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# Changes in Content of Cytochrome $P450_{17\alpha}$, Cytochrome P450scc, and 3-Hydroxy-3-Methylglutaryl CoA Reductase in Developing Rat Ovarian Follicles and Corpora Lutea: Correlation with Theca Cell Steroidogenesis[1]

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## ABSTRACT

*The following study was undertaken to determine which hormones (luteinizing hormone, LH, and prolactin, PRL) and enzymes (cytochrome $P450_{17\alpha}$, nicotinamide adenine dinucleotide phosphate [NADPH]-cytochrome P450 reductase, 3-hydroxy-3-methylglutaryl [HMG] CoA reductase, cholesterol side-chain cleavage cytochrome P450 [P450scc], and adrenodoxin) were associated with the regulation of androgen biosynthesis by developing rat follicles and corpora lutea in vivo as well as by thecal explants maintained in culture. Immunoblots of soluble cell extracts of small antral (SA), preovulatory (PO), and luteinizing (PO + human chorionic gonadotropin [hCG], 7 h) follicles, newly formed corpora lutea (PO + hCG, 24 h), and corpora luteal isolated on Day 15 of pregnancy, demonstrated that cytochrome $P450_{17\alpha}$ was low in SA follicles, selectively increased 4-fold in PO follicles, and decreased to less than 10% within 7 h after hCG. Filter hybridization assays using a $^{32}$P-labeled cytochrome $P450_{17\alpha}$ cDNA probe demonstrated that changes in the content of $P450_{17\alpha}$ mRNA exhibited a pattern similar to that of the enzyme. Conversely, immunoblots for other microsomal enzymes either exhibited no change (NADPH cytochrome P450 reductase) or a transient increase after the hCG surge (HMG CoA reductase), whereas the mitochondrial enzymes either increased markedly in association with luteinization (cytochrome P450scc) or were increased in a more transient manner (adrenodoxin). The LH-induced loss of cytochrome $P450_{17\alpha}$ in vivo was not associated with loss of androgen biosynthesis when luteinizing theca were placed in culture in medium containing either LH or LH and PRL, suggesting that other hormones, or the presence of other cell types, are required to maintain the decrease in cytochrome $P450_{17\alpha}$ in vivo. Conversely, the LH-induced increase in cytochrome P450scc in vivo was associated with the maintenance of elevated progesterone production by theca in culture, suggesting that cytochrome P450scc may be constitutively expressed in luteinized theca. Thus, thecal cell cytochrome $P450_{17\alpha}$ and the regulation of its content and mRNA by LH are pivotal to the biosynthesis of androgens, the obligatory precursors for estradiol biosynthesis and the consequent development of preovulatory follicles. The molecular basis for the different effects of low versus elevated concentrations of LH on cytochrome $P450_{17\alpha}$, as well as cytochrome P450scc, remain to be determined.*

## INTRODUCTION

The synthesis of estradiol by developing preovulatory (PO) follicles is obligatory for granulosa cell differentiation (Richards, 1980) as well as for triggering the luteinizing hormone (LH) surge from the pituitary (Karsch, 1980). In the rat, small antral (SA) follicles of immature female rats (Richards et al., 1980), diestrous rats (Uilenbroek and Richards, 1979), and pregnant rats (Richards and Kersey, 1979; Bogovich and Richards, 1982) produce minimal amounts of estradiol, despite the presence of an active aromatase system in granulosa cells of these follicles that can convert exogenous androgens to

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estradiol. These observations, combined with the evidence that thecal cells are the primary site of androgen biosynthesis (Fortune and Armstrong, 1977; Bjersing, 1978; Carson et al., 1981; Bogovich and Richards, 1984) led us to propose that the synthesis of estradiol by preovulatory follicles was dependent on the ability of thecal cells to synthesize aromatizable androgens (Richards, 1980; Richards and Kersey, 1979) in response to subtle changes in serum concentrations of LH (Richards et al., 1980; Bogovich et al., 1981; Richards and Bogovich, 1982). We (Bogovich and Richards, 1982) have shown that LH regulates androgen biosynthesis in rat theca cells by stimulating the conversion of progesterone to androstenedione via a single cytochrome P450 enzyme containing both 17α-hydroxylase and 17-20-lyase activities ($P450_{17\alpha}$) (Kominami et al., 1982; Nakajin and Hall, 1981; Zuber et al., 1986b). Development of a long-term thecal cell culture system (Bogovich et al., 1986) allowed us to demonstrate further that either LH or forskolin was able to maintain androstenedione synthesis by theca explants of SA and preovulatory follicles for as long as 30 days (Richards et al., 1986). Taken together, the in vivo and in vitro studies indicate that LH and cyclic adenosine 3′,5′-monophosphate (cAMP) act on theca cells of small antral follicles to increase either the content or activity of cytochrome $P450_{17\alpha}$ in theca of PO follicles.

After the LH surge, luteinizing follicles rapidly lose their ability to synthesize estradiol (Hillensjö et al., 1976; Richards and Kersey, 1979). Estradiol synthesis by functional corpora lutea of pregnant rats is also low despite the presence of a significant amount of aromatase activity (Elbaum and Keyes, 1976). Therefore, the decline in estradiol synthesis, which occurs after the LH surge, appears to be related to a decline in androgen biosynthesis and loss of $P450_{17\alpha}$ activity (Hillensjö et al., 1976; Richards and Kersey, 1979; Suzuki and Tamaoki, 1980, 1983; Johnson and Griswald, 1984; Eckstein et al., 1985; Eckstein and Tsafriri, 1986). Using an antibody against cytochrome $P450_{17\alpha}$ (Nakajin and Hall, 1981), Rodgers et al. (1986) have shown that the content of this enzyme was high in large bovine follicles but was negligible (absent) in corpora lutea, indicating that follicular and luteal cell androgen biosynthesis are related to the content of the $P450_{17\alpha}$ enzyme.

The mechanism by which the LH surge causes the decline in $P450_{17\alpha}$ has not been entirely resolved. Suzuki and Tamaoki (1983) have shown that loss of $P450_{17\alpha}$ activity mediated by the LH surge may involve de novo RNA and protein synthesis. Prolactin (PRL) has been shown to inhibit androgen biosynthesis in serum-free cultures of dissociated ovarian cells (Magoffin and Erickson, 1982a,b). It is possible, therefore, that the PRL surge associated with ovulation, as well as the PRL surges (Smith et al., 1975) and release of placental lactogen (Linkie and Niswender, 1972) that occur during pregnancy suppresses $P450_{17\alpha}$. In Leydig cells, steroids (Nozu et al., 1981) and oxygen free-radicals (Quinn and Payne, 1984, 1985) have been shown to inhibit $P450_{17\alpha}$ activity. More recently, aminoglutethimide has been shown to enhance cAMP-induced synthesis of $P450_{17\alpha}$ in Leydig cell cultures (Hales et al., 1986; Sheela Rani and Payne, 1986), suggesting a steroid-mediated inhibition of $P450_{17\alpha}$ synthesis.

Based on these considerations, the following studies were undertaken to determine 1) if changes in cytochrome $P450_{17\alpha}$ content and mRNA occur during follicular development and luteinization in the rat; 2) if changes in cytochrome $P450_{17\alpha}$ were associated with changes in other enzymes involved in follicular and luteal cell steroidogenesis; 3) if changes in cytochrome $P450_{17\alpha}$ were related to thecal cell androgen biosynthesis; and 4) if PRL or rat placental lactogen, which are known to be elevated at estrus and/or during pregnancy, respectively, has a direct effect on androgen biosynthesis by theca isolated from follicles at defined stages of differentiation. For these analyses, we have used an immature rat model system in which the stages of follicle development are well-defined, the initiation of ovulation and luteinization are precisely timed and highly reproducible, and the functional characteristics of theca cells in culture have been analyzed (Richards and Bogovich, 1982; Richards et al., 1986).

## MATERIALS AND METHODS

### *Animals*

Immature female rats and timed-pregnant rats were obtained from Holtzman Co., Madison, WI.

### *Tissue Isolation*

Small antral (SA) and preovulatory (PO) follicles were isolated from 30-day-old immature rats before and after treatment with a low dose of human

chorionic gonadotropin (hCG, 0.2 IU given twice daily for 2 days; Richards and Bogovich, 1982). Luteinizing follicles were isolated 7 h after an i.v. injection of 10 IU hCG (designated PO + hCG, 7 h; Richards et al., 1986). Newly formed corpora lutea were isolated 24 h after 10 IU hCG (PO + hCG, 24 h). Mature functional corpora lutea were isolated from pregnant rats on Day 15 of gestation.

*Immunoblots*

Intact follicles and corpora lutea isolated as described above were frozen in liquid nitrogen and stored at −70°C until used. Tissues were then homogenized (10 vol/g tissue) in phosphate-buffered isotonic saline (PBS) containing 1% cholate and 0.1% sodium dodecyl sulfate (SDS) (Rodgers et al., 1986) or in 10 mM potassium phosphate, pH 6.8, containing 1 mM ethylenediaminetetraacetate (EDTA) and 10 mM 3-[(3-cholamidopropyl)-dimethylammonio]-1-propane-sulfonate (CHAPS) (Hjelmeland, 1980). Homogenates were sonicated (3 × 10 s) and a soluble cell extract was obtained by centrifugation for 5 min at 4°C in a bench-top microfuge to remove particulate matter. Protein concentrations were determined by the method of Bradford (1976) or Lowry (1951), and equal amounts of protein for each sample were resolved by one-dimensional (1-D) SDS-polyacrylamide gel electrophoresis (PAGE) using 10% acrylamide for all immunoblots except for adrenodoxin, which was resolved on 15% acrylamide gels.

Proteins were electrophoretically transferred (Towbin et al., 1979; Richards et al., 1984) to nitrocellulose filters and incubated with specific rabbit immunoglobulin G (IgG) or serum using 5% milk (non-fat dry milk, Carnation Co., LA) in the buffers and dilutions of IgG and serum, as previously described (Jahnsen et al., 1986; Rodgers et al., 1986). $^{125}$I-labeled goat anti-rabbit IgG (Rodgers et al., 1986) or $^{125}$I-Protein A (Richards et al., 1984) was used as the second antibody ($10^6$ cpm/ml). After appropriate washes, all filters were exposed to X-Omat AR film (Eastman Kodak, Rochester, NY) with intensifying screens at −70°C. Relative changes in protein content were quantitated by densitometric scanning of the autoradiographs.

*Antibodies and Purified Protein Standards*

Cholesterol side-chain cleavage cytochrome P450 (P450scc) and adrenodoxin purified from bovine adrenal cortex were kindly donated by Dr. J. D. Lambeth (Emory University, Atlanta, GA). IgG fractions raised against these proteins were prepared as described previously (Dubois et al., 1981). Antisera generated against cytochrome $P450_{17\alpha}$ (Nakajin and Hall, 1981) purified from porcine testis was generously donated by Dr. P. F. Hall (Worcester Foundation for Experimental Biology, Shrewsbury, MA). Nicotinamide adenine dinucleotide phosphate (NADPH)-cytochrome P450 reductase from porcine liver and the IgG raised against this protein were prepared as previously described and donated by Dr. B. S. S. Masters (Medical College of Wisconsin, Milwaukee, WI). Complex V proteins, which include $F_1$-adenosine triphosphatase, were purified by the methods of Stiggall et al. (1977), and antisera against Complex V proteins were raised in rabbits and kindly donated by Dr. G. A. Breen (University of Texas at Dallas, TX). 3-Hydroxy-3-methylglutaryl-CoA reductase antibody (Chin et al., 1982) was generously provided by Dr. R. G. W. Anderson (University of Texas at Dallas, TX).

*Extraction of RNA*

Total RNA was prepared from isolated theca cells by extraction in 4 M guanidine-isothiocyanate and collected by CsCl-density centrifugation as described by Chirgwin et al. (1979). The RNA was phenol/chloroform-extracted, ethanol-precipitated, resuspended in autoclaved water, and stored at −70°C. RNA was measured spectrophotometrically by absorbance at A260.

*cDNA Probe*

A 1.2-kb $P450_{17\alpha}$ cDNA probe was isolated from a $P450_{17\alpha}$ plasmid (pcD17α-2) (Zuber et al., 1986a) by (PstI) digestion. The cDNA insert was labeled according to the procedure of Feinberg and Vogelstein (1984), using an oligonucleotide primer and [α-$^{32}$P]-cytidine 5′-triphosphate (dCTP) and [α-$^{32}$P]-thymidine 5′-triphosphate (TTP) to a final specific activity of $1 \times 10^8$ cpm/μg DNA.

*Filter Hybridization*

Total RNA (20 μg) was denatured at 65°C for 15 min in 12 times 0.15 M sodium chloride, 0.015 M sodium citrate, pH 7.0 (12 × SCC) containing 16% formaldehyde, applied to Biodyne nylon filters (ICN) according to the instructions by the manufacturer using a slot-blot manifold apparatus, and baked at

80°C for 1 h. Filters were prehybridized at 37°C for 16 h, then hybridized with the nick-translated probe for an additional 16 h at 37°C. Filters proved with $P450_{17\alpha}$ $^{32}$P-cDNA were washed in 2× SCC, 0.1% SDS for 5 min at 20°C (repeated 3 times), and washed in 2× SCC, 0.1% SDS for 15 min at 40°C (repeated once). This was followed by a brief wash in 1× SCC, 0.1% SDS for 15 min at 20°C. The filters were then placed with Kodak XAR-7 or XAR-5 film and intensifying screens at −70°C.

*Theca Explant Culture*

Theca explants were isolated from SA, PO, and luteinizing follicles (PO + hCG, 7 h) and were cultued in 24-multiwell Falcon dishes, as described previously (Richards et al., 1986), in Dulbecco's Modified Eagles Medium (DMEM): Ham's F-12 (F12) medium containing 2% fetal bovine serum (FBS) with or without 5 ng/ml LH (NIH-oLH-23; 2.3 × NIH S1; <0.5% NIH FSH S1) and increasing concentrations of ovine prolactin (oPRL) (NIH-oPRL-15) or with 10 μM forskolin (Calbiochem-Behring Corp., San Diego, CA). The doses of oLH and forskolin were chosen on the basis of previous dose-response (Bogovich et al., 1986) and time-course (Richards et al., 1986) studies showing optimal stimulation of theca cell steroidogenesis. The doses of oPRL were chosen to correspond with those used by other investigators (Magoffin and Erickson, 1982a,b). Media samples were removed on Day 4 and at 3-day intervals thereafter, boiled to inactivate phosphodiesterase for measurements of cAMP, and stored at −70°C until assayed for cAMP, progesterone, and androstenedione.

*Radioimmunoassays*

Steroid and cAMP RIAs were conducted as previously described (Bogovich et al., 1986; Richards et al., 1986). Androstenedione, progesterone, and estradiol were measured using $^{3}$H-labeled ligands obtained from Amersham Corp. (Arlington Heights, IL). Antiserum against androstenedione was generously provided by Dr. G. D. Nordblom (Ligand Assay Laboratory, Department of Pathology, University of Michigan, Ann Arbor, MI). Antiserum against progesterone was generously provided by Dr. Gordon D. Niswender (Department of Physiology and Biophysics, Colorado State University, Fort Collins, CO). The antiserum against estradiol (B930) was kindly provided by the Reproductive Endocrinology Program, The University of Michigan, Ann Arbor, MI. Antiserum against cAMP was generously provided by Dr. J. L. Vaitukaitis (Department of Medicine, Boston University School of Medicine, Boston, MA). All medium samples used to measure cAMP, as well as the cAMP standard, were acetylated before analysis to increase the sensitivity of the assay (Richards et al., 1979). All steroid assays were run on unextracted media. Medium blanks (2% fetal calf serum [FCS]) contained negligible amounts of steroid (progesterone, undetectable; androstenedione and estradiol, <4 pg) and cAMP. All data were analyzed using a linear/log computer analysis provided by a Micromedic 4/200 γ-counter (Micromedia Systems, Horsham, PA).

*Reagents*

Medium 199, DMEM, F12, penicillin-streptomycin, and FCS were purchased from Grand Island Biological Co. (Grand Island, NY). [1,2,6,7-$^{3}$H]Progesterone, [1,2,6,7-$^{3}$H]androstenedione, [2,4,6,7-$^{3}$H]-estradiol-17β, and 2′-O-succinyl-3-[$^{125}$I]iodotyrosine methyl ester of cAMP were obtained from Amersham (Arlington Heights, IL). Crude hCG (2800 IU/mg) was purchased from Organon Special Chemicals (West Orange, NJ). Ovine LH (NIAMDK oLH 23; 2.3 × NIH LH S1; <0.5% NIH FSH S1) and ovine prolactin (NIAMDK-oPRL-15) were obtained from the National Hormone and Pituitary Program (Baltimore, MD).

## RESULTS

*Content of Steroidogenic Enzymes in Follicles and Corpora Lutea*

Table 1 summarizes the number of follicles and corpora lutea collected for the first series of immunoblot assays (Figs. 1–4), the wet weight of tissue in each treatment group, and the mean wet weight of each class of follicle and corpus luteum and the average cell extract protein (μg/μl) obtained by homogenizing each tissue in 10 ml buffer/g wet weight. The results demonstrated that the mean wet weight of follicles increased 2-fold from the SA to PO stage of growth, 1.5-fold 7 h after the LH/hCG surge, and another 1.5-fold by 24 h. Mature corpora lutea were 6.8 times heavier than newly formed corpora lutea and 30 times heavier than small antral follicles. Similar increases were observed in the concentration of cell extract protein per follicle or corpus luteum, with the exception of corpora from

TABLE 1. Wet-weight and protein content of follicles and corpora lutea.

| Tissues[a] | Follicles | | | Corpora lutea (CL) | |
|---|---|---|---|---|---|
| | SA | PO | PO + hCG 7 h | PO + hCG 24 h | Day 15 pregnancy |
| No. ovaries | 28 | 28 | 28 | 24 | 14 |
| No. follicles or CL isolated | 260 | 166 | 141 | 108 | 96 |
| Wet weight (mg) | 34.4 | 48.8 | 60.8 | 62.5 | 378.5 |
| Wet weight (mg) follicle or CL | 0.13 | 0.29 | 0.43 | 0.58 | 3.94 |
| Protein ($\mu$g/$\mu$l)[b] | 4.65 | 7.1 | 6.6 | 8.5 | 11.5 |
| Protein ($\mu$g/follicle or CL) | 6.05 | 20.6 | 28.4 | 49.3 | 453.1 |

[a]SA, small antral follicles; PO, preovulatory follicles; PO + hCG, PO follicles isolated 7 h after 10 IU human chorionic gonadotropin i.v.; corpora lutea isolated 24 h after exposing PO follicles to 10 IU of hCG i.v.; corpora lutea isolated from pregnant rats on Day 15 of gestation.

[b]$\mu$g Protein per $\mu$l of soluble cell extract; all tissues were homogenized in 10 ml buffer per g wet weight.

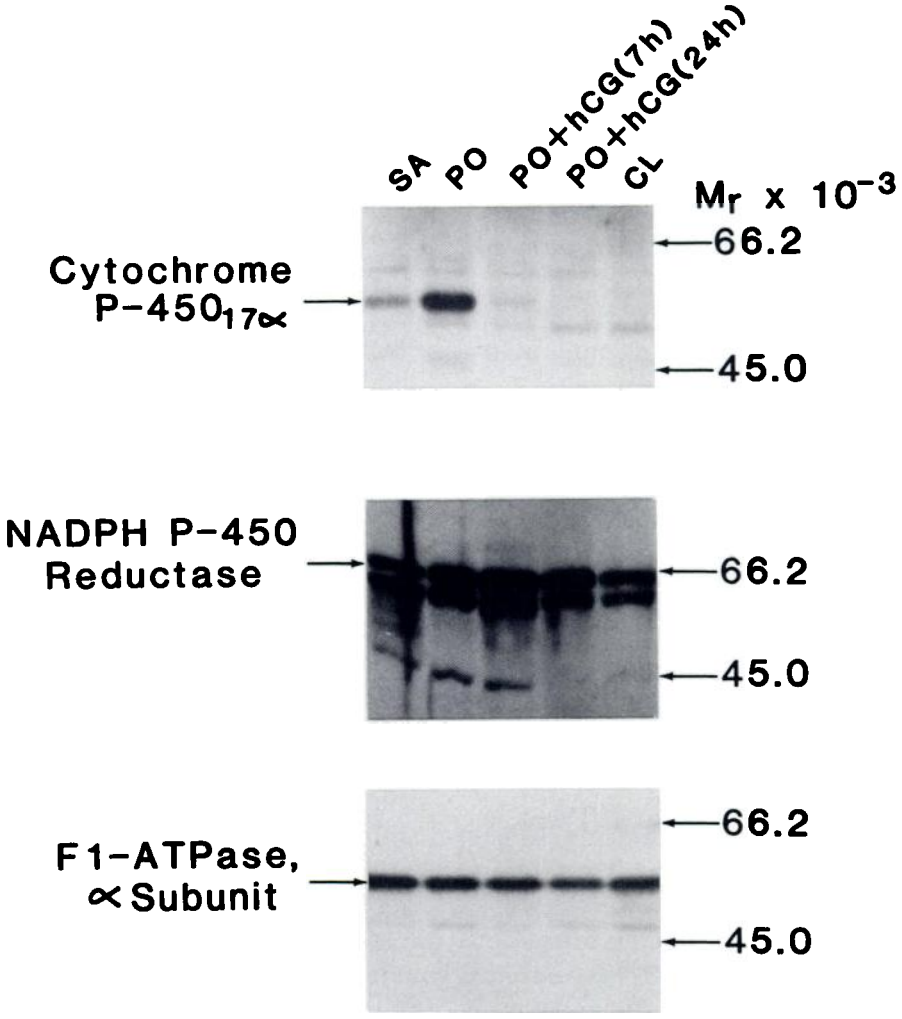


FIG. 1. Autoradiographs of immunoblots of 17$\alpha$-hydroxylase cytochrome P450 (*P450$_{17\alpha}$*), *NADPH P450 reductase*, and *F1-ATPase $\alpha$ subunit*. Soluble cell extracts were prepared from small antral (*SA*), preovulatory (*PO*), and luteinizing (*PO + hCG, 7 h*) follicles as well as from newly formed corpora lutea (*PO + hCG, 24 h*) and corpora lutea (*CL*) of pregnant rats on Day 15 of gestation. Equal amounts of protein (50 $\mu$g) were resolved by one-dimensional SDS-PAGE, electrophoretically transferred to nitrocellulose paper, and incubated with antibodies generated against the indicated proteins. (Abbreviations defined in *Materials and Methods*.)

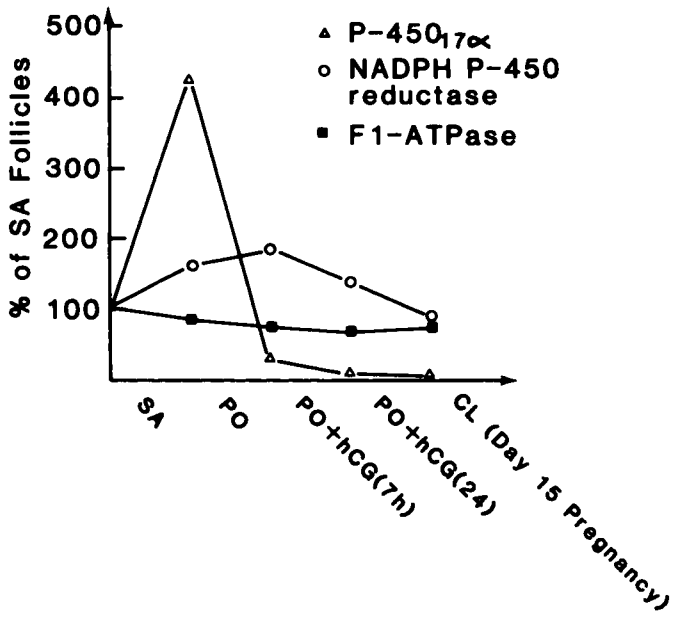


FIG. 2. Quantitation by densitometric scanning of the autoradiographs shown in *Figure 1*. For each protein, the content present in SA follicles was set at 100%.

Day 15 of pregnancy, which contained 70-fold more protein than small antral follicles (compared to the 30-fold increase in size). In all cases, equal amounts of protein were loaded in each lane of all blots to assess the relative change in the total amount of enzyme in follicles as they grew and luteinized.

Immunoblot analyses of the microsomal protein P450$_{17\alpha}$ (Fig. 1) combined with densitometric scanning (Fig. 2) demonstrated that the content (per $\mu$g tissue extract protein) of P450$_{17\alpha}$ was low in small antral follicles and was increased by over 4-fold in preovulatory follicles. In contrast, within 7 h after the LH/hCG surge, the content of cytochrome P450$_{17\alpha}$ decreased 10-fold and was essentially undetectable in newly formed corpora lutea (PO + hCG, 24 h) and in corpora lutea isolated from pregnant rats on Day 15 of gestation (Figs. 1 and 2).

The content (per $\mu$g tissue extract protein) of NADPH cytochrome P450 reductase, the microsomal electron donor for cytochrome P450$_{17\alpha}$ and other cytochromes not directly involved in steroidogenesis, did not change in a manner corresponding to that of the P450$_{17\alpha}$. The content of NADPH cytochrome P450 reductase was 2-fold greater in PO and luteinizing follicles (PO + hCG, 7 h) but otherwise remained unchanged from that of SA follicles (Figs. 1 and 2).

The content (per $\mu$g tissue extract protein) of F1-ATPase, a marker for the inner mitochondrial membrane, was the same in all groups of tissue. This protein was analyzed as a control for other mitochondrial enzymes (shown in Figs. 3 and 4).

Enzymes involved in the synthesis of cholesterol and progestins were analyzed to determine if they might also be associated with changes in androgen biosynthesis (Figs. 3 and 4). HMG-CoA is a microsomal enzyme that regulates a key step in cholesterol biosynthesis (Strauss et al., 1981) and is inhibited by elevated serum and intracellular levels of cholesterol (Brown and Goldstein, 1980). HMG-CoA reductase was present in SA and PO follicles, increased 4.5-fold in luteinizing follicles 7 and 24 h after the LH/hCG surge, and declined in corpora lutea of Day 15 pregnant rats to a concentration similar to that of SA and PO follicles (Figs. 3 and 4).

The mitochondrial proteins, cholesterol side-chain cleavage cytochrome P450 (P450scc) and its electron donor, adrenodoxin, exhibited different patterns. The content (per $\mu$g tissue extract protein) of cytochrome P450scc was increased above that in SA follicles by 2-, 3-, and 4-fold in PO follicles, newly formed corpora lutea, and Day 15 pregnant corpora lutea, respectively (Figs. 3 and 4). In contrast, the content (per $\mu$g tissue extract protein) of adrenodoxin was 2-fold greater in PO than SA and luteinizing follicles, but was less in corpora lutea.

The intrafollicular localization of P450$_{17\alpha}$ was analyzed by isolating theca and granulosa cells from SA, PO, and luteinizing follicles. Soluble cell extracts were then prepared from these cells and analyzed by immunoblotting. As shown in Figure 5, P450$_{17\alpha}$ was demonstrable only in theca cells of PO follicles, thus verifying the tissue and developmental specificity of androgen biosynthesis in the rat follicle.

### *Content of P450$_{17\alpha}$ mRNA*

To determine if these changes in P450$_{17\alpha}$ enzyme content might be related to changes in the mRNA content of this microsomal protein, total RNA was extracted from theca cells of SA, PO, and luteinizing follicles, as well as from corpora lutea, and was hybridized to a bovine P450$_{17\alpha}$ cDNA probe (Zuber et al., 1986a), as described in Materials and Methods. As shown in Figure 6, cytochrome P450$_{17\alpha}$ mRNA content (as a proportion of total isolated RNA) increased in theca of PO follicles and decreased within 7 h in theca of luteinizing follicles and in corpora lutea.

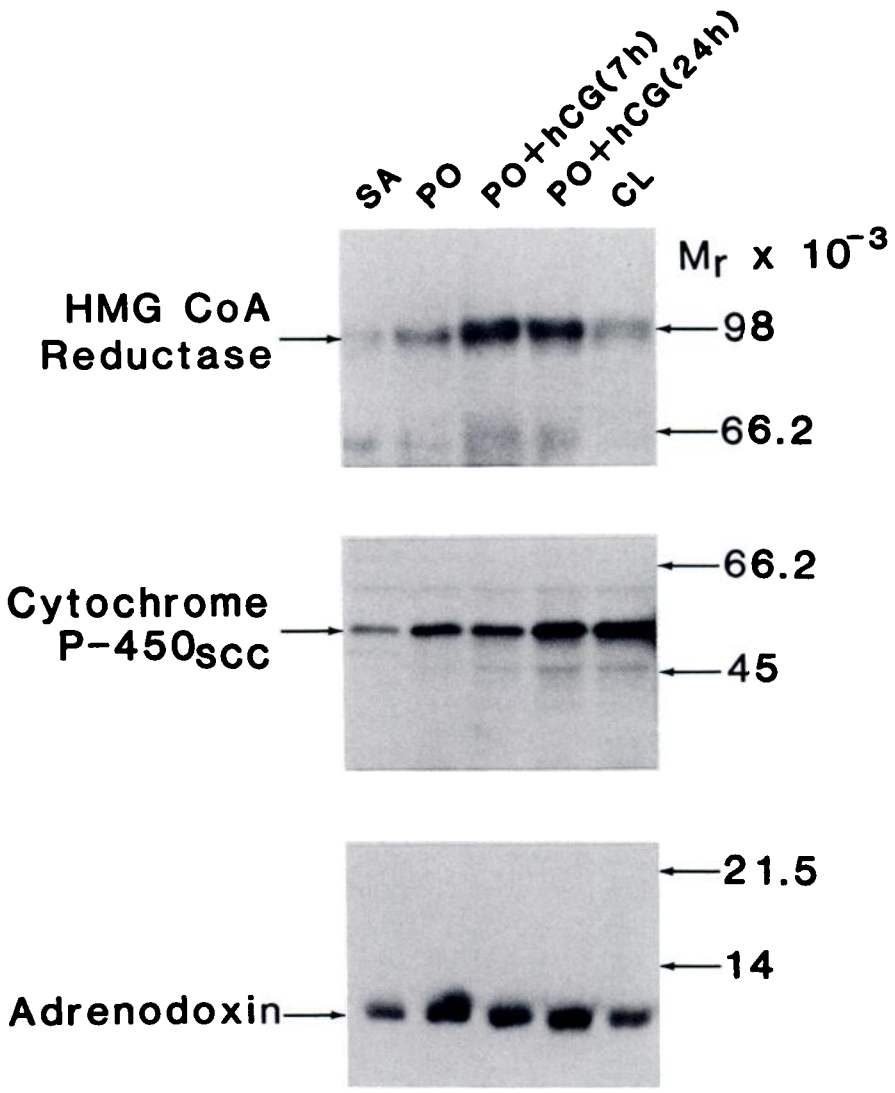


FIG. 3. Autoradiograph of immunoblots of HMG CoA reductase, cytochrome P450scc, and adrenodoxin. (See *legend of Fig. 1* for details.)

*Theca Explants in Culture*

To determine the extent to which LH-stimulated steroid biosynthesis in theca cells of follicles at different stages of development and luteinization might be altered by direct effects of PRL, theca explants of SA, PO, and luteinizing follicles were isolated and cultured for 20 days, as described in Materials and Methods.

*Androstenedione accumulation by theca explant.* The synthesis of androstenedione by SA theca explants was dependent on the presence of LH (Fig. 7) which increased androstenedione concentrations 10–100 times above that observed in theca cultured in media alone. Prolactin alone was ineffective in maintaining androstenedione synthesis by SA theca and by Day 16 appeared inhibitory because androstenedione concentrations were undetectable. PRL at 10 ng/ml partially inhibited the effect of LH in SA theca through Day 12 of culture, but had no effect thereafter (Fig. 7). Doses of prolactin at 100 or 1000 ng/ml caused no further inhibition of the LH response (data not shown).

Similarly, in PO theca, PRL (10–1000 ng/ml) caused a 2- to 4-fold decrease in LH-stimulated androstenedione production. However, this effect was small compared to the 10- to 50-fold increase stimulated by LH. Note also that PO theca (± hormones) produced more androstenedione than SA theca at the initiation of culture (Day 4).

In contrast, PRL at 10, 100, or 1000 ng/ml had no

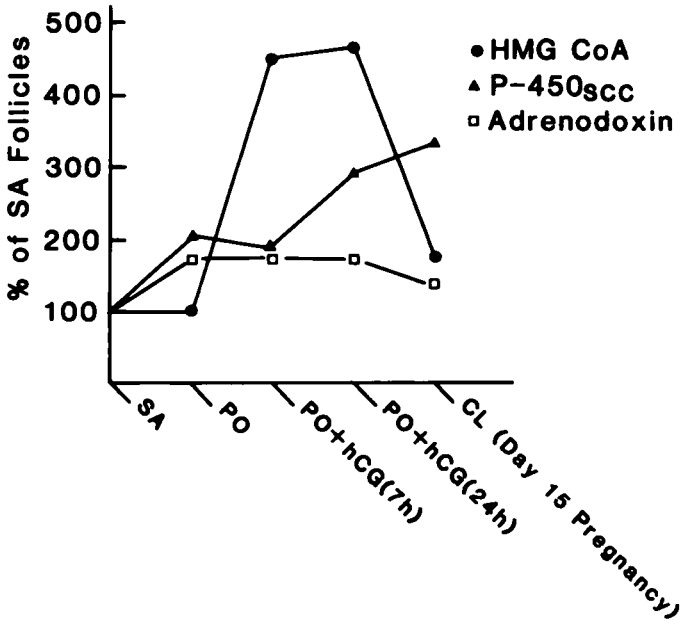


FIG. 4. Quantitation by densitometric scanning of autoradiographs shown in *Figure 3*. For each protein, the content present in SA follicles was set at 100%.

effect on control or LH-stimulated androstenedione production by luteinizing theca. Note that androstenedione production by luteinizing theca was elevated and much less dependent on the presence of LH (Fig. 7).

*Progesterone accumulation by theca explants.* LH was also required to maintain progesterone accumulation by theca of SA and PO follicles (Fig. 8). In contrast to the partial inhibition of LH-stimulated androstenedione synthesis by PRL in SA and PO theca, PRL at 10, 100, or 1000 ng/ml had no effect on LH-stimulated progesterone production by these same explants (Fig. 8). PRL alone did decrease progesterone production in SA and PO theca by Days 10 and 13, respectively, to levels that became undetectable. Conversely, luteinizing theca produced more progesterone than SA or PO theca with or without LH. In addition, PRL acted synergistically with LH to enhance the progesterone synthesis 5- to 10-fold above that observed in theca cultured in media alone, PRL alone, or LH alone.

*Accumulation of cAMP by theca explants.* Despite the pronounced effects of LH on steroidogenesis in theca explants from SA and PO follicles, no demonstrable increase in cAMP was observed in the media in response to LH or LH and prolactin. The small amount of tissue and low amounts of cAMP accumulated, as well as the contribution of cAMP from cell types other than the theca interna, may mask the effect of LH (Richards et al., 1986). The addition of 10 $\mu$M forskolin to the cultures on Day 10 or 13 caused a small but demonstrable increase in cAMP in the media of all groups, including those containing PRL. The increase in cAMP was associated with greater increases in androstenedione production in all groups (Table 2).

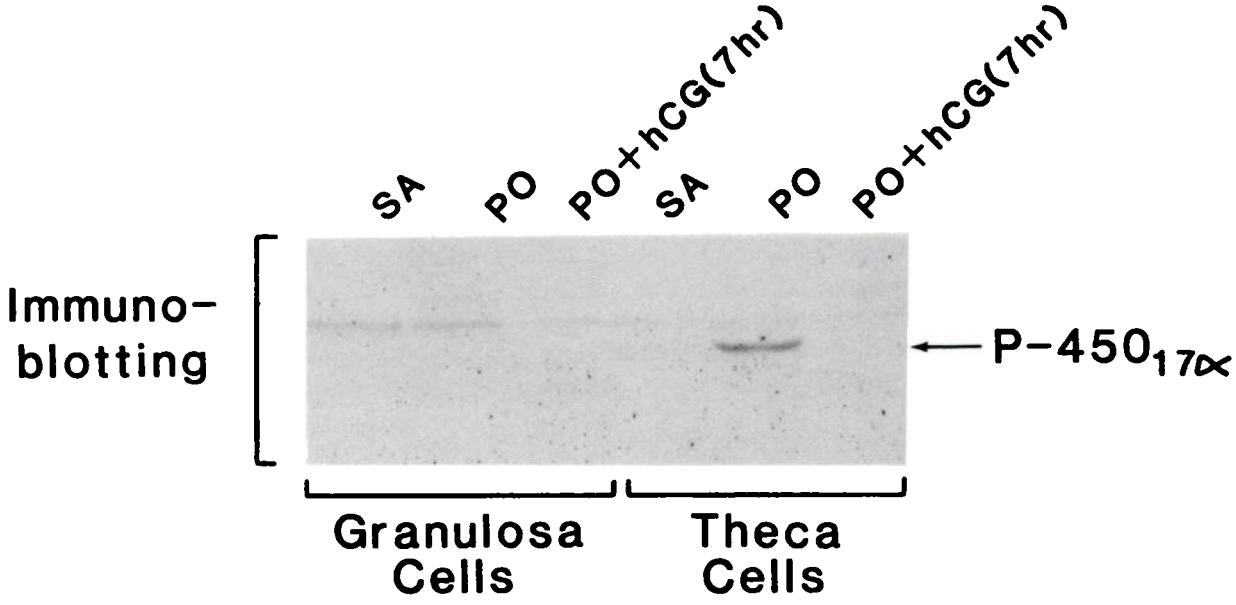


FIG. 5. Autoradiograph of immunoblot of P450$_{17\alpha}$ in theca and granulosa cells of SA, PO, and luteinizing (PO + hCG, 7 h) follicles. Each lane contained 100 $\mu$g of soluble cell extract protein. (See *legend of Fig. 1* for details.)


FIG. 6. P450$_{17\alpha}$ mRNA in theca cells measured by filter hybridization using a $^{32}$P-labeled P450$_{17\alpha}$ cDNA probe. Theca explants from SA, PO, and luteinizing follicles or newly formed corpora lutea were isolated (18 rats per group). The amounts of total RNA extracted from each group was 24.3, 24.3, 35, and 273/μg, respectively. Twenty micrograms of total RNA from each tissue were loaded in each lane. (See *legend of Fig. 1* for details.)

## DISCUSSION

Using an antibody against P450$_{17\alpha}$ (Nakajin and Hall, 1981), we have been able to document in this study that increased androgen biosynthesis by theca cells of PO follicles in vivo (Carson et al., 1981; Bogovich and Richards, 1984) is directly associated with increased content of P450$_{17\alpha}$. Furthermore, using a cDNA probe for P450$_{17\alpha}$ (Zuber et al., 1986a), we have shown that the increase in P450$_{17\alpha}$ enzyme is associated with increased levels of P450$_{17\alpha}$ mRNA. On the basis of these results, it is tempting to speculate that the subtle increases in serum LH and cAMP, which occur during the final stages of PO follicular development, act to stimulate the transcription of the P450$_{17\alpha}$ gene in rat ovarian theca cells. This effect of LH and cAMP on theca cells is analogous to the action of LH in Leydig cells. Studies by Sheela Rani and Payne (1986) and Hales et al. (1986) have shown that cAMP is required to maintain the activity and synthesis of P450$_{17\alpha}$ in mouse Leydig cells in culture. Zuber et al. (1985, 1986a,b) have shown that adrenocoticotropin (ACTH) and cAMP increase the synthesis of P450$_{17\alpha}$ in cultured bovine adrenal cells and that the action of ACTH on P450$_{17\alpha}$ transcription

TABLE 2. Production of cyclic adenosine 3',5'-monophosphate (cAMP) and androstenedione by theca explants.*

| | cAMP, pmole/ml[a] (androstenedione, ng/ml)[b] | | |
|---|---|---|---|
| | Control | LH | LH + PRL |
| SA Theca | | | |
| − Forskolin | 0.41 ± 0.02 (0.08 ± 0.02) | 0.6 ± 0.17 (2.5 ± 0.05) | 0.45 ± 0.08 (3.11 ± 0.3) |
| + Forskolin | 1.44 ± 0.01 (2.96 ± 0.5) | 1.9 ± 0.38 (5.6 ± 0.3) | 1.28 ± 0.02 (5.0 ± 0.8) |
| PO Theca | | | |
| − Forskolin | 0.42 ± 0.03 (0.15 ± 0.05) | 0.34 ± 0.02 (8.5 ± 0.4) | 0.61 ± 0.11 (3.0 ± 0.2) |
| + Forskolin | 1.68 ± 0.20 (14.9 ± 0.2) | 1.91 ± 0.48 (17.2 ± 0.9) | 1.61 ± 0.32 (16.2 ± 0.9) |
| Luteinizing theca | | | |
| − Forskolin | 0.50 ± 0.02 (1.4) | 0.46 ± 0.03 (5.9 ± 0.5) | 0.51 ± 0.16 (6.3 ± 0.4) |
| + Forskolin | 1.08 ± 0.16 (10.7 ± 1.0) | 0.65 ± 0.08 (13.2 ± 0.8) | 1.13 ± 0.23 (9.1 ± 0.3) |

[a]All values for cAMP are shown for Day 16 of culture. cAMP concentrations produced in the absence of forskolin are similar to those measured on Days 4, 7, 10, and 13 for each treatment group (data not shown). Forskolin (10 μM) was added to the media on Days 10 and 13 of culture. Values shown for Day 16 are similar to those observed on Day 13 (data not shown).

[b]Androstenedione from the same cultures is shown in parentheses. Values of androstenedione on other days of culture were similar to those shown in *Figure 7*.

*LH, Luteinizing hormone; PRL, prolactin; SA, small antral follicles; PO, preovulatory follicles.

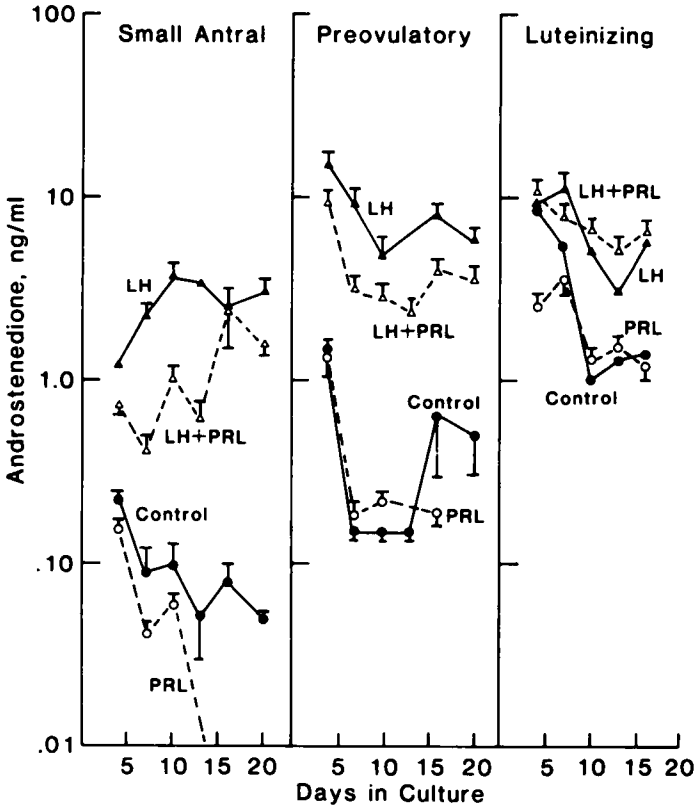


FIG. 7. Androstenedione biosynthesis by theca explants: effects of luteinizing hormone (*LH*) and prolactin (*PRL*). Theca of small antral (*SA*), preovulatory (*PO*), and luteinizing (PO + human chorionic gonadotropin [hCG], 7 h) follicles were isolated. Theca in all treatment groups were cultured individually in Falcon multiwell plates in DMEM: F12 containing 2% fetal bovine serum (FBS) with or without 5 ng/ml oLH or 10 ng/ml oPRL. Media were changed on Day 4 and at 3-day intervals thereafter. Each point represents the mean ± SEM for 8–10 theca. Theca were randomly selected from a large pool of theca collected from follicles isolated from the ovaries of 10–15 animals in each experiment (Richards et al., 1986). Androstenedione was undetectable after Day 13 in theca cultured in the presence of PRL alone. Values of androstenedione in PO theca cultured with PRL alone were not obtained for Day 13 or 20. (Abbreviations defined in *Materials and Methods.*)

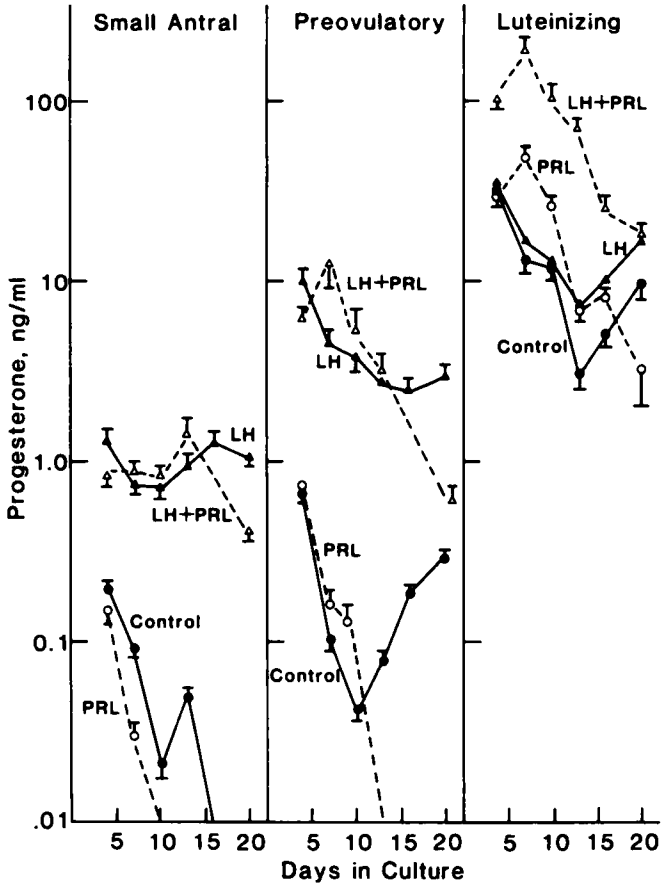


FIG. 8. Progesterone biosynthesis by theca explants: effects of LH and PRL (see *legend of Fig. 7*). Progesterone was undetectable in SA and PO theca after Days 10 and 13, respectively. Progesterone was not measured on Day 16 in PO theca cultured with LH and PRL.

(John et al., 1986) is dependent on protein synthesis. The ability of cAMP to regulate $P450_{17\alpha}$ content in each of these steroidogenic tissues, as well as the ability of cAMP to increase androgen biosynthesis by theca cells in culture, suggests that these tissues may contain a common regulatory pathway, involving the synthesis of a transacting nuclear regulator.

The stimulatory effects of LH and ACTH on gonadal and adrenal $P450_{17\alpha}$, respectively, contrast with the marked decline in androgen biosynthesis, which occurs rapidly in follicles after the LH/hCG surge, precedes ovulation (Fig. 1; PO + hCG, 7 h) and is then maintained during luteinization (Fig. 1). Thus, molecular events set in motion by the LH surge and elevated concentrations of cAMP (Richards et al., 1980) appear to exert different effects on theca cell androgen biosynthesis (Hedin et al., 1983). From the results of this study, the rapid decline in androgen synthesis appears to be associated with a rapid decline (within 7 h after the hCG surge) in the content of $P450_{17\alpha}$ enzyme and its mRNA. These observations, as well as those of Rodgers et al. (1986), indicate that $P450_{17\alpha}$ gene expression is reduced rapidly in luteinizing theca cells in vivo prior to major changes in the amount of total RNA synthesis. The mechanisms by which elevated concentrations of LH and cAMP rapidly reduce $P450_{17\alpha}$ enzyme and mRNA concentrations remain to be determined but may involve de novo RNA and protein synthesis (Suzuki and Tamaoki, 1983) or the actions of specific steroids (Nozu et al., 1981; Hales et al., 1986). Because the amount of total RNA isolated from luteal tissue is increased and because luteal tissue comprises granu-

losa cells, as well as theca cells, the content of P450$_{17\alpha}$ mRNA in luteinized theca could not be accurately determined. Nonetheless, the amount of P450$_{17\alpha}$ mRNA per $\mu$g of total luteal cell RNA appears low.

The decline in androgen biosynthesis and cytochrome P450$_{17\alpha}$ protein content in luteal tissue does not appear to be an irreversible process of differentiation. For example, if theca explants from luteinizing follicles (i.e., those collected 7 h after the LH/hCG surge when cytochrome P450$_{17\alpha}$ protein and mRNA are already reduced) are placed in tissue culture, androstenedione synthesis resumes and is maintained at an elevated rate for at least as long as 20–30 days, even in the absence of LH or agonists that increase cAMP (Fig. 7) (Richards et al., 1986). These results, along with the constitutive synthesis of progesterone in luteinizing theca (Fig. 8) (Richards et al., 1986) suggest that luteinized rat theca in culture are less dependent on cAMP for steroidogenesis or that they produce higher basal concentrations of cAMP in the absence of LH. Furthermore, administration of low doses of hCG to midgestation pregnant rats for 2 days stimulates the accumulation of androgens by corpora lutea during subsequent in vitro incubation (Sridaran et al., 1981), suggesting a potential for androgen biosynthesis in luteinized theca in vivo.

These observations suggest that other hormones or factors that are present in vivo and act to reduce P450$_{17\alpha}$ synthesis and gene expression during early stages of luteinization are absent when theca cells are placed in culture. One hormone that has been shown to rapidly inhibit androgen biosynthesis in collagenase-dispersed ovarian cells isolated from hypophysectomized rats and cultured in serum-free medium is PRL (Magoffin and Erickson, 1982a,b). Using more highly purified preparations of theca cells, we observed that PRL, even at doses as high as 1000 ng/ml, had little or no effect on androgen synthesis by luteinizing theca (Fig. 7). Rather, androgen biosynthesis was maintained in luteinized theca at an elevated level for 20–30 days. PRL did decrease LH-stimulated androgen synthesis by SA and PO theca. However, the effect of PRL in the LH-stimulated cultures was small relative to the marked reduction of androstenedione synthesis that occurred with PRL in the absence of LH (Fig. 7) or in the LH- and PRL-treated ovarian cell cultures of Magoffin and Erickson (1982a,b). Conversely, PRL had no effect on LH-stimulated progesterone synthesis by SA and PO thecal explants and increased progesterone production 5- to 10-fold by luteinizing theca (Fig. 8), whereas PRL suppressed LH-stimulated progestin production in cultures of collagenase-dispersed ovarian cells (Magoffin and Erickson, 1982a,b). Furthermore, we have shown (Table 2) that PRL did not inhibit the stimulation, by forskolin, of androstenedione production or cAMP accumulation in SA, PO, and luteinizing follicles between Days 10 and 16 of culture. Whether or not PRL affected the ability of LH to stimulate cAMP could not be determined, because, unfortunately, the amounts of cAMP released from the small explants were low (Table 2) (Richards et al., 1986). However, since LH is able to maintain progesterone and androstenedione production in the presence of PRL (Figs. 7 and 8), the results indicate that the LH response system itself is largely unaffected by PRL. Taken together, these observations indicate that the effects of prolactin on theca cell steroidogenesis are dependent not only on the type of follicle from which the theca are obtained but also on the presence of other ovarian cell types that may secrete inhibitory factors acting as intermediaries of PRL action.

Changes in the content of other steroidogenic enzymes also occur during follicular growth and luteinization, but the changes are distinct from those of P450$_{17\alpha}$. For example, NADPH cytochrome P450 reductase, the electron donor for P450$_{17\alpha}$ and other microsomal (HMG CoA reductase) and mitochondrial (cytochrome P450scc and adrenodoxin) enzymes all increase in response to the LH/hCG surge. However, whereas the content of cytochrome P450scc continues to increase during luteinization and is highest in corpora lutea of Day 15 pregnant rats, the LH-induced increases in the content of the other enzymes are transient. The transient increase in the content of HMG CoA reductase agrees with changes in activity reported previously (Schuler et al., 1981). Because the content of F1-ATPase remained unchanged in all groups, the increase in the content of cytochrome P450scc represents the increase in P450scc cytochrome protein within mitochondria of luteal cells and not an increase in the size or number of mitochondria per cell.

These changes in content of steroidogenic enzymes are similar to those observed in bovine follicles and corpora luta (Rodgers et al., 1986). However, some differences are also worthy of note. The increase in cytochrome P450$_{17\alpha}$ in preovulatory vs. small antral follicles was 4.5-fold in the rat compared to 0.5-fold

in the bovine. The induction of HMG-CoA reductase, which occurred transiently following the LH/hCG surge in the rat, was higher in the corpus luteum of the cow. The 4- to 5-fold increase in cytochrome P450scc content and the 2-fold increase in adenodoxin in rat luteal tissue was less than the 12-fold and 15-fold increases observed in these enzymes in bovine luteal tissue. F1-ATPase content remained unchanged in rat ovarian tissues, whereas a 3- to 4-fold increase was observed in bovine corpora lutea. These differences suggest that the bovine corpus luteum, compared to the rat corpus luteum, gains an increased number of mitochondria and greater amounts of both cytochrome P450scc and adrenodoxin. This may be required for the production of larger amounts of progesterone necessary to achieve serum concentrations sufficient for specific biological effects in a large mammalian species. Conversely, the greater induction of cytochrome $P450_{17\alpha}$ from small antral to preovulatory follicles in the rat may be associated with a more precise size or functional classification of the rat follicles.

In summary, thecal cell cytochrome $P450_{17\alpha}$ and the regulation of its content and mRNA by LH are pivotal to the biosynthesis of androgens, the obligatory precursors for estradiol biosynthesis and the consequent development of preovulatory follicles. In addition, the dramatic loss of androgen synthesis by luteinizing follicles and corpora lutea in vivo is associated with an LH-induced decrease in the content of cytochrome $P450_{17\alpha}$ and its mRNA. This biophasic regulation of cytochrome $P450_{17\alpha}$ by low vs. elevated concentrations of LH represents a physiological pattern unique to the ovary (compared to the adrenal and testis). Furthermore, the LH-induced loss of cytochrome $P450_{17\alpha}$ in vivo is not retained by luteinizing thecal explants in culture and is not mimicked by the presence of PRL in culture, suggesting that other factors or the interactions of ovarian cell types mediate the LH-induced loss of cytochrome $P450_{17\alpha}$ in vivo. Finally, other microsomal (HMG CoA reductase) and mitochondrial (cytochrome P450scc and adrenodoxin) enzymes appear to increase specifically in association with luteinization (i.e., elevated LH) and either remain elevated (cytochrome P450scc and adrenodoxin) in vivo and in vitro or are increased transiently (HMG CoA reductase).

Thus, cytochrome $P450_{17\alpha}$ and cytochrome P450scc are highly regulated by LH in developing follicles and as a consequence of the LH surge. The molecular basis for the different effects of low vs. elevated concentrations of LH on these two steroidogenic enzymes remains to be determined.

## REFERENCES


Björsing L, 1978. Maturation, morphology and endocrine function of the follicular wall in mammals. In: Jones JE (ed.), The Vertebrate Ovary. New York: Plenum Press, pp. 181–214

Bogovich K, Richards JS, 1982. Androgen biosynthesis in developing ovarian follicles: evidence that luteinizing hormone regulates thecal 17α-hydroxylase and $C_{17\text{-}20}$ lyase activities. Endocrinology 111:1201–08

Bogovich K, Richards JS, 1984. Androgen synthesis during follicular development: evidence that rat granulosa cell 17-keto steroid reductase is independent of hormonal regulation. Biol Reprod 31: 122–31

Bogovich K, Richards JS, Reichert LE Jr, 1981. Obligatory role of luteinizing hormone (LH) in the initiation of preovulatory follicular growth in the pregnant rat: specific effects of human chorionic gonadotropin and follicle stimulating hormone on LH receptors and steroidogenesis in theca, granulosa, and luteal cells. Endocrinology 109:860–67

Bogovich K, Scales LM, Higginbottom E, Ewing LL, Richards JS, 1986. Short term androgen production by rat ovarian follicles and long term steroidogenesis by thecal explants in culture. Endocrinology 118:1379–86

Bradford MM, 1976. A rapid and sensitive method for quantitation of microgram quantities of protein utilizing the principle of protein-due binding. Anal Biochem 72:248–54

Brown MS, Goldstein JL, 1980. Multivalent feedback regulation of HMG CoA reductase, a control mechanism coordinating isoprenoid synthesis and cell growth. J Lipid Res 21:505–17

Carson RS, Kahn LE, Richards JS, 1981. Functional and morphological differentiation of theca and granulosa cells during pregnancy in the rat: dependence on increased basal luteinizing hormone activity. Endocrinology 109:1433–41

Chin DJ, Luskey KL, Anderson RGW, Faust JR, Golstein JL, Brown MS, 1982. Appearance of crystalloid endoplasmic reticulum in compactin-resistant Chinese hamster cells with a 500-fold increase in 3-hydroxy-3-methylglutaryl-coenzyme A reductase. Proc Natl Acad Sci USA 79:1185–89

Chirgwin JM, Przybyla AE, MacDonald RJ, Rutter WJ, 1979. Isolation of biologically active ribonucleic acid from sources enriched in ribonuclease. Biochemistry 18:5294–99

Dubois RN, Simpson ER, Tuckey G, Lambeth JD, Waterman MR, 1981. Evidence for a higher molecular weight precursor of cholesterol side-chain-cleavage cytochrome P450 and induction of mitochondrial and cytosolic proteins by corticotropin in adult bovine adrenal cells. Proc Natl Acad Sci USA 78:1028–32

Eckstein B, Greenbaum O, Cohen S, 1985. Kinetic studies on ovarian C-17,20 lyase activity: effect of luteinizing hormone surge. Endocrinology 117:2376–82

Eckstein B, Tsafriri A, 1986. The steroid C-17,20 lyase complex in isolated Graafian follicles: effects of human chorionic gonadotropin. Endocrinology 118:1266–70

Elbaum DJ, Keyes PL, 1976. Synthesis of 17β-estradiol by isolated ovarian tissues of the pregnant rat: aromatization in the corpus luteum. Endocrinology 99:573–79

Feinberg A, Vogelstein B, 1984. Technique for radiolabeling DNA restriction endonuclease fragments to high specific activity. Anal Biochem 137:266–67

Fortune JE, Armstrong DT, 1977. Androgen production by theca and granulosa isolated from proestrous rat follicles. Endocrinology 100:1341–47

Hales DB, Perkins LM, Payne AH, 1986. cAMP-induced, aminoglutethimide enhanced synthesis of cytochrome P450*17α* in Leydig cell cultures. Biol Reprod 34:140 (Suppl. 1)

Hedin L, Ekholm C, Hillensjö T, 1983. Dose-related effects of lutei-

nizing hormone on the pattern of steroidogenesis and cyclic AMP release in superfused preovulatory rat follicles. Biol Reprod 29: 895–904

Hillensjö T, Bauminger S, Ahren K, 1976. Effect of LH on the pattern of steroid production by preovulatory follicles in PMS-injected immature rats. Endocrinology 99:996–1002

Hjelmeland LM, 1980. A non-denaturing Zwitterionic detergent for membrane biochemistry design and synthesis. Proc Natl Acad Sci USA 77:6368–70

Jahnsen T, Hedin L, Kidd VJ, Beattie WG, Lohmann SM, Walter U, Durica J, Schulz TZ, Schiltz E, Browner M, Lawrence CB, Goldman D, Ratoosh SL, Richards JS, 1986. Molecular cloning, cDNA structure and regulation of the regulatory subunit of type II cAMP-dependent protein kinase from rat ovarian granulosa cells. J Biol Chem 261:12352–61

John ME, John MC, Boggaram V, Simpson ER, Waterman MR, 1986. Transcriptional regulation of steroid hydroxylase genes by corticotropin. Proc Natl Acad Sci USA 83:4715–19

Johnson DC, Griswald T, 1984. Ovarian $C_{17-20}$ lyase: changes in intact and hypophysectomized immature rats treated with pregnant mare's serum gonadotropin. J Steroid Biochem 20:733–40

Karsch FJ, 1980. Seasonal reproduction: a saga of reversible fertility. Physiologist 23:29–38

Kominami S, Shinzawa K, Takemori S, 1982. Purifiation and some properties of cytochrome P450 specific for 17α hydroxylation and $C_{17-20}$ bond cleavage from guinea pig adrenal microsomes. Biochim Biophys Res Commun 109:916–20

Linkie DM, Niswender GD, 1972. Serum levels of prolactin, luteinizing hormone and follicle-stimulating hormone during pregnancy in the rat. Endocrinology 90:632–37

Lowry OH, Rosebrough NJ, Farr AL, Randall RJ, 1951. Protein measurement with the Folin phenol reagent. J Biol Chem 193:265–69

Magoffin DA, Erickson GF, 1982a. Primary culture of differentiating ovarian androgen-producing cells in defined medium. J Biol Chem 257:4507–13

Magoffin DA, Erickson GF, 1982b. Prolactin inhibition of luteinizing hormone-stimulated androgen synthesis in ovarian interstitial cells cultured in defined medium: mechanism of action. Endocrinology 111:2001–07

Nakajin S, Hall PF, 1981. Microsomal P450 from neonatal pig testis. Purification and properties of a C21 steroid side-chain cleavage system (17α hydroxylase-$C_{17-20}$ lyase). J Biol Chem 256:3871–76

Nozu K, Matsuura S, Catt KJ, Dufau ML, 1981. Modulation of Leydig cell androgen biosynthesis and cytochrome P450 levels during estrogen treatment and human chorionic gonadotropin-induced densensitization. J Biol Chem 256:10012–17

Quinn PG, Payne AH, 1984. Oxygen-mediated damage of microsomal P450 enzymes in cultured Leydig cells. J Biol Chem 259:4130–35

Quinn PG, Payne AH, 1985. Steroid product induced, oxygen-mediated damage of microsomal cytochrome P450 enzymes in Leydig cell cultures. J Biol Chem 260:2092–99

Richards JS, 1980. Maturation of ovarian follicles: actions and interactions of pituitary and ovarian hormones on follicular cell differentiation. Physiol Rev 60:51–89

Richards JS, Bogovich K, 1982. Effects of human chorionic gonadotropin and progesterone on follicular development in the immature rat. Endocrinology 111:1429–38

Richards JS, Haddoz M, Tash JS, Walter U, Lohmann SM, 1984. Adenosine 3′,5′-monophosphate-dependent protein kinase and granulosa cell responsiveness to gonadotropins. Endocrinology 114:2190–98

Richards JS, Hedin L, Caston L, 1986. Differentiation of rat ovarian theca cells: evidence for functional luteinization. Endocrinology 118:1660–68

Richards JS, Jonassen JA, Kersey KA, 1980. Evidence that changes in tonic LH secretion determine the growth of preovulatory follicles in the rat. Endocrinology 107:641–48

Richards JS, Jonassen JA, Rolfes AI, Kersey KA, Reichert LE Jr, 1979. Adenosine 3′,5′-monophosphate, luteinizing hormone receptor, and progesterone during granulosa cell differentiation: effects of estradiol and follicle stimulating hormone. Endocrinology 104: 765–73

Richards JS, Kersey KA, 1979. Changes in theca and granulosa cell function in antral follicles developing during pregnancy in the rat: gonadotropin receptors, cyclic AMP and estradiol-17β. Biol Reprod 21:1185–1201

Rodgers RJ, Waterman MR, Simpson ER, 1986. Cytochrome P450scc, P450*17α*, adrenodoxin and reduced nicotinamide adenine dinucleotide phosphate cytochrome P450 reductase in bovine follicles and corpora lutea. Changes in specific contents during the ovarian cycle. Endocrinology 118:1366–74

Schuler LA, Toaff ME, Strauss III JR, 1981. Regulation of ovarian cholesterol metabolism: control of 3-hydroxy-3-methylglutaryl coenzyme A reductase and acyl coenzyme A: cholesterol acyl transferase. Endocrinology 108:1476–82

Sheela Rani CS, Payne AH, 1986 Adenosine 3′,5′-monophosphate-mediated induction of 17α-hydroxylase and $C_{17-20}$ lyase activities in cultured mouse Leydig cells in enhanced by inhibition of steroid biosynthesis. Endocrinology 118:1222–28

Smith MS, Freeman ME, Neill JD, 1975. The control of progesterone secretion during the estrous cycle and early pregnancy in the rat: prolactin, gonadotropin and steroid levels associated with rescue of the corpus luteum of pregnancy. Endocrinology 96:219–26

Sridaran R, Basuray R, Gibori G, 1981. Source and regulation of testosterone secretion in pregnant and pseudo-pregnant rats. Endocrinology 108:855–61

Stiggall Golante YM, Hatefi Y, 1977. Preparation and properties of an ATP-Pi exchange complex (complex V) from bovine heart mitochondria. J Biol Chem 253:956–64

Strauss III JF, Schuler LA, Rosenblum MF, Tankaka T, 1981. Cholesterol metabolism by ovarian tissue. Adv Lipid Res 18:99–157

Suzuki K, Tamaoki B-I, 1980. Postovulatory decrease in estrogen production is caused by the diminished supply of aromatizable androgen to ovarian aromatase. Endocrinology 107:2115–16

Suzuki K, Tamaoki B-I, 1983. Acute decrease by human chorionic gonadotropin of the activity of preovulatory ovarian 17α-hydroxylase and $C_{17-20}$ lyase is due to decrease of microsomal cytochrome P450 through de novo synthesis of ribonucleic acid and protein. Endocrinology 113:1985–91

Towbin H, Staehelin T, Gordon J, 1979. Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications. Proc Natl Acad Sci USA 76:4350–61

Tsai-Morris C-H, Knox G, Luna S, Dufau ML, 1986. Acquisition of estradiol-mediated regulatory mechanism of steroidogenesis in cultured fetal rat Leydig cells. J Biol Chem 261:3471–74

Uilenbroek JThJ, 1985. Effect of LH on progesterone and estradiol production in vivo and in vitro by preovulatory rat follicles. J Reprod Fertil 74:303–10

Uilenbroek JThJ, Richards JS, 1979. Ovarian follicular development during the rat estrous cycle: gonadotropin receptors and follicular responsiveness. Biol Reprod 20:1159–65

Zuber MX, John ME, Okamura T, Simpson ER, Waterman MR, 1986a. Bovine adrenal cytochrome P450*17α*: regulation of gene expression by ACTH and elucidation of primary sequence. J Biol Chem 261:2475–82

Zuber MX, Simpson ER, Hall PF, Waterman MR, 1985. Effects of adrenocorticotropin on 17α synthesis in bovine adrenocortical cells. J Biol Chem 260:1842–48

Zuber MX, Simpson ER, Waterman MR, 1986b. Expression of bovine 17α-hydroxylase cytochrome P450 cDNA in non-steroidogenic (COS1) cells. Science 234:1258–61