Hormonal Regulation of Testicular Luteinizing Hormone Receptors

EFFECTS ON CYCLIC AMP AND TESTOSTERONE RESPONSES IN ISOLATED LEYDIG CELLS

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TSUNEO TSURUHARA, MARIA L. DUF AU, 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 choleragen were retained by interstitial cells from hCG-treated rats. The testosterone responses to hCG, choleragen, 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, choleragen, 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 LH1 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 dibutyl cyclic AMP and choleragen 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 androgenic 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 adenylyl cyclase (6-8) have been shown to result from loss of LH1 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 coll 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 (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 the testes were decapsulated for determination of tissue-bound hCG, measurement of available cyclic-AMP receptor sites, and solubilized preparations, and of 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 (15), 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, III.), and 0.1% bovine serum albumin with or without 100 IU of hCG; 50 µl of 212-hCG (200,000 cpm, 5 to 10 ng) in phosphate-buffered saline 0.1% bovine serum albumin and 100 µl of testis homogenate. Three serial 1:1 dilutions of the homogenate were incubated in triplicate at room temperature for 15 to 18 hr, then diluted with 5 µl 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 γ spectrometer. The specifically bound radioactivity was converted to nanograms of bound hormone per testis, and compared to 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° for 15 min, by radioimmunoassay of hormone released into the supernatant as previously described (10). From the total of the tissue-bound hormone could be recovered by this method.

**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 34°, or at room temperature for 16 h. These conditions have been previously shown to be adequate to reach equilibrium of the binding reaction. Nonspecific binding of 212-hCG was determined for each dose level of labeled hormone by incubation in control tubes containing an excess (100 IU) of unlabeled hormone. At the end of the incubation period, 2-ml aliquots of cold phosphate-buffered saline solution were added to all tubes, followed by centrifugation at 1500 x g for 15 min at 4°. The supernatant solutions were aspirated and discarded, and 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 106 cells) with 200 µl of 103 to 1010 µl cyclic AMP (LH/212-hCG) in 50 µm free phosphate buffer, pH 6.8, containing 10 mM theophylline, 10 mM magnesium acetate, and 10 mM mercaptoethanol, and 100 µl of buffer containing 10-5 M 1-methyl-3-isobutylxanthine. After incubation at 6° for 2 or 16 hr, cyclic AMP bound to protein kinase was isolated by adsorption to cellulose filters (26). Nonspecific binding, determined as previously described (10). From 80 to 85% of the bound hormone could be recovered by this method.

**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

**Determination of LH/hCG Receptor Sites**

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 µg/ml of saline. Testicular LH/hCG receptors were determined by incubating serial dilutions of homogeneate with saturating concentration of labeled hormone, employing 212-hCG labeled hCG by enzymatic radioiodination (29). The following reagents were added to glass tubes (12 x 75 mm) with shaking at 100 cycles/min. After 30 to 60 min, the supernatant was used for binding studies. Aliquots of the 212-hCG in 50 mM phosphate buffer, pH 6.8, containing 10 µM cyclic 3HAMP (25).

For testosterone determinations, incubation media were diluted 1:10 to 1:25 in phosphate-buffered saline, pH 7.4, and subjected to radioimmunoassay as previously described (17). Radioimmunoassay of pregnenolone was carried out with the antisem and procedure described by Pietro et al. (18). Radioimmunoassay of cyclic AMP was performed by a modification of the method of Steiner et al. (19) as previously described (15).
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 6 days and a rise to just above control levels at 8 days. Administration of 200 ng of hCG produced 30% reduction of LH receptors at 6 days, and a further reduction by 60% at 4 days, followed by recovery after 6 to 7 days. After 1 µg 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 µg 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 a generally similar profile when compared with binding studies to intact cells, although the recovery of receptors after the 1-µg dose was slightly slower in the homogenate.

Following administration of 0.1 to 1 µg 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 µg was followed by considerable receptor occupancy, with almost complete saturation of the LH/hCG receptors 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 treated animals with 0.2 µg of hCG, despite a 25% reduction in LH receptors. A more marked reduction in the cyclic AMP response was observed after the 1-µg dose, and no cyclic AMP response could be elicited after the 10-µg dose. After 4 days, following further decreases of receptor sites, a marked decrease of the maximum cyclic AMP response in cells from the testes of the 0.2-µg group. After 6 days, despite almost complete recovery of receptors in the 0.2- and 1-µg 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-µg doses. The cyclic AMP response of the 10-µg 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 µg of hCG and were markedly depressed in cells from rats given 1 and 10 µg.
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 were 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 I](http://www.jbc.org/)

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

* Per cent of control.
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 $2 \times 10^9 \text{ M}^{-1}$.

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.
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 to evoke a maximum steroid response *in vitro* (33). In the male rat, it is likely that the low circulating level of gonadotropin (10 nM LH) causes occupancy of only a small fraction of the total testicular receptor sites (1 pmol/g of testis). In the animals treated with the highest dose of hCG, the initial loss of available receptors was largely due to occupancy by the administered hormone at 24 h, as shown previously, and by the 2nd day a major loss of total receptor sites was evident, as shown in Fig. 3.

The loss of LH receptors after hCG treatment was less marked in detergent-extracted preparations than in the corresponding membrane-rich particulate fraction. This difference in available sites suggests that a proportion of the declining receptor population is masked or occluded within the membrane and rendered free for interaction with hormone when solubilized with nonionic detergent. It is likely that the initial occupancy of receptor sites at the membrane level is followed by internalization of the complexes (and adjacent free receptors) by endocytosis and subsequent degradation after association with lysosomes. Such changes would be similar in sequence and also probably in mechanism to the process of adsorptive pinocytosis by which solutes bound to membrane recognition sites are interiorized with the pinocytic vesicle (34). Thus, recent studies have suggested that some of the LH bound to Leydig cells was internalized and eventually degraded by lysosomes (35), and similar changes were observed in ovarian tissue where the trophic hormone was localized by autoradiography to the plasma membrane and cytoplasmic dense bodies regarded as lysosomes (36).

Further observations in luteinized ovarian cells have revealed that the hormone-receptor complexes initially formed at the cell membrane are later internalized and become associated with a particulate fraction of the cytoplasm (37). Evidence has also been presented for the internalization of cell-bound epidermal growth factor and loss of the specific receptors after endocytosis of the growth factor-receptor complex (38). These various examples of endocytosis of hormone-receptor complexes are part of the more general activity of the vacuolar system responsible for pinocytosis followed by processing, digestion, and recycling of the ingested compounds (34). The extent to which this process is specifically activated by hormonal ligands, and whether it represents more than the expression of a universal cell response to membrane-binding of extracellular ligands, has yet to be established. However, the function of such a process to effect feedback regulation of the receptor population has been clearly demonstrated in studies on the low density lipoprotein receptor of cultured fibroblasts (39) and could account for the chronic lowering of receptor concentration in cells exposed to high hormone levels for prolonged periods.

The changes in hCG-induced cell responses following receptor loss were generally in keeping with the expected effects of impairment of the hormone activation sequence. Thus, cyclic AMP production was proportional to receptor content and closely followed the loss of receptors at Days 2 and 4. (It is important to note that in these experiments, the acute desensitization of cyclic AMP responses a few hours after hCG treatment, and corresponding to the initial phase of 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 the 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 levels were never attained at higher stimulatory doses of the trophic hormone, and this obscured the anticipated change in sensitivity. The failure to achieve such maximal steroid responses 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 steroidogenic process was not overcome by stimulation with dibutyril 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 phosphokinase 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 pregnenolone synthesis in the groups given 0.2 and 1 μg of hCG indicates the adequate availability of cholesterol side chain cleavage enzyme activity. These results have demonstrated that the gonadotropin-induced second lesion in the steroidogenic 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 responsiveness to hormone, with consequent loss of cyclic AMP production 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. However, 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, resulting 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 responsiveness 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 desensitization and receptor loss. Our findings demonstrate that the results of receptor regulation on target-cell function are complex, and that temporally related changes in both proximal and distal receptor-mediated responses contribute to the overall process of cell desensitization.

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REFERENCES
1. Gavin, J. R., Roth, J., Neville, D. M., De Meyts, P., and Buell, D. N. (1974) Proc. Natl. Acad. Sci. U. S. A. 71, 84-88
2. Keabian, J. M., Zatz, M., Romero, J. A., and Axelrod, J. (1975) Proc. Natl. Acad. Sci. U. S. A. 72, 3795-3798
3. Mukherjee, C., and Caron, M. J. (1975) Proc. Natl. Acad. Sci. U. S. A. 72, 1945-1949
4. Hinkle, P. M., and Tashjian, A. H., Jr. (1975) Biochemistry 14, 3845-3851
5. Loveniak, M. A., and Roth, J. (1976) J. Biol. Chem. 251, 3720-3729
6. Hunzicker-Dunn, M., and Birnbaumer, L. (1975) Endocrinology 90, 185-197
7. Bockaert, J., Hunzicker-Dunn, M., and Birnbaumer, L. (1976) J. Biol. Chem. 251, 3653-3663
8. Conti, M., Harwood, J. P., Hausch, A. J. W., Dufau, M. L., and Catt, K. J. (1975) J. Biol. Chem. 251, 7729-7731
9. Hausch, A. J. W., Dufau, M. L., and Catt, K. J. (1976) Biochem. Biophys. Res. Commun. 75, 1145-1151
10. Hausch, A. J. W., Dufau, M. L., and Catt, K. J. (1973) Proc. Natl. Acad. Sci. U. S. A. 70, 592-595
11. Sharpe, R. M. (1976) Nature 264, 644-646
12. Haour, F., and Saez, J. M. (1977) Mol. Cell. Endocrinol. 7, 17-24
13. Chen, D. I., and Payne, A. H. (1977) Biochim. Biophys. Res. Commun. 74, 1589-1596
14. Dufau, M. L., Mandelson, C., and Catt, K. J. (1974) J. Clin. Endocrinol. Metab. 39, 610-613
15. Mandelson, C., Dufau, M., and Catt, K. J. (1975) J. Biol. Chem. 250, 8918-8923
16. Conn, P. M., Tsuhrara, T., Dufau, M. L., and Catt, K. J. (1977) Endocrinology 101, 639-642
17. Dufau, M. L., Catt, K. J., and Tsuhrara, T. (1972) Endocrinology 90, 1032-1040
18. DiPietro, D. L., Brown, R. D., and Strott C. A. (1972) J. Clin. Endocrinol. Metab. 35, 729-735
19. Steiner, A. L., Parker, C. W., and Kipnis, D. M. (1972) J. Biol. Chem. 247, 1106-1113
20. Dufau, M. L., Podesta, E., and Catt, K. J. (1975) Proc. Natl. Acad. Sci. U. S. A. 72, 1271-1275
21. Catt, K. J., Kotelevtseva, J. M., and Dufau, M. L. (1976) in Methods in Receptor Research (Blecher, M., ed) Vol. 1, pp. 175-244, Marcel Dekker, New York.
22. Dufau, M. L., and Catt, K. J. (1973) Nature New Biol. 242, 246
23. Dufau, M. L., Charreau, E. H., and Catt, K. J. (1973) J. Biol. Chem. 248, 9879-9882
24. Charreau, E. H., Dufau, M. L., and Catt, K. J. (1974) J. Biol. Chem. 249, 4189-4195
25. Dufau, M. L., Tsuruhara, T., Horner, K. A., Podesta, E. J., and Catt, K. J. (1977) Proc. Natl. Acad. Sci. U. S. A. 74, 3419-3423
26. Tao, M., Salas, M. L., and Lipman, P. (1970) Proc. Natl. Acad. Sci. U. S. A. 67, 408-412
27. Scatchard, G. (1949) Ann. N. Y. Acad. Sci. 51, 660-665
28. Ketelslegers, J.-M., Knott, G. D., and Catt, K. J. (1975) Biochemistry 14, 3075-3083
29. Knott, G. D., and Reece, D. K. (1972) in Proceedings of the Online 72 International Conference, Vol. 1, pp. 497-499, Brunel University, England
30. Knott, G. D., and Shrager, R. I. (1972) in Computer Graphics: Proceedings of the SIGGRAPH Computers in Medicine Symposium, Vol. 6, pp. 135-142, No. 4, Association for Computing Machinery, New York
31. Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J. (1951) J. Biol. Chem. 193, 265-275
32. Raff, M. C. (1976) Nature 259, 265-266
33. Catt, K. J., and Dufau, M. L. (1973) Nature New Biol. 244, 219-222
34. Steinman, R. M., and Cohen, Z. A. (1976) in Biogenesis and Turnover of Membrane Macromolecules (Cook, J. S., ed) pp. 1-14, Raven Press, New York
35. Ascoli, M., and Puett, D. (1977) FEBS Lett. 75, 77-82
36. Chen, T. T., Abel, J. H., McClellan, M. C., Sawyer, H. R., Diekman, M. A., and Niswender, G. D. (1977) Cytobiologie 14, 412-420
37. Conn, P. M., Conti, M., Harwood, J. P., Dufau, M. L., and Catt, K. J. (1977) J. Cell Biol. 75, HM-916
38. Carpenter, G., and Cohen, S. (1976) J. Cell Biol. 71, 159-171
39. Brown, M. S., and Goldstein, J. L. (1976) Science 191, 150-191
Hormonal regulation of testicular luteinizing hormone receptors. Effects on cyclic AMP and testosterone responses in isolated Leydig cells.

T Tsuruhara, M L Dufau, S Cigorraga and K J Catt

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