Modulation of Leydig Cell Androgen Biosynthesis and Cytochrome P-450 Levels during Estrogen Treatment and Human Chorionic Gonadotropin-induced Desensitization*

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The mechanisms responsible for gonadotropin-induced testicular desensitization were analyzed by direct assay of androgen biosynthetic enzymes and cytochrome P-450 in microsomes and cytosol from testes of adult male rats. Animals were treated with single subcutaneous doses of human chorionic gonadotropin (hCG) (2 and 10 μg) to induce a post-stimulation decrease in testosterone production, previously shown to result from reduced conversion of progesterone to androgen. In addition, the larger dose of hCG caused an earlier steroidogenic defect due to impaired formation of pregnenolone from endogenous precursors. Dose-dependent inhibition of both 17α-hydroxylase and 17,20-desmolase activities by 30% and 90% was observed after treatment with 2 and 10 μg of hCG, respectively. In contrast, hCG treatment caused no change in the activities of 3β-hydroxysteroid dehydrogenase and 17β-hydroxysteroid dehydrogenase. The reductions in microsomal enzyme activity were accompanied by a comparable decrease in cytochrome P-450 levels. Similar correlations between the microsomal cytochrome P-450 content and the same enzymatic activities were observed after hypophysectomy. Treatment of hypophysectomized rats with 17β-estradiol (0.1 to 20 μg) reduced testosterone responses to hCG in a dose-dependent manner (by 20 to 60%), and caused further decreases of microsomal enzymatic activities and of the levels of cytochrome P-450, but not of 3β-hydroxy- and 17β-hydroxysteroid dehydrogenases. The dependence of 17α-hydroxylase and 17,20-desmolase on cytochrome P-450 levels was indicated by the constancy of specific activity of the microsomal enzymes when expressed in terms of cytochrome P-450. The similarity of estrogen-dependent lesions to those produced by hCG treatment further indicates the involvement of endogenous estrogen in the development of the microsomal enzymatic lesions in gonadotropin-induced desensitization of testicular androgen production.

The maintenance of Leydig cell function and androgen secretion is dependent upon luteinizing hormone, which is released from the pituitary gland in a pulsatile manner and circulates in relatively low concentrations (about 10^-10 M). Intermittent stimulation by LH1 is responsible for supporting the differentiated function of the Leydig cell, and induction and/or maintenance of LH receptors and steroidogenic enzymes occurs with near-normal or slightly increased levels of gonadotropin (1, 2). Further increases in circulating hormone levels, from either endogenous or exogenous sources, causes negative regulation of testicular LH receptors and Leydig cell responses (3-5). Although elevations of circulating gonadotropin initially stimulate testosterone secretion by testicular Leydig cells, the steroidogenic response to LH or hCG is followed by a period during which the Leydig cells are desensitized (or refractory) to subsequent stimulation with LH/hCG in vitro (3-6). Gonadotropin-induced loss or down-regulation of LH receptors occurs at a later stage and is preceded by a "late" steroidogenic lesion of the androgen biosynthetic pathway (after pregnenolone formation) following moderate elevations of gonadotropin, and by an additional "early" lesion in steroidogenesis (before pregnenolone formation) after marked elevations of gonadotropin (5-9).

We have recently suggested that the gonadotropin-induced late defect in testosterone production is predominantly due to decreased 17α-hydroxylase and 17,20-desmolase activities, and that these changes are due to an inhibitory effect of intracellular estrogen formed from androgen during the initial elevation of testosterone secretion during hormone action (5-10). The site of the early defect has been localized as prior to pregnenolone formation (5, 8), but the nature of the lesion(s) has not yet been defined. This early lesion is not estrogen-dependent, and precedes the marked down-regulation of receptors (6-8). Although estrogen inhibition of testosterone secretion is due in part to negative feedback on pituitary LH secretion, direct inhibitory effects of estradiol on the tests have been observed in hypophysectomized rats (11-15). The estradiol content of Leydig cells is markedly increased after a single dose of hCG (7), and such elevations are followed by nuclear translocation of the estrogen receptor (8). The occurrence of such changes before the steroidogenic lesion suggests that estrogen could be responsible for the enzymatic lesions of the androgen biosynthetic pathway. Furthermore, the 17α-hydroxylase/17,20-desmolase lesions caused by hCG treatment have been shown to be prevented by the anti-estrogen, Tamoxifen (7-9). The present studies were conducted to analyze the enzyme activities of microsomal enzymes (17α-hydroxylase and 17,20-desmolase) in Leydig cells with selective late steroidogenic lesions induced by hCG, and to explore the dependence of such lesions upon the microsomal levels of cytochrome P-450. The similarity of these lesions to those produced by exogenous estrogen indicates the importance of human chorionic gonadotropin; HPLC, high pressure liquid chromatography.
testicular estrogen in the development of steroidogenic defects in hormone-stimulated Leydig cells.

MATERIALS AND METHODS

Treatment of Animals and Leydig Cell Preparation—Adult male rats (200 to 250 g) were obtained from Charles River Laboratories. Gonadotropin-induced desensitization of Leydig cells was elicited by subcutaneous injection of hCG (Pregnyl, Organon) of which 10 IU was equivalent to 1 µg of purified hCG in 250 µl of phosphate-buffered saline, pH 7.4. Animals were killed by decapitation 2 days after hCG treatment unless otherwise indicated. Hypophysectomized rats (50 days of age) and intact control rats were purchased from Hormone Assay Laboratories (Chicago, IL), and hormone treatments were initiated 3 days after surgery. Subcutaneous injections were performed for 3 days with selected doses of estradiol-17β (0.01 to 20 pg) dissolved in 200 µl of sesame oil, or with hCG (1 µg). Animals were killed by decapitation 24 h after the last injection. In some experiments, testicular Leydig cells were prepared by incubation of the decapsulated gonads with collagenase as previously described (16). The isolated interstitial cells were incubated at 34°C for 3 h with 100 ng of hCG in the presence of MIX for measurement of cAMP and testosterone production. When progesterone production was to be measured, its further metabolism was inhibited by 8% by incubation of Leydig cells with cyanoacetone (10-5 M) and amilorol (10-5 M). Leydig cell number was determined by counting at least 500 cells in a chamber after hematoxylin staining for As-3P-hydroxysteroid dehydrogenase (17). LH/hCG receptors were determined by binding analysis with 3H-hCG, prepared by enzymatic radioiodination as previously described (18).

Preparation of Microsomes—After decapsulation, the testes were dispersed with a 1:10 glass homogenizer in 5 volumes of 0.25 M sucrose solution containing 20 mM Tris-HCl (pH 7.4). The homogenates were centrifuged at 9,000 g in a Sorvall refrigerated centrifuge, and the supernatants were subsequently sedimented for 1 h at 105,000 × g in a Beckman ultracentrifuge model L5-75. The pelleted microsomes were washed twice with 0.25 M sucrose, 20 mM Tris-HCl buffer (pH 7.4) containing 1 mM EDTA and 150 mM KCl and resuspended in 250 mM potassium phosphate buffer (pH 7.4) containing 30% glycerol. Protein was determined by the method of Lowry et al. (19) using bovine serum albumin as standard.

Measurement of Cytochrome P-450—Quantitation of cytochrome P-450 was performed by analysis of the carbon monoxide absorption spectrum (20). Microsomes were suspended in 250 mM potassium phosphate buffer (pH 7.4) containing 0.2% Emulgen 911 (Kao-Atras, Tokyo, Japan) and 20% glycerol (2.5 mg of protein/ml), and were equally divided between two cuvettes having an optical path of 1 cm. One cuvette was slowly bubbled with CO for 10 s, and the other with N2. The CO-absorption spectrum was recorded 1 min after addition of a few grains of sodium dithionite, in an Aminco DW-2 recording spectrophotometer equipped with a high intensity light source. The concentration of cytochrome P-450 was estimated using an extinction coefficient of 900 M⁻¹ cm⁻¹ for the absorption difference between 450 nm and 490 nm. In all cases, the CO-450 band was converted back to CO-420 band, representing formation of a breakdown product of the cytochrome P-450. For total concentration of cytochrome P-450, therefore, the amount of cytochrome P-420 determined using an extinction coefficient of 110 M⁻¹ cm⁻¹ for the change between 420 and 490 nm was added to the concentration of cytochrome P-450. Reaction rates were linear with time and protein concentration during the incubation period. During measurement of cytochrome P-450, the breakdown of cytochrome P-450 in rat tests usually occurred immediately after the addition of sodium dithionite. This indicated that such conversion was not due to contamination with oxyhemoglobin which would be converted to carbon monoxide hemoglobin with increase in absorbance at about 420 nm (21). The content of cytochrome P-420 represented 20 to 40% of the total content of reduced CO-spectra 1 min after the addition of sodium dithionite. The initial extent of cytochrome P-450 obtained by the sum of the contents of cytochrome P-420 and P-450 was in agreement with that measured in the presence of NADPH, which prevented breakdown of the P-450 enzyme (22).

Enzyme Assays—The microsomes (3 to 4 mg of protein) were incubated with selected 14C-labeled steroids (150 nmol) and cofactors (3 nmol) in 1 ml of Krebs-Ringer bicarbonate buffer (pH 7.4) at 37°C for 30 min. Rates of reaction were linear with time and protein concentration during time period. For 3β-hydroxysteroid dehydrogenase, the reactions used were 34,45 isomerase. [4, 14C]pregnenolone and NAD+ were used as substrate and cofactor. [4, 14C]Progesterone, 17α-[4, 14C]hydroxyprogesterone, and 14,17β-androstenedione were incubated in the presence of NADPH for assay of 17α-hydroxylase, 17,20-desmolase, and 17β-hydroxysteroid dehydrogenase activities, respectively. The reaction was terminated by the addition of 2 ml of methylene chloride, and the metabolites were separated by HPLC using Partisil-10 column as described below, or by silica gel thin layer chromatography.

High Pressure Liquid Chromatography—Steroid analysis were performed on a Hewlett Packard 108413 liquid chromatography system equipped with UV detector; peak areas and retention times were determined with a Hewlett Packard 7890BLC laboratory data system. A Partisil PXS 10 column, 25 cm × 4.6 mm, (Whatman, Clifton, NJ) operated at ambient temperature was used to separate steroids, which were eluted with methylene chloride/acetonitrile/isopropanol alcohol (86:9:2) at a flow rate of 1 ml/min. UV absorbance was monitored at 240 nm. Fig. 1 shows a typical HPLC chromatogram of the separation of mixed standards of progesterone, 17α-hydroxyprogesterone, androstenedione, and testosterone. Retention times and peak areas were highly reproducible.

Thin Layer Chromatography—After aliquots of the extract were taken for HPLC analysis, the remainder of the extract was analyzed by TLC on Whatman LK5DF silica gel plates. The samples were mixed with aliquots of a standard mixture of progesterone, 17α-hydroxyprogesterone, androstenedione, and testosterone, and chromatographed on the plates with benzene/acetonitrile (85:15). After development, the positions of standard steroids were marked under UV light, and the radioactive metabolites were detected by autoradiography using X-ray films (Kodak XR), which were then developed in a Kodak X-omat rapid developer. The labeled steroids in each radioactive band were extracted from the silica gel with methanol, and crystallized to homogeneity following reverse isotope dilution to constant specific activity.

HPLC PROFILE OF MIXED STANDARD STEROIDS

<table>
<thead>
<tr>
<th>COLUMN: PARTISIL-10</th>
<th>SOLVENT:</th>
<th>CH3CL2:CH3CN:PROPANOL</th>
<th>88:10:2</th>
</tr>
</thead>
<tbody>
<tr>
<td>PROGESTERONE (P)</td>
<td>ANDROSTENEDIONE (A)</td>
<td>FLOW RATE:</td>
<td>1 ml/min</td>
</tr>
<tr>
<td>TESTOSTERONE (T)</td>
<td>17α-OH-PROGESTERONE (17βP)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

FIG. 1. High pressure liquid chromatography profile of standard steroids. Mixed standards of progesterone, 17α-hydroxyprogesterone, androstenedione, and testosterone were resolved on a Partisil-10 column by isocratic elution with CH3Cl2/CH3CN/propanol (88:10:2). UV absorbance was monitored at 240 nm.
RESULTS

Steroidogenesis in Gonadotropin-desensitized Leydig Cells—Leydig cells from animals treated with low doses of hCG (up to 1 μg), showed no change in maximal testosterone production in vitro, although LH/hCG receptors were reduced by 50%. The production of pregnenolone, measured in the presence of enzyme inhibitors to prevent its further metabolism, was markedly increased by the 1-μg dose of hCG. Cells from animals treated with 2.5 μg of hCG showed about 40% reduction of testosterone responses to hCG stimulation in vitro, with accumulation of both progesterone and 17α-progesterone, but with no significant change in pregnenolone formation (Fig. 2). These results suggested that the late steroidogenic lesions are also present in the Leydig cells of animals treated with the lower dose of hCG (1 μg). Such cells were able to maintain a normal testosterone response to hCG stimulation in vitro, but only with the accumulation of steroid intermediates such as 17α-hydroxyprogesterone and progesterone. Higher doses of hCG (5 to 10 μg) caused marked impairment of pregnenolone formation, with consequent reduction in the testosterone response, indicating the presence of an additional lesion prior to pregnenolone formation.

Steroid Synthesis in Testicular Microsomes of Gonadotropin-desensitized Rats—When testicular microsomes of intact control rats were incubated with [4-14C]progesterone, 17α-hydroxyprogesterone was the major metabolite and only small quantities of androstenedione and testosterone were found. In microsomes from testes of rats treated with 2 μg of hCG, partial inhibition of the conversion of progesterone to 17α-hydroxyprogesterone was observed (Fig. 3, left); such inhibition was more marked in animals treated with 10 μg of hCG. An early absorbance peak that eluted before progesterone was present in the HPLC profile of all samples, and was not accompanied by radioactivity. The conversion of 17α-hydroxyprogesterone to androstenedione and testosterone was also markedly reduced in microsomes from animals treated with 2 and 10 μg of hCG (Fig. 3, middle). In contrast, the conversion of androstenedione to testosterone was not reduced.

FIG. 2. Effect of hCG treatment on LH receptor content and steroidogenic capacity of the Leydig cell. Cells prepared from testes of groups of six animals killed 48 h after subcutaneous injection of 1 to 10 μg of hCG were incubated in the absence or presence of 100 ng of hCG, a supramaximal steroidogenic dose. The hCG receptor content determined in each experimental group was expressed as the number of sites per Leydig cell. Data are the mean ± S.D. from triplicate incubation.

FIG. 3. High pressure liquid chromatography of steroids synthesized from radioactive precursors by microsomes of gonadotropin-desensitized testis. Microsomes prepared from testes of normal and hCG-treated animals (2 and 10 μg) were incubated with [4-14C]progesterone (left); 17α-[4-14C]hydroxyprogesterone (center); and [4-14C]androstenedione (right) (150 nmol) and cofactors at 37 °C for 30 min as described under "Materials and Methods." The metabolites formed during incubation were extracted and analyzed by HPLC. Fractions containing 0.5 ml of effluent were collected and the radioactivity of metabolites was measured (---). Absorbance of the Δ5 steroids was monitored at 240 nm (—). The positions of the standard steroids are indicated by arrows. P, progesterone; A, androstenedione; 17P, 17α-140H-progesterone; T, testosterone.

FIG. 4. Microsomal steroidogenic enzyme activities from control and gonadotropin-desensitized Leydig cells. Data are the mean ± S.D. of enzyme assays from triplicate incubations. 3β-hydroxysteroid dehydrogenase, 3β-OH DHG; 17α-hydroxyprogesterone, 17α-14OH-DHG; 17α-hydroxylase, 17α-OHase; 17-20-DSm; 17β-hydroxy steroid dehydrogenase, 17β-OH DHG; Δ5-pregnenolone, Δ5P; Δ4-progesterone, Δ4P; 17α-hydroxyprogesterone: 17P; androstenedione, A; testosterone, T.
Hormonal Modulation of Microsomal Steroidogenesis

DECREASE OF MICROSMAL CYTOCHROME P-450 IN GONADOTROPIN-DESENSITIZED TESTES

Fig. 5. Measurement of microsomal cytochrome P-450 in control and gonadotropin-desensitized testes. The CO-difference spectrum of microsomal suspension from testes of control and animals treated with a single subcutaneous dose of hCG (2 and 10 μg) was determined. The microsomal fraction was divided between two cuvettes and the CO-difference spectrum was recorded 1 min after addition of sodium dithionite.

Table I

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Cytochrome P-450</th>
<th>17α-Hydroxylase</th>
<th>C17-20-desmolase</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.126 (100)</td>
<td>26.9 ± 1.8</td>
<td>15.8 ± 1.1</td>
</tr>
<tr>
<td>2 μg hCG</td>
<td>0.064 (50.8)</td>
<td>13.8 ± 0.6</td>
<td>8.4 ± 0.5</td>
</tr>
<tr>
<td>10 μg hCG</td>
<td>0.027 (21.4)</td>
<td>5.4 ± 0.3</td>
<td>4.5 ± 0.3</td>
</tr>
</tbody>
</table>

*Numbers in parentheses equal % of control.

Fig. 6. Effect of estradiol treatment upon cyclic nucleotide production (left) and steroidogenesis (right) in Leydig cells prepared from hypophysectomized animals treated with estradiol (0.01 to 20 μg) as described under "Materials and Methods". Leydig cells were isolated from testes of control and estrogen-treated animals and incubated in the absence or presence of 100 ng of hCG. Data are the mean ± S.D. from quadruplicate incubations.
The enzymatic changes and reduction of cytochrome P-450 corresponding to those observed during hCG-induced desensitization. Levels brought about by estradiol treatment were very similar or its metabolites caused defects in 17α-hydroxylase and 17α,20-desmolase activities in testicular microsomes, with a corresponding reduction of cytochrome P-450 levels in testicular desmolase activities in testicular microsomes, with a correspondence to normal levels. These results demonstrate that estradiol administered decreases were dose-related, and no changes were noted in pregnenolone formation. The effects of estradiol treatment were similar to those observed after administration of low doses of hCG (2.5 μg), which caused the late steroidogenic lesion distal to progesterone (Fig. 2). No changes were observed in cyclic AMP production by Leydig cells from estradiol-treated animals (Fig. 6).

In order to localize the nature of the steroidogenic lesion caused by estradiol treatment, the levels of microsomal cytochrome P-450 and enzyme activities directly related to androgen synthesis were measured. The cytochrome P-450 content of microsomes of hypophysectomized animals was about one-tenth that of intact rats, and was restored to the intact level by treatment with hCG for 3 days. Estradiol treatment caused further reduction of cytochrome P-450 levels in testicular microsomes, and the activities of 17α-hydroxylase and 17,20-desmolase were also reduced below the levels observed in hypophysectomized controls. The latter changes correlated well with the decreases of cytochrome P-450. The enzymatic activities of 3β-hydroxysteroid dehydrogenase and 17β-hydroxysteroid dehydrogenase were not affected by estradiol treatment, although both activities were decreased by 40 to 50% by hypophysectomy Table II. Treatment of hypophysectomized rats with 1 μg of hCG for 3 days caused recovery of all enzyme activities except 17β-hydroxysteroid dehydrogenase to normal levels. These results demonstrate that estradiol or its metabolites caused defects in 17α-hydroxylase and 17,20-desmolase activities in testicular microsomes, with a corresponding reduction in microsomal cytochrome P-450 content.

The enzymatic changes and reduction of cytochrome P-450 levels brought about by estradiol treatment were very similar to those observed during hCG-induced desensitization.

Table II

Effect of estradiol and hCG on microsomal cytochrome P-450 and steroidogenic enzymes in hypophysectomized rats

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Cytochrome P-450</th>
<th>3β-OH Dehydrogenase</th>
<th>17α-Hydroxylase</th>
<th>C17,20-Desmolase</th>
<th>17β-OH Dehydrogenase</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (Normal)</td>
<td>0.198*</td>
<td>97.3 ± 8.1</td>
<td>24.4 ± 1.8</td>
<td>13.8 ± 1.2</td>
<td>4.5 ± 0.6</td>
</tr>
<tr>
<td>Hypox</td>
<td>0.022</td>
<td>50.5 ± 4.3</td>
<td>2.5 ± 0.2</td>
<td>1.5 ± 0.2</td>
<td>2.5 ± 0.4</td>
</tr>
<tr>
<td>Hypox*</td>
<td>0.009</td>
<td>50.3 ± 3.6</td>
<td>1.1 ± 0.1</td>
<td>0.7 ± 0.1</td>
<td>2.1 ± 0.4</td>
</tr>
<tr>
<td>-1 μg estradiol</td>
<td>0.007</td>
<td>55.6 ± 4.1</td>
<td>0.8 ± 0.1</td>
<td>0.5 ± 0.1</td>
<td>2.1 ± 0.3</td>
</tr>
<tr>
<td>Hypox</td>
<td>-20 μg estradiol</td>
<td>124.3 ± 9.5</td>
<td>22.6 ± 1.7</td>
<td>15.4 ± 0.9</td>
<td>2.8 ± 0.5</td>
</tr>
<tr>
<td>Hypox</td>
<td>-1 μg hCG</td>
<td>124.3 ± 9.5</td>
<td>22.6 ± 1.7</td>
<td>15.4 ± 0.9</td>
<td>2.8 ± 0.5</td>
</tr>
</tbody>
</table>

* Mean of closely agreeing duplicates.
* Treatment 3 days after hypophysectomy (Hypox); mean ± S.D.

450 and P-420 levels. The concentrations of cytochrome P-450 that were calculated are summarized in Table I, and are compared the enzymatic activities of 17α-hydroxylase and 17,20-desmolase. The decrease in cytochrome P-450 level caused by treatment with gonadotropin was correlated with the reduction of both enzyme activities, suggesting that the decrease of cytochrome P-450 were responsible for the 17α-hydroxylase/17,20-desmolase lesion in Leydig cells induced by gonadotropin desensitization.

Effect of Estradiol Treatment upon Steroidogenesis in Hypophysectomized Rats—Administration of estradiol to hypophysectomized rats caused a marked reduction in the testosterone response of Leydig cells to hCG in vitro. The observed decreases were dose-related, and no changes were noted in pregnenolone formation. The effects of estradiol treatment were similar to those observed after administration of low doses of hCG (2.5 μg), which caused the late steroidogenic lesion distal to progesterone (Fig. 2). No changes were observed in cyclic AMP production by Leydig cells from estradiol-treated animals (Fig. 6).

In order to localize the nature of the steroidogenic lesion caused by estradiol treatment, the levels of microsomal cytochrome P-450 and enzyme activities directly related to androgen synthesis were measured. The cytochrome P-450 content of microsomes of hypophysectomized animals was about one-tenth that of intact rats, and was restored to the intact level by treatment with hCG for 3 days. Estradiol treatment caused further reduction of cytochrome P-450 levels in testicular microsomes, and the activities of 17α-hydroxylase and 17,20-desmolase were also reduced below the levels observed in hypophysectomized controls. The latter changes correlated well with the decreases of cytochrome P-450. The enzymatic activities of 3β-hydroxysteroid dehydrogenase and 17β-hydroxysteroid dehydrogenase were not affected by estradiol treatment, although both activities were decreased by 40 to 50% by hypophysectomy Table II. Treatment of hypophysectomized rats with 1 μg of hCG for 3 days caused recovery of all enzyme activities except 17β-hydroxysteroid dehydrogenase to normal levels. These results demonstrate that estradiol or its metabolites caused defects in 17α-hydroxylase and 17,20-desmolase activities in testicular microsomes, with a corresponding reduction in microsomal cytochrome P-450 content.

The enzymatic changes and reduction of cytochrome P-450 levels brought about by estradiol treatment were very similar to those observed during hCG-induced desensitization.

Discussion

Direct assay of microsomal enzymes in the androgen biosynthetic pathway of castrated hCG-treated rats has shown that the accumulation of precursors such as progesterone and 17α-hydroxyprogesterone is caused by dose-dependent defects of 17α-hydroxylase and 17,20-desmolase activities. In contrast, other enzymes, including 17β-hydroxysteroid dehydrogenase and 3β-hydroxysteroid dehydrogenase, were not affected in the desensitized testes. These results have demonstrated that the effects of 17α-hydroxylase and 17,20-desmolase are responsible for the distal steroidogenic lesions in the Leydig cell. These two enzymes have been previously shown to be related to microsomal cytochrome P-450, which requires NADPH and molecular oxygen for enzyme activity (23). It has also been suggested that the levels of cytochrome P-450 in interstitial cells, and consequently 17α-hydroxylase activity, are controlled by gonadotropin (22, 25). In the desensitized rat testes, the content of cytochrome P-450 was markedly decreased and was paralleled by reduction of the enzymatic activities of 17α-hydroxylase and 17,20-desmolase.

Previous studies have shown that the half-lives of 17α-hydroxylase and 17,20-desmolase enzyme activities (2.3 and 3.4 days, respectively) in the rat testis were similar to that of cytochrome P-450 (3.3 days), estimated from the decay curves after hypophysectomy (22). Such a similarity in half-lives provides indirect evidence that these two steroidogenic enzymes are dependent on cytochrome P-450. It was also of interest that the testicular microsomal cytochrome P-450 has the ability to metabolize benzopyrene, and that this activity was decreased in desensitized testicular microsomes, correlating with the reductions in 17α-hydroxylase and 17,20-desmolase (26). However, chronic treatment with phenobarbital or 3-methylcholanthrene did not induce the testicular microsomal cytochrome P-450 as reported in other tissues. In the present study, the correlation between the decrease of cytochrome P-450 levels and the reduction of 17α-hydroxylase and 17,20-desmolase enzyme activities after hypophysectomy, and the similarity of their responses to hCG treatment, were also consistent with this proposal. In addition, both 17α-hydroxylase and 17,20-desmolase were shown to have a constant activity per nmol of cytochrome P-450 during the marked changes produced by hCG treatment, while microsomal protein content was unaltered by desensitization. Therefore, it is likely that the decrease in microsomal cytochrome P-450 is responsible for the reduction of enzyme activities, and consequently for the distal lesion in androgen synthesis.

In previous studies, a decrease in microsomal enzyme activities has been noted in animals treated with 50 μg of hCG (26). This large dose of hCG invariably causes a marked decrease of gonadotropin receptors to undetectable levels, and an additional early lesion in the biosynthetic pathway prior to pregnenolone (5–9). In the present study, we have characterized the enzymatic changes in Leydig cells that selectively exhibit the microsomal lesions while maintaining a substantial number of LH receptors. This approach has permitted a precise definition of the nature and extent of the enzymatic
lesion, and has provided a valid comparison with the model that includes the early lesion and major receptor down-regulation. It has been demonstrated recently that the distal steroidogenic lesions are prevented by the antiestrogen Tamoxifen (7–10), and that exogenous estrogens directly reduce androgen secretion in hypophysectomized rat (12–15) and mouse testis (11). The participation of estrogen in the desensitization process was further suggested by the increased production of estradiol by the Leydig cells of LH-stimulated testes (7, 10) and by the presence of estradiol receptors in the rodent testis (7, 29, 30).

In hypophysectomized rats, estrogen treatment reduced testosterone responses to gonadotropin by the Leydig cell in vitro, with no decrease in cyclic AMP and pregnenolone formation. This steroidogenic lesion produced by exogenous estrogen was similar to that caused by low doses of hCG. Recently, such direct effects of estrogen have been also demonstrated during culture of Leydig cells, and could be prevented by Tamoxifen (31). Furthermore, in the present study, estrogen treatment caused decreases in 17α-hydroxylase and 17,20-desmolase activities and microsomal P-450 levels, but not in 3β-hydroxysteroid dehydrogenase activity. The similarity of such estrogen-dependent lesions to those observed in animals with testes desensitized by hCG, coupled with previous finding on the prevention of the lesions by Tamoxifen, clearly indicates an involvement of endogenous estrogen in the development of steroidogenic defects in gonadotropin-receptor desensitized Leydig cells. These observations also further indication of the potential importance of endogenous estrogen as an intra-testicular modulator of androgen bio synthesis and testosterone secretion (8).

Acknowledgment—We express our thanks to Dr. M. Negishi for his helpful advice in the measurement of cytochrome P-450.

REFERENCES