Primary Culture of Differentiating Ovarian Androgen-Producing Cells in Defined Medium*

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A method for maintaining primary cultures of ovarian cells in serum-free medium in which the metabolic functions of the androgen-producing cells can be studied is described. Freshly dispersed cells from ovaries of hypophysectomized immature rats contained interstitial cells with specific, high affinity $^{125}$I-human chorionic gonadotropin (hCG) binding sites which were functionally coupled to adenylate cyclase but not to steroid synthesis. Culturing the interstitial cells with luteinizing hormone (LH) or hCG, but not follicle-stimulating hormone or prolactin, there was a 200-fold increase in steroid synthesis. Of the total steroid secreted, 96% was androgen, of which 98% was androsterone. The stimulation of androsterone synthesis by LH or hCG was dose-dependent ($ED_{50} = 2 \pm 0.2$ ng/ml for both hormones). A time course study with a saturating dose of LH showed that androsterone production was low at days 0–3, increased rapidly to maximum levels at day 4, and remained maximal through day 10. The LH effect on androsterone synthesis was mimicked in a dose-dependent manner by prostaglandin E$_2$, cholera toxin, and 8-bromo cAMP. Treatment with actinomycin D reversibly inhibited LH-stimulated androsterone biosynthesis. Experiments in vitro using hCG induced a similar pattern of androgen response indicating our defined in vitro system has physiological relevance. These in vitro results show that physiological concentrations of LH or hCG specifically induce the differentiation of the ovarian androgen-producing cells in a manner similar to that observed in vivo. This defined culture system should provide an excellent model for studying the mechanisms and control of ovarian androgen biosynthesis.

The ovary contains a population of cells which synthesize and secrete androgens (1–3). Cells located in both the theca interna and the interstitial compartment of the ovary (4–8) contain luteinizing hormone receptors (9, 10) which are functionally coupled to androgen biosynthesis (7–9). In the female rat, the existence of specialized populations of interstitial cells is physiologically important because the androgens they secrete provide the metabolic precursors for the biosynthesis of estrogens (11), which are essential for normal reproductive function.

Despite the importance of ovarian androgens, surprisingly little is known about the basic biochemical events involved in the regulation of androgen synthesis by interstitial cells. This gap in our knowledge is due in part to the absence of a suitable tissue culture system for analyzing, under defined conditions, the mechanisms and controls of ovarian androgen biosynthesis. In the present study we describe a primary cell culture model of differentiating ovarian androgen-producing cells. With this serum-free system, we show that physiological concentrations of LH specifically induce the functional differentiation of the ovarian androgen-producing cells and suggest that the process involves cyclic AMP and gene activity.

MATERIALS AND METHODS

Animals—Immature female rats (Sprague-Dawley, 21 days old) were hypophysectomized by Curtis Johnson Laboratories (Bridgeview, IL) and delivered on the second postoperative day. They were given physiological saline and a mixture of bread, milk, and dog food ad libitum and were sacrificed by cervical dislocation 4 or 5 days posthypophysectomy. In one experiment, beginning at day 25 animals were injected subcutaneously once daily for 1–3 days with hCG (13 IU in 0.9% saline) or vehicle after which they were killed and the ovaries processed for histology or acute biochemical studies.

Reagents—Highly purified ovine LH (Papoff G3-256DA; LH potency = 2.75 NIH-LH-S1 units/mg; FSH potency <0.001 NIH-FSH-S1 units/mg) and highly purified ovine FSH (Papoff G4-150; FSH potency = 50 NIH-FSH-S1 units/mg; LH potency <0.01 NIH-LH-S1 units/mg) were provided by Dr. H. Papoff, University of California, San Francisco. Highly purified hCG (CR-121; 13,450 IU/mg) was provided by Dr. R. E. Canfield through the Center for Population Research, National Institute of Child Health and Human Development. Highly purified ovine prolactin (IDB-67C) was provided by Dr. B. D. Burleigh, University of Texas, Houston. Prostaglandin E$_2$ was provided by Dr. R. E. Canfield through the Center for Population Research, National Institute of Child Health and Human Development. Highly purified ovine prolactin (IDB-67C) was provided by Dr. B. D. Burleigh, University of Texas, Houston. Prostaglandin E$_2$ was provided by Dr. R. E. Canfield through the Center for Population Research, National Institute of Child Health and Human Development.

Cell Dispersal—Ovarian cells were dispersed as previously described (12). Briefly, each ovary was cut into 4–6 pieces, washed twice with 10 ml of Medium 199, and incubated for 90 min (37 °C) in 0.1

* This work was supported by National Institute of Child Health and Human Development Research Center Grant HD-12303. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

† This research was submitted in partial fulfillment of the requirements for the Doctor of Philosophy degree at the University of California, San Diego.

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1 The abbreviations used are: hCG, human chorionic gonadotropin; LH, luteinizing hormone; FSH, follicle-stimulating hormone; RIA, radioimmunoassay; 8-Br-cyclic AMP, 8-bromo cyclic AMP.
ml/ovary of collagenase-DNase solution (4 mg/ml of collagenase, 10 mg/ml of DNase, 10 mg/ml of bovine serum albumin in Medium 199). The pieces of ovary were flushed through Pasteur pipets with successively smaller orifices every 30 min. The dispersed cells were then centrifuged at 250 × g for 5 min and washed 3 times with McCoy's medium, and the final pellet was resuspended in a known volume of McCoy's medium. Samples (50 μl) of the cell suspension were diluted with 50 μl of trypsin blue stain and aliquots were counted in a hemacytometer. The dispersal procedure yielded 2.5 × 10⁶ cells/ovary of which 50–80% were viable.

Short Term Incubation—The acute steroidogenic responsiveness was assessed by incubating 2 × 10⁶ viable cells in glass test tubes with and without increasing concentrations of purified hCG (0.1–1000 ng/ml) in 0.5 ml of Medium 199, 0.1% bovine serum albumin for 4 h at 37 °C in an air atmosphere. After the incubation, the media were frozen (−20 °C) until assayed for steroid hormones by RIA.

Cell Culture—Aliquots (50 μl; 1 × 10⁶ viable cells) were pipetted into Falcon tissue culture dishes (35 × 10 mm) containing 1 ml of McCoy's 5a medium supplemented with 100 units/ml of penicillin, 100 μg/ml of streptomycin sulfate, and 2 mM L-glutamine with and without purified gonadotropins. The ovarian cells were cultured up to 10 days in a humidified 95% air, 5% CO₂ incubator at 37 °C. Unless otherwise specified, the medium was changed at 48-h intervals at 37 °C. The pieces of ovary were flushed through Pasteur pipets with successive smaller orifices every 30 min. The dispersed cells were then centrifuged at 250 × g for 5 min and washed twice with ice-cold phosphate-buffered saline buffer and air-dried. To determine the fraction of cells with LH/hCG receptors, dispersed cells (1 × 10⁶) were incubated with 125I-labeled hCG (Pregnyl). The final incubation volume was 100 μl of Medium 199, 0.1% bovine serum albumin, 1 mg/ml of bovine serum albumin, pH 7.2 and the final pellets counted in a γ-spectrometer. The dissociation constant (KD) and binding capacity were determined by Scatchard plot analysis (20).

Autoradiography—Ovaries were removed from hypophysectomized immature rats and immediately frozen (unfixed) to a cryotome specimen holder cooled with dry ice. Frozen sections (10 μm) were incubated with 125I-labeled hCG (10 ng; 40,000–50,000 cpm/ng) for 1 h at 37 °C. Nonspecific binding was determined by incubating sections with 125I-unlabeled hCG (Pregnyl). After the incubation, the sections were washed twice with ice-cold phosphate-buffered saline buffer and air-dried. To determine the fraction of cells with LH/hCG receptors, dispersed cells (1 × 10⁶) were incubated with 125I-labeled hCG (10 ng) for 1 h at 37 °C. After the incubation, the cells were washed twice with ice-cold phosphate-buffered saline buffer, smeared onto microscope slides, and air-dried. Slides were coated with NTB-2 nuclear track emulsion (Kodak) and exposed for 14 days. The slides were developed and stained with 1% eosin (40% ethanol, pH 5.5). The percentage of labeled cells was determined by counting 500 or more cells in randomly selected fields from 5 separate preparations.

Electron Microscopy—Freshly collected ovarian tissue from con-

![Fig. 1](http://www.jbc.org/)
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Statistical Analysis—Statistical analyses were done using analysis of variance and Student's t-test. Dose-response curves were analyzed and the ED\textsubscript{50} was calculated using the curve-fitting program of Jaffe et al. (21).

RESULTS

hCG Binding, Cyclic AMP and Steroid Synthesis by Freshly Dispersed Cells—Fig. 1A shows that freshly dispersed cells from ovaries of hypophysectomized immature rats contain a single class of high affinity LH receptors (K\textsubscript{d} = 1.0 \pm 0.08 \times 10^{-10} M; binding capacity = 3113 \pm 263 sites/cell).

TABLE I
Steroid metabolites produced by cultured ovarian cells

<table>
<thead>
<tr>
<th>Steroid</th>
<th>Steroid concentration (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
</tr>
<tr>
<td>Androgens</td>
<td></td>
</tr>
<tr>
<td>Androstenediol</td>
<td>5.8 \pm 0.4</td>
</tr>
<tr>
<td>pregnenolone</td>
<td>0.02 \pm 0.06</td>
</tr>
<tr>
<td>Testosterone</td>
<td>0.6 \pm 0.1</td>
</tr>
<tr>
<td>Dehydroepiandrosterone</td>
<td>0.2 \pm 0.01</td>
</tr>
<tr>
<td>Progestins</td>
<td></td>
</tr>
<tr>
<td>pregnenolone</td>
<td>8.9 \pm 0.3</td>
</tr>
<tr>
<td>Progesterone</td>
<td>&lt;0.2</td>
</tr>
<tr>
<td>pregnenolone</td>
<td>&lt;0.2</td>
</tr>
<tr>
<td>Estrogens</td>
<td></td>
</tr>
<tr>
<td>Estradiol + Estriol</td>
<td>2.1 \pm 0.1</td>
</tr>
<tr>
<td>Estradiol + Estriol</td>
<td>0.2 \pm 0.02</td>
</tr>
</tbody>
</table>

FIG. 2. Effect of hCG on cyclic AMP and androgen production. Freshly dispersed cells (2 x 10\textsuperscript{6} viable cells/tube) from ovaries of hypophysectomized immature rats were incubated with increasing concentrations of hCG (0-1000 ng/ml) for 4 h to determine steroid responses or 1 h with 0.1 mM 3-isobutyl-1-methylxanthine to determine cyclic AMP responses. The data are the means \pm S.E. of 3 experiments with triplicate incubations per experiment.

FIG. 3. LH/hCG-stimulated androsterone synthesis. A, time course and specificity of LH-stimulated androgen biosynthesis. Dispersed cells (1 x 10\textsuperscript{6} viable cells/dish) from ovaries of hypophysectomized immature rats were cultured for 10 days in the presence and absence of 100 ng/ml of highly purified LH, hCG, FSH, or prolactin (PRL). The medium was changed every 2 days at which time fresh hormones were added. Androsterone in the media was measured by RIA. The data represent the means \pm S.E. of 6 experiments with triplicate incubations per experiment. B, dose-response curves for LH and hCG stimulation of androgen biosynthesis. Dispersed cells (1 x 10\textsuperscript{6} viable cells/dish) were cultured for 4 days in the presence of increasing concentrations of LH or hCG (10\textsuperscript{2}-10\textsuperscript{8} ng/ml). The medium was changed and fresh hormones were added at 2 days. Androsterone was measured in media collected at day 4 by RIA. The data represent the means \pm S.E. of 2 experiments with triplicate incubations per experiment.
Autoradiographic analysis showed that the $^{125}$I-hCG binding sites were localized exclusively in the theca and secondary interstitial cells (Fig. 1, B and C) and that these cells represented 29 ± 1% of the total cell population. When the binding

data represent the means of triplicate incubations per experiment. Androsterone in the media was measured by RIA. The data represent the means ± S.E. of 2 experiments with triplicate incubations per experiment.

**Fig. 4.** Effect of delayed addition of LH on the time course of LH-stimulated androgen biosynthesis. Dispersed cells (1 x $10^6$ viable cells/dish) were cultured for 8 days (144 h). At 0, 2, or 4 days, LH (100 ng/ml) was added to the cultures. The medium was changed every 2 days at which time fresh LH was added to the appropriate dishes. Androsterone in the media was measured by RIA. The data represent the means ± S.E. of 2 experiments with triplicate incubations per experiment.

**Fig. 5.** Effect of actinomycin D on the detailed time course of LH-stimulated androgen biosynthesis. Dispersed cells (4 x $10^6$ viable cells/dish) were cultured for 144 h in the absence (control) and presence of LH (100 ng/ml). The medium was changed at 48-h intervals, at which time fresh LH was added. At 48 h actinomycin D (0.1 gg/ml) was added to appropriate dishes. At 72 h all dishes were washed thoroughly and recultured with LH alone. At the times indicated the medium was collected from groups of dishes and assayed for androsterone by RIA. The data represent the means ± S.E. of 2 experiments with triplicate incubations per experiment.

**Fig. 6.** Effect of prostaglandin E$_2$, cholera toxin, and 8-bromo cyclic AMP on androgen biosynthesis. Dispersed cells (4 x $10^6$ viable cells/dish) were cultured for 4 days with increasing concentrations of prostaglandin E$_2$ (1 x $10^{-10}$ ng/ml), cholera toxin (1 x $10^{-10}$ ng/ml), or 8-bromo cyclic AMP (10$^{-7}$ 10$^{-2}$ M). The medium was changed at 2 days and fresh hormones were added. Androsterone in the media collected at 4 days was measured by RIA. Data represent the means ± S.E. of 3 experiments with triplicate incubations per experiment.

**Fig. 7.** Effect of in vivo hCG treatment on steroidogenesis in vitro. Hypophysectomized immature rats were injected subcutaneously with hCG (13 IU/day; @ and D) or saline (A and B) for 1-3 days. Dispersed cells (2 x $10^6$ viable cells/tube) were incubated in Medium 199 (0.5 ml; 25 mm 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid, 0.1% bovine serum albumin with @ and A) with and without (O and D) hCG (100 ng/ml) for 4 h (37 °C). After the incubation, the media were assayed for steroid hormones by RIA. The data represent the means ± S.E. of 3 experiments with triplicate incubations per experiment.

capacity was corrected for the percentage of labeled cells, the thecal and interstitial cells contained an average of 10,734 ± 907 LH/hCG receptors/cell.

Fig. 2 shows the acute effects of hCG on cyclic AMP and steroid production by freshly dispersed cells. Increasing concentrations of hCG (0-1 pg/ml) caused a dose-related increase in cyclic AMP accumulation; the minimum, maximum, and half-maximum effective doses of hCG were 3, 100, and 6.2 ng/ml, respectively. In contrast, hCG failed to stimulate steroid production above control levels during the 4-h incubation.
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(Fig. 2); <300 pg of androsterone, androstanediol, and androstenedione, progesterone, 20α-dihydroprogesterone, and estrogen were secreted during a 4-h incubation. Collectively, these results indicate that the freshly dispersed ovarian cells contain specific LH/hCG receptors which are functionally coupled to adenylate cyclase but not steroidogenesis and that the primary target of LH/hCG action is the theca and secondary interstitial cells.

LH Stimulation of Androgen Synthesis in vitro—In contrast to the acute studies, steroidogenesis was stimulated by prolonged LH treatment in tissue culture. Table I shows the steroid metabolites secreted by control and LH-treated cells in culture. The principal steroids produced by control cells were pregnenolone > androsterone > 20α-dihydroprogesterone; other steroids were very low (<1 ng/ml). Cells treated with LH showed a 78-fold stimulation of total steroidogenesis with increases in all 11 steroids. By far the major steroid metabolites were androgens (96% of total steroid produced) with androsterone > 5α-androstane-3α,17β-diol > androstenedione. Of the total steroid secreted, progestins constituted only 4% with pregnenolone > 17α-hydroxyprogesterone > 17α-hydroxypregnenolone. Only trace levels of estrogen were produced.

Because androsterone is the major steroid formed in response to LH stimulation, only data on this hormone will be presented in the following in vitro studies.

Time Course, Specificity, and Dose-Response of LH-Stimulated Androsterone Synthesis—The time course and specificity of the androsterone response are shown in Fig. 3A. During a 10-day time course study, control (untreated) cells

Fig. 8. Histology and ultrastructure of control and hCG-treated ovaries. A, control ovary from a 27-day hypophysectomized rat showing a follicle with granulosa cells (G), 3-4 layers of fibroblast-like theca cells (T), and well developed secondary interstitial tissue (I) (×500). B, typical theca cell from control ovary showing nucleus (N) with abundant heterochromatin, rough endoplasmic reticulum (rer), and mitochondria (M) with laminar cristae (×17,500). C, secondary interstitial cell from control ovary; nucleus (N), mitochondria (M) with tubular cristae, smooth endoplasmic reticulum (ser), and multiple lipid (L) inclusions (×20,000). D, ovary from hypophysectomized rat after 3 days of hCG treatment; follicle with granulosa cells (G), hypertrophied theca interna (T) with islands of highly differentiated cells (arrow), and secondary interstitial cells (I) (×500). E, theca cell from hCG-treated ovary containing nucleus (N) with little heterochromatin, lipid (L) inclusions, abundant smooth endoplasmic reticulum (ser), and mitochondria (M) with tubular cristae (×17,500). F, secondary interstitial cell from hCG-treated ovary showing lipid (L) inclusions, abundant smooth endoplasmic reticulum (ser), and mitochondria (M) with tubular cristae (×20,000).
produced low levels (<6 ng/ml each 2-day period) of androsterone. In the presence of LH (100 ng/ml) or hCG (100 ng/ml), androstenedione production was low (6 ± 0.4 ng/ml) at day 2, increased dramatically (200-fold) to maximum levels (1265 ± 21 ng/ml) at day 4, and remained maximal through day 10 of culture. The stimulation of androstenedione synthesis was specific to LH and hCG since FSH (100 ng/ml) and prolactin (100 ng/ml) were without effect.

As shown in Fig. 2B, the stimulatory effects of LH and hCG were dose-related; the minimum, maximum, and half-maximum effective doses were 0.3, 10, and 2 ± 0.2 ng/ml (8.2 × 10⁻¹⁰ M), respectively, for both hormones.

Characterization of the Latency Phase—Fig. 4 shows that the latency phase of 4 days between the addition of LH and the appearance of androstenedione in the medium was obligatory; if LH was not added until day 2 or 4, then the appearance of androstenedione in the medium was delayed until day 6 or 8, respectively. When LH was added at day 4, androstenedione levels at day 8 were approximately half those at comparable times after LH addition at day 0 or 2, suggesting the ovarian cells were less responsive to LH stimulation at day 4. The mechanism by which the cells become less responsive is unclear.

Results of a detailed study of the latency period for LH stimulation of androstenedione synthesis are shown in Fig. 5. No significant change in androstenedione production was observed in the LH-treated cultures until after 72 h of incubation. At 76 h, androstenedione began to accumulate in the medium, then increased very rapidly reaching maximal concentration at 96 h. Treatment with actinomycin D during the latency period inhibited the LH stimulation of androstenedione production. In this experiment, LH-stimulated cells were exposed for 24 h to 0.1 µg/ml of actinomycin D beginning at 48 h, washed thoroughly at 72 h, and then recultured with LH alone. As shown in Fig. 5, exposure to actinomycin D completely prevented the expected LH stimulation of androstenedione synthesis at 96 h; however, when the inhibitor was removed, androstenedione concentration increased rapidly to maximum levels after a 24-h latency period.

Effect of Cyclic AMP Analogues and Adenylate Cyclase Activators—Fig. 6 shows that prostaglandin E₂, cholera toxin, and 8-Br-cyclic AMP stimulated dose-related increases in androstenedione synthesis; the ED₅₀ values were prostaglandin E₂ = 54 ± 4 ng/ml (1.5 ± 0.1 × 10⁻⁵ M), cholera toxin = 14 ± 3 ng/ml (1.7 ± 0.4 × 10⁻⁶ M); 8-Br-cyclic AMP = 3.3 × 10⁻⁴ M. The maximum responses stimulated by prostaglandin E₂ and 8-Br-cyclic AMP were equal to those stimulated by LH/hCG while the response to cholera toxin was only 70% of the maximum amount stimulated by LH or hCG.

Effect of in Vivo hCG Administration on the Structure and Function of the Interstitial Cells—To determine if the in vitro observations have physiological relevance, hypophysectomized immature rats were injected with hCG for 0–3 days after which ovarian androgen synthesis was assessed. As shown in Fig. 7, cells from control (saline-treated) ovaries secreted little or no androgen in the absence or presence of hCG (100 ng/ml). In contrast, after a latency phase of 24 h, the cells primed in vivo with hCG responded to in vitro hCG stimulation by showing a sharp increase in androstenedione and androstenedione production (Fig. 7). Although the androstenedione response by the hCG-primed cells remained high the androstenedione response declined markedly after 3 days of hCG priming in vivo.

Histological studies showed that ovaries of hypophysectomized immature rats contained preantral follicles (~30% of which showed evidence of atresia), and a well developed secondary interstitial tissue located in the interfollicular

stoma (Fig. 8A). The secondary interstitial cells (Fig. 8C) exhibited ultrastructural characteristics typical of differentiated steroid-secreting cells. The theca interna surrounding the healthy follicles was comprised of 3–4 layers of fibroblast-like cells (Fig. 8D). After 3 days of hCG treatment in vivo, the theca cells showed morphologic alterations and exhibited the ultrastructure characteristic of active steroid-secreting cells (Fig. 8E). Secondary interstitial cells also responded to hCG-treatment by exhibiting increased prominence and vesiculation of smooth endoplasmic reticulum and an increase in lipid inclusions (Fig. 8F).

Discussion

The results of the present function-structure study confirm and extend previous observations that the rat ovary contains a population of androgen-producing cells (2, 3) which are located in the theca interna and stromal tissue (4, 8). The ovarian androgens are of major physiological importance for estrogen biosynthesis (11), follicular progesterone production (22, 23), atresia (24), and the onset of puberty (25). Despite the importance of the ovarian theca and interstitial cells, relatively little is known about the regulatory mechanisms which control their physiological responses. We now report that isolated theca and interstitial cells are able to respond to physiological concentrations of LH in serum-free medium with the expression of their normal differentiated functions. We anticipate that this defined primary culture model will be valuable in elucidating the regulatory mechanisms involved in the development and function of the ovarian androgen-producing cells.

Our in vitro data show that LH and hCG, but not FSH or prolactin, are able to induce androgen synthesis. This highly specific effect of LH/hCG in stimulating the interstitial cells in vitro is consistent with previous in vivo findings (26, 27) and supports the classical concept that ovarian androgen biosynthesis is primarily regulated by LH/hCG (26–28). The obligatory latent period of 3 days between the addition of LH and the appearance of androgens in the medium may represent the time necessary for the ovarian cells to synthesize important molecules required for androgen synthesis. Our observation that actinomycin D (an inhibitor of RNA synthesis) completely blocked the LH stimulation of androgen synthesis is consistent with this possibility. The nature of the LH-induced molecules is unknown; however, previous metabolic studies with homogenates of ovaries from hypophysectomized immature rats have provided evidence that LH selectively increases the cholesterol side chain cleavage complex (29), 17α-hydroxylase and C₁₇-α desmolase (30) activities in vivo. Therefore, it is not unreasonable to propose that in our system, the androgenic response of the cultured interstitial cells to LH/hCG involves differential gene expression which results in the de novo synthesis of new molecules involved in the activation of key rate-limiting steps in the synthesis of androgens. Experiments are now in progress to test this hypothesis.

The demonstration that the LH/hCG effect on androgen synthesis was mimicked by cyclic nucleotide analogues and several natural activators of adenylate cyclase (31, 32) suggests a primary role for cyclic AMP in the hormone induction of androgen biosynthesis. This finding is not surprising since numerous previous studies have shown that the mechanism of action of LH/hCG in the stimulation of ovarian (33) and testicular (34) steroid production is mediated by cyclic AMP. The maximal androgen responses stimulated by cholera toxin and 8-Br-cyclic AMP appeared to be reduced, raising the possibility that cellular responses other than cyclic AMP may also be involved in the LH induction of androgen biosynthesis.
The physiological significance of prostaglandin-stimulated androgen-producing cell differentiation is unknown but prostaglandin E₂ is present in follicular fluid (36) and therefore may influence theca and interstitial cell development and function.

The principal steroid metabolites produced by the cultured interstitial cells were 5α-reduced androgens; androsterone > 5α-androstane-3α,17β-diol. This 5α-reduction of androgens in culture is physiological because it is well established that the ovaries of the prepubertal rat contain an active 4-ene-5α-reductase (25, 31, 32, 36) located in the microsomal fraction (37) of the ovarian interstitial cells (38), the activity of which has been shown to be increased specifically by LH (30, 39). The physiological relevance of our in vitro system was again supported by our demonstration that administration of hCG in vivo induced the ultrastructural differentiation of the theca and interstitial cells which was accompanied (after a latency phase) by a selective stimulation of androgen synthesis. The reason that the latency period of hCG action in vivo is shorter than that observed in vitro is unclear.

In conclusion, the results described here demonstrated that LH/hCG induces the functional differentiation of the ovarian androgen-producing cells in serum-free medium in a manner comparable to that observed in vivo. The metabolic process is complex and seems to involve hormone-regulated gene expression. With the availability of this primary cell culture system, it should be possible to define the biochemical mechanisms and controls of hormone-regulated steroidogenesis during the differentiation of the ovarian androgen-producing cells.

Acknowledgments—We thank Dr. S. S. C. Yen for his interest in this work, Dr. H. Papkoff for kindly providing purified LH and FSH, Dr. B. D. Burleigh for providing purified prolactin, Dr. R. E. Canfield and the Center for Population Research of the National Institute of Child Health and Human Development for supplying purified hCG, Dr. W. H. Moger for supplying the androsterone antiserum, Dr. E. Samojlik for providing the 5α-androstane-3α,17β-diol antiserum, N. Moss for assistance with RIA and C. Hofeditz for expert electron microscopy.

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