Hormonal Regulation of Testicular Luteinizing Hormone Receptors

EFFECTS ON CYCLIC AMP AND TESTOSTERONE RESPONSES IN ISOLATED LEYDIG CELLS*

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TSUNEKO TSURUHARA, MARIA L. DUFATU, SELVA CIGORRAGA, AND KEVIN J. CATT

From the Endocrinology and Reproduction Research Branch, National Institute of Child Health and Human Development, National Institutes of Health, Bethesda, Maryland 20014

Hormone-induced regulation of luteinizing hormone (LH) receptors and desensitization of target cell responses were studied in purified Leydig cells prepared from testicular interstitial tissue of rats treated with 0.2, 1, and 10 µg of human chorionic gonadotropin (hCG). The extent and rate of LH receptor loss after hCG were dose-related, with maximum decreases of 50, 80, and 95% at 4, 2, and 1 days after treatment with 0.2, 1, and 10 µg of hCG, respectively. After 10 µg of hCG, testicular LH receptors were almost completely abolished for 4 days, rose slightly at 6 days, and returned to normal at 10 days. Cyclic AMP responses to stimulation by hCG in vitro were generally reduced in proportion to receptor loss, with no change in sensitivity to gonadotropin. However, cyclic AMP responses to cholera toxin were retained by interstitial cells from hCG-treated rats. The testosterone responses to hCG, cholera toxin, and dibutyryl cyclic AMP were enhanced in interstitial cells from rats treated with 0.2 µg of hCG and were markedly depressed in cells from rats given 1 and 10 µg of hCG. The reduced maximum testosterone responses of partially desensitized interstitial cells could not be raised to the control levels by high concentrations of hCG in vitro at 4 days, but were restored after 6 days, with a 2-fold decrease in sensitivity to hCG. The enhanced testosterone responses to hCG, cholera toxin, and dibutyryl cyclic AMP observed after the low dose of hCG (0.2 µg) reflect physiological stimulation of Leydig cell function, whereas reduced testosterone responses after higher doses are manifestations of receptor loss and desensitization of steps in the steroidogenic pathway.

Measurement of occupied receptors showed that minor degrees of occupancy were followed by major loss of receptor sites. The decrease in LH receptors was less marked in detergent-solubilized preparations, suggesting that the extensive receptor loss observed in isolated cells was partly due to sequestration or internalization of binding sites. The loss of LH receptors in isolated Leydig cells persisted for several days after 1- and 10-µg doses of hCG, indicating that occupied LH receptors were processed or degraded rather than vacated and reutilized.

The transient loss of testosterone responses to dibutyryl cyclic AMP and cholera toxin after high doses of hCG revealed the presence of a postcyclic AMP activation block as a consequence of extensive receptor loss. Steroidogenesis in the desensitized Leydig cell (2 days after 1 µg of hCG) was inhibited at a point beyond the activation of cholesterol side chain cleavage enzyme, as indicated by the normal or elevated pregnenolone responses to hCG in the presence of impaired testosterone production. These experiments have shown that loss of LH receptors is of major importance in the mechanism of sustained Leydig cell desensitization. In addition, the development of a biosynthetic defect in the steroidogenic pathway contributes to the marked loss of androgen responses to gonadotropin and to cyclic AMP of endogenous or exogenous origin. Thus, desensitization induced by a trophic hormone leads to loss of distal hormone-dependent biosynthetic functions, as well as the proximate lesion in receptor concentration and coupled cyclic AMP production.

Several hormones have been shown to regulate the concentration of their specific receptor sites on the surface of target cells (1-5). This regulation most commonly leads to loss of receptor sites, with little or no change in the binding properties of the residual receptors. The consequences of hormone-induced receptor loss upon target cell function have not been examined in detail, although several reports have established that "desensitization" of adenylate cyclase to hormonal stimulation is correlated with reduction of specific receptors (2, 3). The desensitizing effects of gonadotropin upon ovarian adenylate cyclase (6-8) have been shown to result from loss of LH receptors (8), and negative regulation of testicular LH receptors by gonadotropin (9-13) is accompanied by reduced responses of cyclic AMP and testosterone production in excised testes during hormone stimulation in vitro (8-13).

The detailed analysis of biochemical responses in the interstitial cells of testes from animals given desensitizing doses of gonadotropin requires elucidation of the properties of Leydig cells during the induction and spontaneous reversal of receptor loss. For this purpose, we have examined the hormonal regulation of LH receptors, cyclic AMP, and testosterone
Production in purified Leydig cells prepared from the rat testis. These studies have revealed changes in the sensitivity as well as in the maximum responsiveness of androgen production after receptor loss, and also the presence of a postreceptor block in steroidogenesis during the period of maximum desensitization.

MATERIALS AND METHODS

Hormone Treatment and Isolation of Desensitized Leydig Cells

Adult male rats (200 to 250 g) obtained from Charles River Laboratories, Wilmington, Mass., were given intravenous injections of hCG via the external jugular vein. The hCG employed for desensitization was obtained from Organon as Pregnyl (3000 IU/mg), and administered as doses of 2, 10, and 100 IU in 100 µl of Dulbecco's phosphate-buffered saline. These doses were equivalent to 0.2, 1, and 10 µg of purified hCG with biological activity of 10,000 IU/mg. Animals were killed by decapitation at selected intervals, and testes were decapsulated for determination of tissue-bound hCG, measurement of available (free) LH/hCG receptor sites in testis homogenates and solubilized preparations, and preparation of isolated Leydig cells for measurements of LH receptors and gonadotropin in vitro.

Testicular interstitial cells were prepared by collagenase digestion of decapsulated testes from adult rats as previously described (14, 15). The interstitial cells were further fractionated by density gradient centrifugation in Metrizamide as previously described (16), giving purified cell preparations containing more than 90% Leydig cells by morphological criteria. The purified Leydig cells were washed once and resuspended in Medium 199 (Microbiological Associates, Bethesda, Md.) containing 0.1% bovine serum albumin (Armour Pharmaceutical, Kankakee, Ill.). The proportion of incubation medium to cells was usually equivalent to 3 ml/testis, or about 2 x 10^6 purified Leydig cells/ml. Cell concentrations were determined by counting at least 500 cells in a Levy ultramicro counting chamber.

Incubation of Isolated Leydig Cells

Incubation of 2-ml aliquots of the Leydig cell suspension was performed in 20-ml polyethylene counting vials (Packard) in the presence of 0.125 mM 1-methyl-3-isobutylxanthine. Additions of hCG, cholera toxin, or dibutyryl cyclic AMP were made as 100-μl aliquots to three to six sample vials and incubations were performed at 34°C under 95% O_2/5% CO_2 with shaking at 100 cycles/min. After incubation at 34°C for 12 min and the supernatant solution was saved for assay of pregnenolone production after receptor loss, and also the presence of a postreceptor block in steroidogenesis during the period of maximum desensitization.

Radioimmunoassay as previously described (17). Radioimmunoassay Calculations

A parallel series of incubation vials was used to prepare cell aliquots to three to six sample vials and incubations were performed at 0°C. The cells plus incubation medium were transferred into glass tubes containing 100 µl of 10 mM theophylline, placed into a boiling water bath for 10 min, and kept frozen until analyzed. The supernatants were aspirated and discarded, 3 ml of cold phosphate-buffered saline were added to all tubes, followed by centrifugation at 1500 x g for 15 min at 4°C. The supernatant solutions were aspirated and discarded, 3 ml of cold phosphate-buffered saline solution were added to all tubes, followed by centrifugation at 1500 x g for 15 min at 4°C. The supernatant solutions were aspirated and discarded, 3 ml of cold phosphate-buffered saline solution was added to each cell pellet, and the centrifugation step was repeated. After aspiration of the supernatants, the cell-bound radioactivity present in each tube was determined.

Binding Studies to Soluble Receptors — The gonadotropin receptors of testes from control and hCG-treated animals were solubilized by extraction of the 120 to 27,000 g fraction of fragmented interstitial cells with Triton X-100 as previously described (22-24). The detergent-solubilized preparation was centrifuged for 3 h at 360,000 x g, the supernatant solution was aspirated and discarded, 3 ml of cold phosphate-buffered saline solution was added to each cell pellet, and the centrifugation step was repeated. After aspiration of the supernatants, the cell-bound radioactivity present in each tube was determined.

Determination of Cyclic AMP Binding Capacity

Cyclic AMP binding studies were performed as previously described (25) by incubation of 100-μl aliquots of Leydig cell extract (equivalent to 2 to 3 x 10^6 cells) with 200 µl of 10^-15 M cyclic [3H]AMP bound to protein kinase as previously described (10). From 80 to 90% of the bound hormone could be recovered by this method.

Binding Studies in Testis Homogenates — Decapsulated testes were homogenized in phosphate-buffered saline (1 ml/testis for 1 min) in a Waring Blender at 13,000 rpm. After centrifugation at 20,000 x g for 15 min, the pellet was resuspended in 40 ml and centrifuged again, then weighed and dispersed at a final concentration of 100 to 200 mg/ml of saline. Testicular LH/hCG receptors were determined by incubating serial dilutions of homogenate with saturating concentration of labeled hormone, employing 125I-labeled hCG prepared by enzymatic radioiodination (20). The following reagents were added to glass tubes (12 x 75 mm), containing 100 µl of phosphate-buffered saline. Three serial 1:1 dilutions of the homogenate were incubated in triplicate at room temperature for 15 to 15 h, then diluted with 5 ml of ice-cold phosphate-buffered saline and centrifuged at 1500 x g for 15 min. The sediments were washed once, and the tissue-bound radioactivity was determined in a y spectrometer. The specifically bound radioactivity was converted to nanograms of bound hormone per testis, and was corrected for the specific activity and maximum binding activity of each tracer preparation (21).

Determination of Tissue-bound hCG — Receptor-bound hCG was determined after heating aliquots of testis homogenates at 65°C for 15 min, by radioimmunoassay of hormone released into the supernatant as previously described (14, 15). From 80 to 90% of the tissue-bound hormone could be recovered by this method.

Equilibrium binding data were analyzed by the Scatchard method (27) or by an equation relating the concentration of bound ligand to the total cyclic AMP concentration (28). An interactive computer program with differential equation solving ability was used to

Calculations of Receptor Concentration

Equilibrium binding data were analyzed by the Scatchard method (27) or by an equation relating the concentration of bound ligand to the total cyclic AMP concentration (28). An interactive computer program with differential equation solving ability was used to
perform all curve fitting and calculations (29, 30). Such programs were executed on a PDP-10 time sharing computer, with graphic output facilities, via a Tektronic terminal 4010-1. Protein concentrations were determined by the method of Lowry et al. (31) employing bovine serum albumin as standard.

**RESULTS**

**hCG-induced Changes in LH Receptor Concentration**

During binding studies with \(^{125}\)I-hCG in control and "desensitized" Leydig cells prepared from animals treated with increasing doses of hCG, the extent of receptor loss was both dose-dependent and time-related (Fig. 1). The lowest dose of hCG employed (100 ng) had no significant effect on receptor concentration, other than a slight fall at 5 days and a rise to just above control levels at 6 days. Administration of 200 ng of hCG produced 30% reduction of LH receptors at 2 days, and a further reduction by 60% at 4 days, followed by recovery after 6 to 7 days. After 1 \(\mu\)g of hCG, receptor sites decreased by 30% at Day 1, then fell by 70% at 2 days, and remained low for 4 days. Binding of \(^{125}\)I-hCG recovered significantly at the 6th day, to 80% of the control value, and returned to normal at Day 7. After 10 \(\mu\)g of hCG, available LH receptors dropped rapidly to very low levels, remained low or undetectable for several days, and began to recover 5 days after injection. However, restoration of receptor content to the control value was not complete until Day 14 (Fig. 1). A parallel experiment on the LH/hCG binding capacity of homogenized Leydig cells from treated and control animals gave almost complete receptor occupancy, with almost complete saturation of the gonadotropin receptors was present at 2 days after the 6-pg dose was slightly slower in the homogenate.

Following administration of 0.1 to 1 \(\mu\)g of hCG, no detectable occupancy of the gonadotropin receptors was present at 2 days, when receptor number had fallen to about 30% of the control value. In contrast, injection of 10 \(\mu\)g was followed by considerable receptor occupancy, with almost complete saturation at 24 h. After this high dose of hCG, significant occupancy was still present at 2 days (about 25% of total LH receptors), and declined thereafter but was still detectable at 10 days (Fig. 2). The early disappearance of available LH/hCG binding sites at the high desensitizing dose was attributable to occupancy by the administered hCG. Following the subsequent loss of receptor sites, recovery of binding capacity was very slow and did not reach the control value until Day 14.

Comparative studies were performed to evaluate the binding affinity and capacity of Leydig cells (Fig. 3A) and solubilized LH/hCG receptors (Fig. 3B) from control and hCG-treated animals killed 3 days after receiving the desensitizing dose of hCG.

Scatchard analysis of equilibrium binding studies gave linear plots in all studies, indicating a single set of binding sites and no changes in binding affinity during desensitization. The reduction in number of hCG receptor sites in cells and extracts was not accompanied by a change in the affinity of the LH/hCG receptors from the control value of \(2 \times 10^{10}\) M\(^{-1}\). In both cases, a dose-dependent decrease in receptor sites was observed, but the reduction was significantly less marked in the solubilized receptors, suggesting that receptor sites were occluded or internalized during the desensitization process (Table I).

**Responses of Receptor-depleted Leydig Cells to hCG in Vitro**

The cyclic AMP responses to increasing concentrations of hCG from 0.01 to 10 ng/ml in vitro were reduced in proportion to receptor loss with no changes on the sensitivity to hCG (Fig. 4). After 2 days, there was no reduction of the maximum cyclic AMP response in cells from animals treated with 0.2 \(\mu\)g of hCG, despite a 25% reduction in LH receptors. A more marked reduction in the cyclic AMP response was observed after the 1-\(\mu\)g dose, and no cyclic AMP response could be elicited after the 10-\(\mu\)g dose. After 4 days, following further decreases of receptor sites, a marked decrease of the maximum cyclic AMP response was observed in cells from the testes of the 0.2-\(\mu\)g group. After 6 days, despite almost complete recovery of receptors in the 0.2- and 1-\(\mu\)g groups, the cyclic AMP responses were not completely regained and remained reduced by 10 and 50% of control values for the 0.2- and 1-\(\mu\)g doses. The cyclic AMP response of the 10-\(\mu\)g group was partially recovered by Day 6, to 25% of the control value.

Testosterone responses to hCG in vitro were enhanced in interstitial cells from rats treated with 0.2 \(\mu\)g of hCG and were markedly depressed in cells from rats given 1 and 10 \(\mu\)g.
of hCG. The maximum in vitro testosterone responses of desensitized interstitial cells could not be raised to those of control cells by high concentrations of hCG at 2 and 4 days (Fig. 4). By 6 days, the steroid dose-response curves had returned to normal in cells from animals treated with 0.2 and 1 μg of hCG and showed a 2-fold higher ED₅₀ for hCG in cells from rats treated with 10 μg of hCG.

Since hCG-stimulated testosterone responses of desensitized interstitial cells (1 to 10 μg) could not be raised to the maximum control values at 2 and 4 days, the question arose whether the cyclic AMP produced during hormone stimulation in the desensitized state was either unavailable, or insufficient, for stimulation of steroidogenesis. Further experiments were performed to examine this question, by determining the stimulation of testosterone production by choleragen and dibutyryl cyclic AMP (Fig. 5). During stimulation with 10 μM choleragen, testosterone production in Leydig cells from the 0.2-μg treated group was significantly increased over control values at 2 days, and were close to control values at 4 and 6 days. In the groups given 1 and 10 μg of hCG, the maximum testosterone responses were reduced to 45% of the control value at 2 days, then rose to 60 and 75% of the control value at 4 and 6 days. At the same time, the cyclic AMP responses to choleragen did not differ from the controls in any of the hCG-treated groups (Table II).

The testosterone responses to 1 mM dibutyryl cyclic AMP were generally similar to those evoked by choleragen. The responses in cells of rats treated with 0.2 μg of hCG were again increased above the control value, whereas those of the 1- and 10-μg treated groups were markedly reduced at 2 days, to about 25% of the control value in normal cells, and returned nearly to the control values at 4 and 6 days. The inability of excess cyclic AMP, both choleragen-stimulated and exogenous in origin, to evoke maximum testosterone levels for a period of 2 to 3 days indicated that the block in steroidogenic responses to hCG was not simply an immediate consequence of receptor loss and must be located beyond the level of cyclic AMP formation.

To determine whether the defect in steroid response was related to a change in protein kinase content, further studies were performed to measure the availability and occupancy of

<table>
<thead>
<tr>
<th>Preparation</th>
<th>Leydig cells</th>
<th>Soluble receptors</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(binding sites/cell)</td>
<td>(fmol/mg protein)</td>
</tr>
<tr>
<td>Control</td>
<td>13,000 ± 312 (100)*</td>
<td>98 ± 4.9 (100)</td>
</tr>
<tr>
<td>hCG 0.2 μg</td>
<td>6,500 ± 200 (48)</td>
<td>83 ± 6.8 (85)</td>
</tr>
<tr>
<td>1 μg</td>
<td>4,200 ± 163 (31)</td>
<td>51 ± 9.0 (52)</td>
</tr>
<tr>
<td>10 μg</td>
<td>930 ± 233 (6.8)</td>
<td>24 ± 7.0 (24)</td>
</tr>
</tbody>
</table>

* Per cent of control.

Table I

Concentration of LH receptors in isolated intact Leydig cells and in solubilized cell extracts

Each value is the mean ± S. D. of the receptor concentrations derived by computer analysis (26) of forty data points from equilibrium binding studies performed on pooled cells from four groups of eight animals.
the cyclic AMP receptor protein in 2-day desensitized Leydig cells. Measurements of cyclic [3H]AMP binding to the receptor protein in Leydig cell extracts showed no significant differences in binding capacity between controls and the group treated with 1 µg of hCG, during 2-h incubation with the tritiated nucleotide. The number of available cyclic AMP receptors was slightly higher in the group treated with 0.2 µg of hCG, and was slightly reduced in the 10-µg hCG-treated group (Fig. 6, left). Binding assays with cyclic [3H]AMP were also performed with incubation for 16 h to permit exchange of endogenously occupied receptors with the added radioactive cyclic AMP, to determine the total number of cyclic AMP receptor sites in addition to the available binding sites measured after the 2-h incubation (25). By this method, no difference was observed between the control group and the 10-µg treated group, and significant increases in cyclic AMP receptors were observed in the 0.2 and 1 µg treated groups (Fig. 6, right). No differences in the binding affinity of the cyclic AMP receptor was observed among the several groups, the mean association constant being $2 \times 10^9$ M$^{-1}$.

The absence of a change in the number of cyclic AMP receptors in the desensitized state suggested that the lesion in steroidogenic response was probably located beyond the protein kinase level. Therefore, the functional integrity of the early portion of the steroid biosynthetic pathway was evaluated by measuring the activity of the cholesterol side chain cleavage enzyme system in the normal and receptor-depleted Leydig cells. This was performed by assay of pregnenolone production in Leydig cells from animals treated 2 days earlier with 0.2, 1, and 10 µg of hCG (Fig. 7). The maximum pregnenolone production in response to hCG in vitro was slightly elevated from control levels after the 0.2-µg dose of hCG and was markedly increased in the 1-µg group. These responses were in contrast with the notable reductions in testosterone production after such hCG doses in this and previous experiments (Figs. 4 and 5). The basal pregnenolone values were progressively increased with rising hormone dose in each of the hCG-treated groups, with maximum increase in the 10-µg treated group. In the latter, no increase in pregnenolone production was elicited by incubation with hCG in vitro, unlike the prominent elevations observed in cells from animals treated with lower doses of hCG.

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**TABLE II**

<table>
<thead>
<tr>
<th>Cholera-</th>
<th>Control</th>
<th>0.2 µg</th>
<th>1.0 µg</th>
<th>10 µg</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 µg</td>
<td>12 ± 1.5</td>
<td>14 ± 2.3</td>
<td>14 ± 1.5</td>
<td>30 ± 3.4</td>
</tr>
<tr>
<td>10 µg</td>
<td>174 ± 2.5</td>
<td>178 ± 1.3</td>
<td>195 ± 6.2</td>
<td>216 ± 16</td>
</tr>
</tbody>
</table>

Each value is the mean ± S. D. of the cyclic AMP response (picomoles/10⁶ cells) to choleragen (10 µg/ml) in cells derived from groups of four animals at each dose level, 2 days after hCG administration.

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**Fig. 5.** Testosterone response of Leydig cells to 1 mM dibutyryl cyclic AMP (top) and 10 µg of choleragen (bottom) at 2, 4, and 6 days after hCG treatment (0.2, 1, and 10 µg). Values are expressed as a percentage of the control value (mean ± S. D., n = 6).

**Fig. 6.** Left, specific binding of cyclic [3H]AMP (10⁻¹¹ to 10⁻⁷ M) by cyclic AMP receptor protein of Leydig cells from control and hCG-treated rats. Binding assays were performed for 2 h at 6°C, 2 days after treatment with 0.2, 1, and 10 µg of hCG. Right, specific binding of cyclic [3H]AMP (10⁻¹¹ to 10⁻⁷ M) to cyclic AMP receptors of Leydig cells during incubation with cell extracts for 16 h at 6°C.

**Fig. 7.** Pregnenolone production by Leydig cells from control and hCG-treated animals determined 2 days after treatment with 0.2, 1, and 10 µg of hCG. Synthesis of pregnenolone from endogenous precursors was measured during stimulation with hCG in the presence of cyanoketone and spironolactone. Results are the mean ± S. D. of quadruplicate determinations.
The present observations in isolated Leydig cells have significantly extended previous findings on the regulation of testicular and ovarian LH receptors and responses following administration of exogenous gonadotropin (9–11). The phenomenon of gonadal receptor regulation by the homologous hormone is consistent with the process observed in cells bearing receptors for insulin (1), growth hormone (5), catecholamines (3), and other ligands (32). In the Leydig cell system, the preparation of isolated cells following the in vitro effects of gonadotropin on receptor concentration has allowed the functional consequences of the receptor regulation to be analyzed in considerable detail.

In this study, administration of hCG, employed as an analogue of the endogenous luteinizing hormone, caused marked loss of receptors from the Leydig cells and consequent changes in the hormone responsiveness of these cells in vitro. Loss of LH receptors was not observed when the hCG dose was as low as 100 ng, but became detectable after 200 ng of hCG and increased in a dose-dependent manner after 1 and 10 μg of hCG. The level of receptor occupancy caused by the lowest effective dose (200 ng of hCG) was not detectable at 48 h by elution and assay of the bound hormone, yet led to a 60% loss of receptors on the 4th day after injection. The intermediate dose (1 μg) caused about 8% occupancy after 24 h, as previously shown (10), and produced more extensive receptor loss. After the highest dose (10 μg), which caused near-maximum receptor occupancy at 24 h (10), the degree of occupancy was still significant at 48 h and declined over the next few days. The consequent loss of receptors from isolated Leydig cells was maximum at Day 4 and returned almost to the normal level by Day 8. The loss of receptors in each of the lower-dose groups (0.2 and 1 μg of hCG) is clearly in considerable excess of the level of occupancy caused by the administered hormone, and the major fall in receptors occurred at a time when occupancy was undetectable or minimal. This indicates that an active process of receptor regulation was initiated at the cell membrane level when a small proportion of the receptor population had been occupied by the homologous hormone or its active analogue. The Leydig cell is known to possess about 15,000 LH receptors (16), and occupancy of less than 1% of these is adequate for effects on LH receptor occupancy (10), had already occurred before the actual loss of receptors and responses studied from 2 to 6 days after the administration of hCG.) The recovery phase of the cyclic AMP response was relatively slow and lagged behind the return of the LH receptors. Thus, after 6 days the maximum cyclic AMP responses of cells from the groups given 0.2 and 1 μg of hCG had not returned to normal, despite complete recovery of the LH receptor population. Since stimulation of cyclic AMP responses by cholera toxin was normal or increased throughout the period of receptor loss and recovery, it is likely that a coupling defect exists between newly formed receptors and adenylate cyclase during the initial phase of the recovery process. Earlier studies in normal Leydig cells have shown a close correlation between receptor occupancy and cyclic AMP formation during stimulation by gonadotropins in vitro (15).

The corresponding changes in testosterone responses of cells after hCG-induced receptor loss were more complex, and also bore a more interesting relationship to the known physiological action of the trophic hormone in maintaining Leydig cell function and androgen secretion. An important finding in this regard was the increased testosterone responses seen at 2 and 4 days in cells of rats treated with the lowest dose of hCG (200 ng), despite the significant fall in LH receptors and cyclic AMP responses of these cells. This effect reveals that the process of receptor regulation that occurs at all levels of occupancy is followed by enhancement of the steroidogenic pathway when small doses of hormones are given to simulate...
physiological gonadotropin levels in vivo. By contrast, Leydig
cells from animals treated with higher doses of hCG showed
marked reduction of maximum steroid responses at 2 and 4
days.
Since the Leydig cell has an abundance of spare receptors,
such marked reductions in receptors would be expected to
cause a shift to the right in the dose-response curve, with
increased ED50 for hCG reflecting a decrease in sensitivity to
gonadotropin hormone. However, maximum testosterone lev-
els were never attained at higher stimulatory doses of the
tropic hormone, and this obscured the anticipated change in
sensitivity. The failure to achieve such maximal steroid re-
sponses could indicate that the cyclic AMP produced was not
available for stimulating subsequent responses, that a marked
reduction of the relevant protein phosphokinase had taken
place, or that a more distal lesion had occurred in one or more
of the enzymes regulating the steroidogenic pathway.

Stimulation of the Leydig cells with choleragen elicited
cyclic AMP responses which were comparable with the control
groups, but despite such cyclic AMP increases the production
of testosterone remained markedly reduced. Also, the steroido-
genic lesion was not overcome by stimulation with dibutyryl
cyclic AMP in concentrations that evoked steroidogenesis in
normal Leydig cells. Since the cyclic AMP binding studies
showed no loss of binding to cyclic AMP-dependent phospho-
kinase in the desensitized Leydig cells, it appears unlikely
that cyclic AMP and protein kinase are limiting factors in the
impaired steroid response. Further, the finding that hormone
stimulation in vitro produced a significant increase in pre-
genolone synthesis in the groups given 0.2 and 1 μg of hCG
indicates the adequate availability of cholesterol side chain
clavage enzyme activity. These results have demonstrated that
the gonadotropin-induced second lesion in the steroido-
genic pathway lies beyond the side chain cleavage enzyme in
cells with moderate degrees of receptor depletion. In marked
contrast, in the 10-μg group where membrane-bound receptors
were reduced to about 6% (Table 1), the in vitro pregnenolone
response to hCG was completely abolished. This indicates that
the most extensive loss of receptors, and presumably of
coupled responses, results in loss of the processes necessary to
maintain steroidogenic enzymes including the cholesterol side
chain cleavage enzyme.

These studies have shown that the responses of target cells
after hormone-induced receptor loss undergo a series of
changes that result in the overall process referred to as
desensitization. The earliest change, not examined in the
present work, is the rapid loss of adenylate cyclase responsive-
ness to hormone, with consequent loss of cyclic AMP produc-
tion in the intact cell. The initial desensitization of adenylate
cyclase is an immediate consequence of receptor occupancy
and occurs much earlier than the true loss of receptors
examined in this report. This process has been described
previously in the luteal cells of the ovary (6-8) and the Leydig
cells of the testis (10), as well as in several other tissues (2, 3).
The later consequences of receptor occupancy include the
loss of receptors noted in this and earlier reports, and the
related impairment of cyclic AMP and more distal responses
in the target cell. Two components of this delayed effect can
be distinguished and probably operate to different extents in
specific target cells. The first and most obvious of these is the
loss of ability to activate adenylate cyclase and cyclic AMP
production, in proportion to the loss of receptor sites which
mediate the hormonal signal. This change probably occurs in
all receptor-depleted target cells, and in the absence of other
changes in cell function would result mainly in a relative loss
in sensitivity of responses to the homologous hormone. How-
ever, a second effect of receptor depletion occurs in certain
target cells, such as those of the testis and ovary. In these
tissues, additional changes occur at more distal points in the
cellular metabolic pathways and modify the capacity of the
cells to respond to hormonal stimuli. Such a change is the
defect in steroidogenesis observed in the present study, result-
ing in loss or reduction of the maximum steroid response to
saturating hormone concentrations. This defect will probably
prove to be characteristic of target cells for trophic peptide
and protein hormones that regulate the state of differentiation
as well as the acute responses of the target cell. By this
reasoning, the loss of steroidogenesis in receptor-depleted
Leydig cells would represent the result of impaired trophic
action by LH in vivo during the initial phases of desensitiza-
tion and receptor loss. Our findings demonstrate that the
results of receptor regulation on target-cell function are com-
plex, and that temporally related changes in both proximal
and distal receptor-mediated responses contribute to the over-
all process of cell desensitization.

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