-stimulated progesterin production appears to be the result of a synergistic increase in pregnenolone biosynthesis and 20a-hydroxysteroid dehydrogenase activity, resulting in substantial increases in 20a-hydroxy pregn-4-en-3-one but not progesterone production. The effects of EGF were shown to be time-dependent. The concept of a direct action of EGF on rat granulosa cells is reinforced by the demonstration of high affinity (Kd ~ 3 x 10^{-10} M), low capacity (~5,000 sites/cell) EGF binding sites in these cells. Thus EGF interacts with specific granulosa cell receptors to stimulate progesterin but not inhibit estrogen biosynthesis.

EGF1 stimulates mitogenesis in a variety of cell types (1-3). Our preliminary results demonstrated that in vitro treatment of rat granulosa cells with EGF inhibits the FSH-induced increase in estrogen production (4). In addition, Mondshein and Schomberg (5) reported that EGF inhibits the FSH-induced increase in luteinizing hormone receptor content of cultured rat granulosa cells. Similarly, we have shown that EGF inhibits the human chorionic gonadotropin-induced increase in testosterone production by cultured rat Leydig cells (4), while Ascoli (6) has reported that EGF decreases luteinizing hormone receptor content in a clonal strain of murine Leydig tumor cells. Thus, EGF modulates steroidogenesis and gonadotropin receptor content in cultured gonadal cells.

The present investigation extends our earlier studies on the EGF modulation of granulosa cell functions. Specifically, we have characterized the granulosa cell EGF receptor and examined the effect of EGF on progesterin and estrogen biosynthesis.

**EXPERIMENTAL PROCEDURES**

*Reagents and Hormones—Ovine FSH (NIH-FSH-S13; FSH activity 15 X 10^6 NIH-FSH-S1 units/mg; LH activity 0.05 X 10^6 NIH-LH-S1 units/mg; prolactin activity < 0.1% by weight), ovine prolactin (NIH-P-S13; prolactin activity 30 IU/mg; LH and FSH activities < 0.1% by weight), and porcine relaxin (3,000 units/mg) were from the National Pituitary Agency, National Institute of Arthritis, Metabolism, and Digestive Diseases. Purified mouse EGF, bovine fibroblast growth factor, mouse nerve growth factor, and antiserum to mouse EGF were obtained from Collaborative Research, Inc., Waltham, MA. Gonadotropin-releasing hormone was the generous gift of Dr. N. C. Ling, Salk Institute, La Jolla, CA. Highly purified ovine LH (Papoff G3-222B; LH activity 2.75 X 10^6 NIH-LH-S1 units/mg; FSH activity < 0.001 X NIH-FSH-S1 units/mg) was kindly provided by Dr. H. Papoff, University of California, San Francisco. Human chorionic gonadotropin (hCG CR-121; 13,450 IU/mg) was from Dr. R. E. Canfield, Columbia University, New York. Highly purified single component bovine insulin (lot 615-075-256) was generously provided by Dr. R. E. Chance, Lilly Research Laboratories, Indianapolis, IN.

McCoy's 5a medium (modified; without serum), penicillin-streptomycin solution, L-glutamine, and trypan blue stain were obtained from Grand Island Biological Co., Santa Clara, CA. Bovine serum albumin (fraction V), 20a-OH-P, progesterone, pregnenolone, and androstenedione were purchased from Sigma. Diethylstilbestrol was obtained from Steraloids, Inc., Wilton, NH. Cyanoketone was kindly provided by J. A. Campbell, The Upjohn Co.

[7-H]Pregnenolone (19.3 Ci/mmol), 20a-[1,2-3H]OH-progesterone (55.7 Ci/mmol), and [4,13-C]progesterone (51.0 mCi/mmol) were obtained from New England Nuclear. Labeled steroids were purified before use by thin layer chromatography using the system chloroform/ether (5:1, v/v). Precast silica plates were obtained from MCB Manufacturing Chemists, Inc., Cincinnati, OH.

Granulosa Cell Cultures—Immature female Sprague-Dawley rats (21-23 days old) were hypophysectomized by Curtis Johnson Laboratories, Chicago, IL, and delivered on the third postoperative day. Silastic capsules (10 mm) containing diethylstilbestrol were implanted at the time of surgery. Hypophysectomized animals were given a mixture of bread, milk, tap water, and physiological saline (0.9% NaCl solution) ad libitum.

Four to six days after surgery, granulosa cells were obtained from the hypophysectomized rats as described previously (7). These cells were cultured in Falcon tissue culture dishes (35 x 10 mm) (2-4 x 10^6 viable cells/dish) in 1 ml of McCoy's 5a medium supplemented with 2 mM L-glutamine, 100 units/ml of penicillin, 109 μg/ml of strepto-
mycin sulfate, and 100 ng androstenedione. Androstenedione was included in the culture medium to serve as the aromatase substrate and to synergize with FSH in stimulating progesterone production (8, 9, 10). FSH and EGF were diluted in sterile culture medium and added in 50-μl aliquots. Cells were cultured at 37 °C in a humidified 8% air, 5% CO2 incubator. At the end of the incubation, media were collected and stored at -20 °C until analyzed for progesterone, 20a-OH-P, and estrogen contents by radioimmunoassays.

To examine pregnenolone production, granulosa cells were incubated for 2 days with the appropriate hormones. Media were then replaced with fresh medium containing 10-5 M cyanoacetone, an inhibitor of 3β-hydroxysteroid dehydrogenase (11), and the cells were incubated for 25 min. After the incubation, the appropriate hormones were added back to the cultures and the cells were reincubated for 4 h. Media were analyzed for pregnenolone contents by radioimmunoassay.

Radioimmunoassays—Medium progesterone and estrogen contents were measured using specific antisera (12). Medium 20a-OH-P contents were measured using specific antiserum supplied by Ralph Schwall, University of California, San Diego. The 20a-OH-P antiserum 316 cross-reacts <0.1% with progesterone, C19, C21, and other C21 steroids. Medium pregnenolone contents were measured with specific antisera supplied by Dr. P. Chrousos, National Institutes of Health, Bethesda, MD; this antiserum cross-reacts <0.4% with progesterone, 20α-OH-P, 17α-hydroxyprogesterone, 11α-hydroxyprogesterone, estradiol, and testosterone. High concentrations (10-6 M) of diethylstilbestrol, androstenedione, or cytochrome P450 in the culture medium do not interfere with the radioimmunoassays.

Enzyme Assays—The assay of 20α-hydroxysteroid dehydrogenase (EC 1.1.1.149) activity was based on a procedure developed by Eckstein et al. (13) and modified by us (14) in which 20α-hydroxysteroid dehydrogenase activity was measured as the rate of conversion of [17β]20α-OH-P to [17β]progesterone.

The assay of 3β-hydroxysteroid dehydrogenase/steroid Δ-isomerase (EC 1.1.1.31/EC 5.3.3.1) activity was based on a procedure developed by Murono and Payne (15) in which 3β-hydroxysteroid dehydrogenase activity was measured as the rate of conversion of pregnenolone to progesterone. Our modification of this procedure has been described elsewhere (16).

EGF Receptor Assay—EGF was iodinated using a method developed by Carpenter and Cohen (17). The reaction mixture contained 5 μg of EGF in 20 μl of distilled water, 1 mM of carrier-free Na125I in 30 μl of 0.5 M sodium phosphate buffer (pH 7.5), and 20 μg of chloramine-T in 10 μl of distilled water. The reaction was stopped in 40 s by the addition of 40 μg of sodium metabisulfite in 10 μl of distilled water. After adding 500 μl of the elution buffer (0.06 M sodium phosphate buffer with 0.075 M sodium chloride, pH 7.5), the mixture was passed through a Sephadex G-25 column to separate 125I-EGF from free iodine. The labeled EGF was stored at -20 °C in the presence of 0.1% bovine serum albumin. The specific activity of 125I-EGF, measured by self-displacement with specific EGF antisera, was 143 ± 17 pCi/μg (N = 3), or ~130,000 to 190,000 cpm/ng. The maximum binding capacity of the tracer in the presence of excess EGF receptor was determined to be 30 ± 2% (N = 3), as measured by the radioreceptor assay using increasing amounts of rat liver homogenate (18).

In vivo FSH treatment increases 125I-EGF binding capacity in granulosa cells by ~2-fold (see “Results”). Thus, granulosa cells were obtained from the ovaries of immature, hypophysectomized, diethylstilbestrol-treated rats which had been injected subcutaneously twice daily for 2 days with FSH (6.7 μg/0.1 ml of saline). In addition, samples of liver, kidney, spleen, and cerebellum were obtained to examine the tissue specificity of 125I-EGF binding. The granulosa cells or minced tissues were briefly homogenized (~60 s in ice-cold Dulbecco's phosphate-buffered saline (pH 7.2) using a glass-Teflon homogenizer. Three aliquots of cell suspension or tissue homogenate were incubated in tubes coated with bovine serum albumin for 16 h at 22 °C with 125I-EGF (~180,000 cpm) with or without excess (50 ng) unlabeled EGF. At the end of the incubation, the reaction mixtures were diluted with 2.5 ml of ice-cold buffer and centrifuged at 1,500 × g for 30 min at 4 °C. After the pellet was resuspended with the buffer and recentrifuged, radioactivity in the pellet was determined by counting in a γ-spectrometer. After correcting for the radioactivity bound in the presence of excess unlabeled EGF, the amount of specifically bound EGF was determined. Less than 0.1% of the tracer bound to tubes in the absence of tissue.

To determine the dissociation constant (Kd) of the ovarian EGF receptors, the radioreceptor assay was performed as described above. Ovarian granulosa cells were incubated with ~90,000 cpm of 125I-EGF in the presence or absence of varying amounts of unlabeled EGF. After determination of specific binding, the data were plotted in a Scatchard plot (18). The slope and intercept of the resultant line were calculated using a linear regression program.

Protein and DNA Determinations—The Bio-Rad protein assay (Bio-Rad Co.) was used to measure protein content (19) using bovine γ-globulin as the standard. DNA content was determined according to the method of Burton (20).

Statistical Analyses—A four-parameter logistic curve-fitting program was used to obtain dose-response curves (21). A linear regression program was used to approximate the Scatchard plot. Other statistical analyses were performed using the Student’s t test. Comparisons with p ≥ 0.05 were not considered significant.

RESULTS

Effects of EGF and FSH on Progestin and Estragen Production by Cultured Granulosa Cells—To examine the effect of EGF on ovarian steroidogenesis, granulosa cells were incubated in medium alone (control), or with increasing concentrations of EGF, FSH (10 ng/ml), or EGF plus FSH. The medium concentration of 20α-OH-P was low in control cells while FSH treatment stimulated 20α-OH-P production from <0.3 ng/ml to 24.9 ng/ml (Fig. 1A). Similarly, EGF treatment stimulated 20α-OH-P production in a dose-dependent manner with 10 ng of EGF/ml simulating 20α-OH-P production to 14.3 ng/ml. Furthermore, concomitant treatment with FSH and increasing concentrations of EGF resulted in a synergistic stimulation of 20α-OH-P production. The synergistic effect of EGF was due to dose-dependent with an ED50 value of ~0.4 ng of EGF/ml.

In the same cultures, medium progesterone was low (<0.2 ng/ml) in control cells, while FSH treatment stimulated progesterone production by >80-fold (Fig. 1B). In addition, EGF treatment alone increased progesterone production in a dose-dependent manner with an ED50 value of 0.802 ng of EGF/ml.

![Fig. 1. Effects of EGF and FSH on steroidogenesis by cultured granulosa cells.](image-url)
and with a maximal stimulation of ~3 ng/ml of progesterone. In contrast to the synergistic action of EGF on FSH-stimulated 20α-OH-P production, treatment with increasing concentrations of EGF did not affect FSH-stimulated progesterone production.

FSH treatment increased estrogen production from ~0.09 ng/ml to 2.54 ng/ml (Fig. 1C) while treatment with EGF alone did not affect estrogen production. Concomitant treatment with EGF inhibited the stimulatory effect of FSH in a dose-dependent manner and the ED₅₀ value was determined to be 0.206 ng of EGF/ml. High concentrations of EGF inhibited FSH-stimulated estrogen production by ~70%.

Time Course of Action of EGF and FSH upon Steroidogenesis by Cultured Granulosa Cells—To examine the time course of action of EGF and FSH, granulosa cells were incubated with medium alone (control), EGF (3 ng/ml), FSH (10 ng/ml), or EGF plus FSH. Media from sets of quadruplicate cultures were removed throughout a 48-h incubation period to study the time-dependent changes in the accumulation of 20α-OH-P, progesterone, and estrogen in the media. As shown in Fig. 2A, treatment with either FSH or EGF for 48 h stimulated 20α-OH-P production to 33.5 or 10.1 ng/ml, respectively. The synergistic stimulation of 20α-OH-P production by concomitant FSH and EGF treatment was evident after 34 h of treatment.

In the same cultures, FSH treatment stimulated progesterone production from 2.11 ng/ml at 10 h of incubation to 36.4 ng/ml at 48 h. In a similar temporal pattern, treatment with EGF alone resulted in an increase in progesterone production to 2.65 ng/ml after 48 h. In contrast, concomitant treatment with EGF did not further augment FSH action.

As shown in Fig. 2C, the concentration of medium estrogen in FSH-treated cells increased from <0.1 ng/ml after 10 h of incubation to 3.85 ng/ml after 48 h of incubation. In contrast, an EGF inhibition of FSH-stimulated estrogen production was observed after 24 h of incubation, resulting in an 84% decrease in estrogen production at 48 h. Treatment with EGF alone did not affect estrogen biosynthesis.

Effects of EGF and FSH on Pregnenolone Production by Cultured Granulosa Cells—The observation that concomitant treatment with EGF and FSH results in a dose-dependent increase in pregnenolone production from 0.75 ng of EGF/ml and a maximal stimulation of ~30 ng of pregnenolone/ml. Concomitant treatment with FSH and increasing concentrations of EGF resulted in a synergistic stimulation of pregnenolone production. This effect was dose-dependent with an ED₅₀ value of ~0.3 ng of EGF/ml.

Effects of EGF and FSH on 3β-Hydroxysteroid Dehydrogenase Activity in Cultured Granulosa Cells—In an earlier study (16), we demonstrated that FSH treatment of cultured granulosa cells increases the activity of 3β-hydroxysteroid dehydrogenase, which converts pregnenolone to progesterone. To examine the effect of EGF on 3β-hydroxysteroid dehydrogenase activity, granulosa cells were incubated for 2 days in medium alone (control), with FSH (10 ng/ml), increasing concentrations of EGF, or FSH plus EGF. The enzyme activities of the treated cells were determined as described under "Experimental Procedures." As shown in Fig. 4, FSH treatment increased enzyme activity by ~4-fold. Likewise, treatment with increasing concentrations of EGF resulted in a dose-dependent increase in 3β-hydroxysteroid dehydrogenase activity with an ED₅₀ of 0.843 ng of EGF/ml and a maximal increase of 4.7-fold as compared to the untreated controls. Furthermore, treatment with increasing concentrations of EGF in the presence of FSH resulted in a maximal 30% increase in enzyme activity as compared to the FSH-treated cells.

Effects of EGF and FSH on 20α-Hydroxysteroid Dehydrogenase Activity of Cultured Granulosa Cells—The observation that concomitant treatment with EGF and FSH results in synergistic increases of both pregnenolone and 20α-OH-P biosynthesis with no alteration in progesterone production suggested that EGF treatment may increase the activity of 20α-hydroxysteroid dehydrogenase, which converts progesterone to 20α-OH-P. To examine this possibility, granulosa cells were incubated for 2 days with medium alone (control), FSH (10 ng/ml), increasing concentrations of EGF, or FSH plus EGF. The enzyme activities of the treated cells were determined as described under "Experimental Procedures." As shown in Fig. 5, FSH treatment resulted in a slight increase in enzyme activity. In contrast, treatment with increasing concentrations of EGF alone resulted in a dose-dependent increase in 20α-hydroxysteroid dehydrogenase activity with an ED₅₀ value of 0.75 ng of EGF/ml. When cultures were treated with FSH and high concentrations of EGF (1 to 10 ng of EGF/ml), further synergistic increases in 20α-hydroxysteroid dehydrogenase activity were detected.

Effect of EGF and FSH on Cellular Protein and DNA Content of Cultured Granulosa Cells—To examine the possible mitogenic effects of EGF and FSH on granulosa cells, cells were incubated for 2 days in medium alone (control), or

![Fig. 2](image-url) Time course of action of EGF and FSH upon steroidogenesis by cultured granulosa cells. Granulosa cells (2 x 10⁶ viable cells/dish) were cultured in medium alone (control), with EGF (3 ng/ml), FSH (10 ng/ml), or EGF plus FSH for various intervals. Medium concentrations of 20α-OH-P (A), progesterone (B), and estrogen (C) were measured by radioimmunoassays. Data points represent mean ± S.E. of four replicate cultures.
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Fig. 3 (left). Effects of EGF and FSH upon pregnenolone production. Granulosa cells (2 × 10^5 viable cells/dish) were cultured in medium alone (control), with FSH (10 ng/ml), increasing concentrations of EGF, or FSH plus EGF. Pregnenolone production was evaluated as described under “Experimental Procedures.” Data points represent mean ± S.E. of three determinations after pooling triplicate cultures.

Fig. 4 (center). Effects of EGF and FSH on 3β-hydroxysteroid dehydrogenase activity of cultured granulosa cells. Granulosa cells (4 × 10^5 viable cells/dish) were cultured for 2 days in medium alone (control), with FSH (10 ng/ml), increasing concentrations of EGF, or FSH plus EGF. Enzyme activities were determined as described under “Experimental Procedures.” Data points represent mean ± S.E. for four determinations after pooling triplicate cultures.

Fig. 5 (right). Effects of EGF and FSH on 20α-hydroxysteroid dehydrogenase activity of cultured granulosa cells. Granulosa cells (~4 × 10^5 viable cells/dish) were cultured for 2 days in medium alone (control), with FSH (10 ng/ml), increasing concentrations of EGF, or FSH plus EGF. Enzyme activities were determined as described under “Experimental Procedures.” Data points represent mean ± S.E. for three determinations after pooling triplicate cultures.

In several cases, the variation was less than the symbols indicated.

Fig. 6. Effects of EGF and FSH on protein and DNA contents of cultured granulosa cells. Granulosa cells were incubated in medium alone (control), with EGF (5 ng/ml), FSH (10 ng/ml), or EGF plus FSH. After 2 days, media were removed, cells were scraped from the dishes, and protein and DNA contents were determined as described under “Experimental Procedures.” Left, data points represent mean ± S.E. for four samples (~2 × 10^5 viable cells/dish). Right, data points represent mean ± S.E. for three cultures (~10^6 viable cells/dish).

with EGF (5 ng/ml), FSH (10 ng/ml), or EGF plus FSH. As shown in Fig. 6 (right), neither EGF nor FSH treatment increased DNA content, indicating a lack of mitogenic effect of these agents in the cultured granulosa cells. In contrast, treatment with either EGF or FSH resulted in ~50% increase in cellular protein. Furthermore, concomitant treatment with both hormones resulted in ~93% increase in cellular protein. Thus, EGF and FSH increased cellular protein in an apparently additive manner.

Determination of Optimal Incubation Conditions for the EGF Receptor Assay—To determine the optimal incubation time for the radioreceptor assay, aliquots of granulosa cells were incubated for various periods of time with [125I]-EGF in the presence or absence of excess unlabeled EGF at 22 °C as described under “Experimental Procedures.” As shown in Figures 7A, the amount of specifically-bound EGF increased during the first 4 h of incubation reaching a plateau by 16 h. While several other incubation temperatures were examined (4 and 37 °C; data not shown), maximal specific binding in this system occurred at 22 °C. Thus, the EGF radioreceptor assays were performed at 22 °C for 16 h. Since in vivo FSH treatment increases the granulosa cell EGF binding capacity (picograms of [125I]-EGF bound/granulosa cell, controls = 11.9

FIG. 7. Determination of optimal incubation time and tissue concentration for the EGF binding assay. Granulosa cells were obtained from immature, hypophysectomized, estrogen-treated rats after 2 days of FSH treatment in vivo. A, the cells were incubated with labeled EGF in the absence or presence of excess unlabeled EGF for various intervals at 22 °C. B, increasing numbers of cells were incubated with labeled EGF in the absence or presence of unlabeled EGF for ~16 h at 22 °C, as described under “Experimental Procedures.” Data points represent mean ± S.E. for three samples.
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± 0.9; FSH-treated = 23.0 ± 0.5, N = 5), all assays used FSH-treated granulosa cells. To determine the optimal tissue concentrations for EGF binding, various aliquots of a granulosa cell preparation were incubated with labeled EGF in the presence or absence of excess unlabeled EGF. As shown in Fig. 7B, specific EGF binding was linear from 26 to 154 ng of protein; the correlation coefficient associated with the line was calculated to be 0.996. All experiments with granulosa cells employed tissue concentrations within this range.

Characterization of EGF Binding—To examine the tissue specificity of EGF binding, tissue homogenates were prepared and specific binding was determined as described under “Experimental Procedures.” Specific EGF binding of homogenates from ovary, liver, and kidney tissues were determined to be 28 ± 4, 627 ± 32, and 22 ± 5 pg/mg of protein, respectively (n = 6). In contrast, homogenates of spleen and cerebellum did not bind 125I-EGF in a specific manner.

To determine the dissociation constant (Kd) of the granulosa cell EGF receptor, granulosa cells were incubated with labeled EGF in the presence or absence of increasing concentrations of unlabeled EGF as described under “Experimental Procedures.” Scatchard analysis (Fig. 8) of the displacement curve indicated that the Kd of the granulosa cell EGF binding site is 2.74 ± 0.51 × 10⁻¹⁰ m or 1.67 ± 0.31 ng/ml (n = 3) and the binding capacity was determined to be 4860 ± 930 binding sites/cell (n = 3). To further examine the specificity of EGF binding, granulosa cells were incubated with labeled EGF alone or in the presence of excess unlabeled EGF or various other peptides and protein hormones. As shown in Table I, only unlabeled EGF significantly displaced 125I-EGF from the granulosa cell binding sites, whereas co-incubation with fibroblast growth factor, nerve growth factor, and other hormones did not affect 125I-EGF binding.

**DISCUSSION**

The present results demonstrate that 1) EGF binds to rat granulosa cells with high affinity and specificity; 2) in contrast to the inhibitory effect of EGF on FSH-stimulated estrogen production, EGF enhances FSH-stimulated 20α-OH-P production in a synergistic manner (this stimulatory effect appears to be related to an increase in pregnenolone biosynthesis and the stimulation of 20α-hydroxy-steroid dehydrogenase activity); 3) EGF treatment alone stimulates 20α-OH-P and progesterone production, as well as 20α-hydroxy-steroid dehydrogenase and 3β-hydroxy-steroid dehydrogenase activities; and 4) while EGF is not mitogenic in the cultured rat granulosa cells, EGF treatment increases the protein content of the granulosa cells.

EGF modulates FSH action in a disparate manner in cultured granulosa cells. EGF inhibits FSH-stimulated increases in estrogen production (4) and luteinizing hormone receptor content (5), while stimulating FSH-induced 20α-OH-P production synergistically (Figs. 1 and 2). These divergent effects of EGF on FSH-stimulated steroidogenesis indicate that estrogen production and progesterin production are regulated through different pathways. The notion that EGF can exert divergent effects upon endocrine cells is also supported by the observation that EGF treatment results in an increase in prolactin synthesis and a decrease in growth-hormone synthesis in a cultured pituitary tumor cell line (22).

The EGF enhancement of FSH-stimulated 20α-OH-P production is not accompanied by apparent changes in progesterone production but rather appears to be the result of alterations in several steroidogenic enzymes. This is characterized by a synergistic stimulation of pregnenolone biosynthesis and a synergistic increase in 20α-hydroxy-steroid dehydrogenase activity. Furthermore, EGF treatment enhanced FSH-stimulated 3β-hydroxy-steroid dehydrogenase activity by ~30%.

In the present study, treatment with EGF alone was shown to stimulate progesterone and 20α-OH-P (but not estrogen) production by cultured granulosa cells. These observations strengthen the concept that the cellular mechanisms controlling estrogen and progesterin production are regulated differently in rat granulosa cells. The EGF-induced increase in progesterin production appears to be the result of increased pregnenolone biosynthesis, as well as increases in the activities of 20α-hydroxy-steroid dehydrogenase and 3β-hydroxy-steroid dehydrogenase. EGF-stimulated pregnenolone production may be the result of increased side chain cleavage activity, although one cannot exclude the possibility that EGF stimulates enzymes involved in cholesterol biosynthesis. The observation that EGF stimulates progesterin biosynthesis in granulosa cells from these pre-antral follicles places EGF in a unique category with FSH because only FSH and several cAMP-inducing agents (such as cholera toxin and prostaglandin E₂) are capable of enhancing progesterin production in these rela-
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EGF has been found in various biological fluids (1-3). Byyny et al. (57) have reported that the concentration of EGF in the plasma of female mice is ~1.2 ng/ml, a concentration which is effective in the present rat granulosa cell culture system. Although EGF acts as a mitogen for a variety of cell types, our results demonstrate that this “growth factor” regulates steroidogenesis of rat granulosa cells. Thus, EGF may play an important endocrine role in controlling ovarian development by regulating the differentiation and steroidogenesis of granulosa cells.

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