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Desensitization to gonadotropins in cultured Leydig tumor cells involves loss of gonadotropin receptors and decreased capacity for steroidogenesis

(choriogonadotropin/hormone receptors/hormone action/target cell responses/desensitization)

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ABSTRACT **The ability of human choriogonadotropin (hCG) to regulate its receptors and target cell responses has been studied in a clonal strain of cultured Leydig tumor cells (MA-10). Exposure of the MA-10 cells to hCG results in decrease in hCG binding activity which is dependent on time and the concentration of hCG. This decrease is due to a change in the number of receptors rather than in the affinity of the receptors, and it is accompanied by a corresponding reduction in the ability of hCG to stimulate steroidogenesis. Exposure of the MA-10 cells to hCG also resulted in a reduction of the steroidogenic responses to cholera toxin and 8-Br-adenosine cyclic 3′,5′-monophosphate. The hCG-induced loss of steroidogenic responses to these stimuli seems to be due to the stimulation of steroidogenesis rather than to the decrease in hCG receptors because it also can be induced when steroidogenesis is stimulated with cholera toxin or 8-Br-adenosine 3′,5′-monophosphate under conditions such that the number of hCG receptors is not reduced.**

It is now well known that the ability of target cells to respond to gonadotropins may be regulated by changes in the number of gonadotropin receptors (see refs. 1 and 2 for reviews). The regulation of gonadotropin receptors by homologous or heterologous hormones has been documented in normal Leydig cells (3–5), granulosa/luteal cells (6–8), and Leydig tumor cells (9, 10).

The study of homologous down-regulation of gonadotropin receptors is of particular interest because it represents a mechanism by which lutropin and human choriogonadotropin (LH and hCG) reduce the cellular response to subsequent hormonal stimulation. Studies on normal Leydig cells have shown that the homologous down-regulation of gonadotropin receptors leads to a reduction in the steroidogenic response of the cells not only to subsequent hormonal stimulation but also to subsequent stimulation with cholera toxin or cyclic AMP. This loss of steroidogenic responses has been called "desensitization" and appears to be due to the decrease in gonadotropin receptors (down-regulation) and a reduction in the activity of the steroidogenic pathway. Leydig cells isolated from rats that had been injected previously with LH or hCG have been shown to display: (*a*) reduced numbers of gonadotropin receptors (1–5); (*b*) decreased activity of LH/hCG-sensitive adenylate cyclase (4, 5); and (*c*) specific lesions in the pathway leading to testosterone biosynthesis (1, 2, 4, 5). The lesions in the steroidogenic pathway are due to a decease in 17α-hydroxylase and 17,20-desmolase activities (11, 12). At high concentrations of hCG, another lesion, localized prior to the formation of pregnenolone, has also been observed (11).

LH/hCG-induced down-regulation of gonadotropin receptors and steroidogenic responses has also been documented in the ovary (6, 7, 13). These cells also show a lesion in the steroidogenic pathway beyond the formation of cyclic AMP (6, 7, 13). This lesion, however, must be different from the principal lesion described in normal Leydig cells because the ovaries synthesize progesterone rather than testosterone. Thus, LH/hCG appear to induce different lesions in the steroidogenic pathways of these two tissues.

The MA-10 line is a clonal strain of Leydig tumor cells that have receptors for hCG and mouse epidermal growth factor (mEGF) (10, 14). These cells respond to hCG, cholera toxin, and 8-Br cyclic AMP with increased progesterone production. It has been reported (10) that exposure of these cells to mEGF results in a substantial reduction in the number of hCG receptors and a corresponding reduction in the ability of hCG to stimulate steroidogenesis. The steroidogenic responses to cholera toxin and 8-Br cyclic AMP, however, were not affected.

We now report that exposure of these cells to hCG also results in a reduction in the number of gonadotropin receptors and a corresponding reduction in the ability of hCG to stimulate steroidogenesis. In contrast to the results obtained with mEGF (10), however, exposure of the cells to hCG also resulted in a reduction in the steroidogenic responses to cholera toxin and 8-Br cyclic AMP. These results show that the functional states induced by homologous and heterologous regulation of gonadotropin receptors in the MA-10 cells are different.

MATERIALS AND METHODS

Hormones and Supplies. hCG (batch CR-121) was obtained from the National Institute of Child Health and Human Development and was iodinated as described (15). Antiserum to progesterone was prepared in this laboratory (16). 8-Br cyclic AMP, cholera toxin, and crude hCG (3000 international units/mg) were obtained from Sigma. All other materials were obtained as described (9, 10, 14–16).

Cell Culture. All experiments were performed with cells from the recently described MA-10 cell line (14). Culture conditions were as described (14), with the exception that stock cultures were subcultured (split ratio, 1:36) once a week. Experimental cultures were plated (split ratio, 1:8) in 6-cm culture dishes containing 5 ml of growth medium and treated as described below.

Experimental Protocol for Desensitization. Three days after plating, the medium was removed and replaced with 4 ml of fresh growth medium. hCG, 8-Br cyclic AMP, or cholera toxin

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Abbreviations: hCG, human choriogonadotropin; mEGF, mouse epidermal growth factor; LH, lutropin; ED_{50}, dose for half-maximal effect.

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was added to the culture plates as 40-fold concentrated solutions in 10 mM sodium phosphate/0.15 M NaCl/ albumin (1 mg/ml), pH 7.4. Sterile conditions were maintained throughout.

At the times indicated in the figure legends, the cultures were removed from the incubator and washed twice with 2 ml of warm Waymouth MB752/1 at pH 7.4 and containing $NaHCO_3$ (1.12 g/liter), 20 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid, gentamycin (40 μg/ml), albumin (1 mg/ml) (referred to below as assay medium). The dishes then received 2–4 ml of assay medium and were used to measure hormone binding and progesterone production.

Hormone binding was measured as described (10, 14), except that nonspecific binding was determined in the presence of crude hCG at 25 international units/ml. Progesterone production was measured after a 4-hr incubation (37°C) in 4 ml of assay medium containing 1 nM hCG, 1.2 nM cholera toxin, or 1 mM 8-Br cyclic AMP. Progesterone was measured by radioimmunoassay in suitable aliquots of the unextracted medium (10, 14–16). The concentrations of steroidogenic stimuli used were shown to stimulate progesterone production maximally under the experimental conditions used (10, 14, 17). All determinations were done in duplicate or triplicate.

Other Methods. Measurements of progesterone in growth medium or in the medium of cells that had been incubated with ^{125}I-labeled hCG (^{125}I-hCG) were done after extraction with diethyl ether as described (14).

The release of surface-bound hCG by exposing the cells to acidic conditions has been described (18). Briefly, after they were washed to remove the free hormone, the dishes received 1.5 ml of ice-cold 50 mM glycine/100 mM NaCl, pH 3.0, and were incubated at 4°C for 2 min. The buffer was then aspirated, and the dishes were washed once with 1 ml of the same buffer (at 4°C). This treatment releases 85–95% of the surface-bound hormone and leaves the cells morphologically and functionally intact, as judged by their ability to bind ^{125}I-hCG and to produce progesterone (18).

All other methods used have been described (10, 14).

RESULTS

HCG-Induced Loss of ^{125}I-hCG Binding. Previous results from this laboratory have shown that upon binding at (37°C) to the MA-10 cells, the receptor-bound hCG is rapidly internalized and subsequently degraded. The time course of this phenomenon is such that most of the cell-associated radioactivity is intracellular by 6 hr of incubation (18).

The binding and internalization of hCG was accompanied by a reduction in the ^{125}I-hCG binding activity (Fig. 1). When the MA-10 cells were incubated with a saturating concentration of hCG (40 ng/ml), a time-dependent reduction in ^{125}I-hCG binding was observed. In this experiment, ^{125}I-hCG binding was measured in washed cells and in washed cells that were briefly exposed to acidic conditions (to remove the surface-bound hormone remaining from the preincubation). This procedure allowed us to determine the contribution of receptor occupancy to the decrease in ^{125}I-hCG binding. The data (Fig. 1) show that, at early time points (2–4 hr), about half of the observed decrease in ^{125}I-hCG binding is due to residual occupancy of the hCG receptors. At later times (14–24 hr), none of the observed decrease is due to residual occupancy. Half-maximal decrease in ^{125}I-hCG binding was observed after 1½ or 2½ hr in washed and acid-treated cells, respectively. After 24 hr of incubation, the binding was decreased by about 95%.

The effect of hCG on the subsequent binding of ^{125}I-hCG was also dependent on the concentration of hormone used. After a 14-hr incubation, half-maximal reduction of ^{125}I-hCG binding

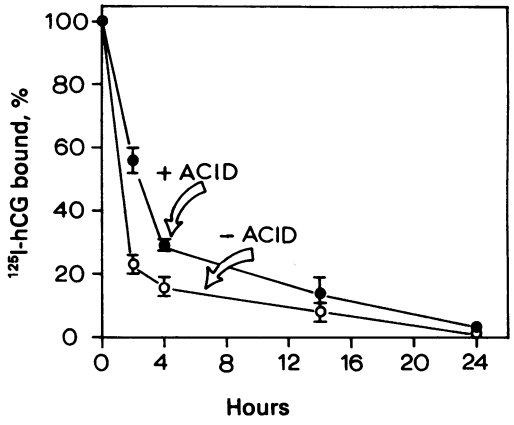

FIG. 1. Time course of the hCG-induced loss of ^{125}I-hCG binding. Cells were incubated (37°C) in 4 ml of growth medium containing hCG at 40 ng/ml. At the times indicated, the growth medium was removed, the cells were washed twice with assay medium, and half of the dishes were treated with acid. ^{125}I-hCG binding was then determined after a 2-hr incubation (37°C) in 2 ml of assay medium containing ^{125}I-hCG at 20 ng/ml. Binding to washed (○) and acid-treated (●) cells is expressed as percentage of their respective controls (i.e., cells treated in the same way, except that they were not exposed to hCG). Under these conditions, washed and acid-treated cells bound (mean ± SEM) 21 ± 1.2 (n = 10) and 17.5 ± 1.0 (n = 3) pg of ^{125}I-hCG per μg of DNA. Each point shown represents the mean ± SEM of three to five independent experiments, except that the 24-hr point is the result of a single experiment.

was observed with hCG at 1 ng/ml and maximal reduction (90–95%) was observed with hCG at 5–10 ng/ml.

Effect of hCG Preincubation on ^{125}I-HCG Binding and Responsiveness. The effects of hCG exposure on the subsequent ability of ^{125}I-hCG to bind to and stimulate progesterone production in the MA-10 cells are shown in Fig. 2 and Table 1. In these experiments, cells were initially incubated either without hCG or with hCG at 1 or 40 ng/ml for 14 hr and then, after washing, were exposed to increasing concentrations of ^{125}I-hCG for 2 hr at 37°C. At this time, specific binding and progesterone production were determined. Exposure to hCG at 1 ng/ml reduced the maximal ^{125}I-hCG binding capacity by 50%; exposure to 40 ng/ml reduced the maximal binding capacity by 94%. The

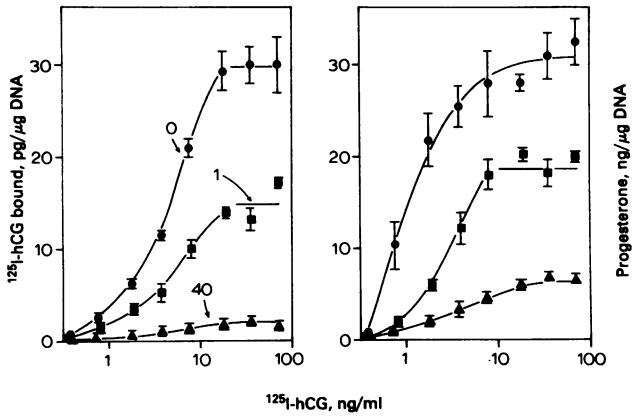

FIG. 2. Dose–responses for ^{125}I-hCG binding and stimulation of progesterone production in hCG-treated cells. Cells were incubated for 14 hr at 37°C in 4 ml of growth medium containing no hCG (●), hCG at 1 ng/ml (■), or hCG at 40 ng/ml (▲). After washing, 2-ml portions of assay medium containing increasing concentrations of ^{125}I-hCG were added. The amount of cell-associated radioactivity (*Left*) and progesterone produced (*Right*) were then determined in duplicate dishes after a 2-hr incubation at 37°C. Each point represents the mean ± SEM of four (control) or two (hCG-exposed) independent experiments.

Table 1. hCG-induced loss of ^{125}I-hCG binding and steroidogenic response

HCG present during preincubation, ng/ml	Maximum*	ED_{50}, M × 10^{10}
	^{125}I-hCG binding	
0	7.8 (100)	1.3 (100)
1	3.9 (50)	1.4 (108)
40	0.13 (5.7)	1.2 (92)
	^{125}I-hCG stimulated progesterone production	
0	29.4 (100)	0.34 (100)
1	18.5 (63)	0.63 (185)
40	6.5 (22)	0.09 (289)

Experimental details are in Fig. 2. All values were calculated by visual inspection of the data plotted in Fig. 2. ED_{50}, 50% effective dose; the numbers in parentheses represent percentages relative to controls.
* Units: for ^{125}I-hCG binding, mol × $10^{16}/\mu g$ of DNA; for progesterone production, ng/μg of DNA.

50% effective dose (ED_{50})¶ for binding after exposure to either concentration of hCG was not altered.

Maximal progesterone production in response to hCG decreased by 37% and 78%, respectively, after preincubation with hCG at 1 or 40 ng/ml. The ED_{50} for progesterone production increased as the maximal binding capacity and progesterone production decreased. The results in Fig. 2 show that most of the loss of maximal progesterone production associated with hCG preincubation can be explained by loss of hCG binding sites. However, coupling between binding and progesterone production also changes as receptor number decreases. In control cells, the ratio of the ED_{50} for binding to the ED_{50} for progesterone production ($ED_{50}b/ED_{50}p$) is 3.9,‖ whereas in cells preincubated with hCG it is 2.2 at 1 ng/ml and 1.2 at 40 ng/ml. This observation suggests that, as these cells lose binding sites, the coupling between binding and progesterone production becomes tighter. In spite of these changes, however, maximal progesterone production was induced at a concentration of ^{125}I-hCG (10 ng/ml) that gave a high degree of receptor occupancy—i.e., 70–80% for the cells exposed to no hCG or to hCG at 1 ng/ml and 100% occupancy for the cells exposed to hCG at 40 ng/ml.

Effects of hCG on Subsequent Steroidogenic Responsiveness to hCG, Cholera Toxin, or 8-Br Cyclic AMP. Cells were preincubated without hCG or with hCG at 1 or 40 ng/ml for 14 hr. At this time, the cells were washed and reincubated for 4 hr with concentrations of hCG, cholera toxin, or 8-Br cyclic AMP that had been shown to induce maximal steroid production (10, 14, 17). Preincubation with hCG at 1 ng/ml caused the production of progesterone to be stimulated 84-fold (over control values) during the 14-hr preincubation and decreased the binding by 43%; preincubation with hCG at 40 ng/ml caused progesterone to be stimulated 116-fold and binding to be reduced by 93%. After the 14-hr exposure to hCG the steroidogenic responsiveness to hCG, cholera toxin, and 8-Br cyclic AMP all were decreased (Table 2). Because cholera toxin and 8-Br cyclic AMP do not require hCG receptors for stimulation of steroidogenesis, it is concluded that the preincubation with hCG affected some process distal to the hCG receptor.

The time course for desensitization of the steroidogenic response by exposure to hCG at 40 ng/ml is shown in Fig. 3. After exposure to hCG, the desensitization of steroidogenesis was most marked when progesterone production was restimulated with hCG, but there also was a loss of responsiveness to cholera toxin and 8-Br cyclic AMP at all time points.

These results show that the hCG-induced desensitization of the steroidogenic responses to hCG is due to at least two factors: (*i*) decrease in hCG receptors; and (*ii*) lesion(s) beyond the formation of cyclic AMP. At late times (14–24 hr), when there is not residual receptor occupancy, the post-cyclic AMP lesions may account for about 50% of the observed reduction in hCG-stimulated progesterone production.

Induction of Desensitization by Cholera Toxin and 8-Br Cyclic AMP. The data presented above suggest that the hCG-induced loss of responsiveness to cholera toxin and 8-Br cyclic AMP may be a direct result of the stimulation of the steroidogenic pathway. To test this possibility, we examined the ability of cholera toxin and 8-Br cyclic AMP to induce a similar loss of steroidogenic responses.

First, cells were incubated with different concentrations of hCG, cholera toxin, or 8-Br cyclic AMP for 14 hr and the amount of progesterone produced was measured. From these results (not shown), we chose concentrations of cholera toxin (2 ng/ml; 24 pM) and 8-Br cyclic AMP (70 μM) that stimulated steroidogenesis to an extent (100- to 150-fold) similar to that observed with a saturating concentration of hCG (40 ng/ml; 1.05 nM).

Next we exposed cells to these concentrations of cholera toxin or 8-Br cyclic AMP for 14 hr and tested their response to a subsequent stimulation with hCG, cholera toxin, or 8-Br cyclic AMP. Both steroidogenic stimuli induced a reduction of steroidogenic responses to hCG, cholera toxin, or 8-Br cyclic AMP (Table 3). The magnitude of this reduction was similar to that induced by exposure to hCG (Table 2) and occurred in the absence of a reduction in ^{125}I-hCG binding. When expressed as

¶ The binding data in Table 1 are expressed as maximal binding and ED_{50}, instead of as the conventional receptor number and K_d, respectively, because the binding of ^{125}I-hCG to intact cells (at 37°C) is not reversible (18). Thus, strictly speaking, one cannot calculate equilibrium binding constants. However, it is worth noting that, when the dose–response for ^{125}I-hCG binding to control cells was measured in the presence of NaN_3 (a compound that inhibits internalization), the maximal binding capacity was 6.8 mol × $10^{-16}/\mu g$ of DNA, and the ED_{50} was 1.8 × 10^{-10} M. Thus, it appears that the binding data shown in Table 1 can be equated with receptor number and K_d.

‖ This ratio is higher than anticipated from previous experience. A better coupling between hormone binding and steroid production with $ED_{50}b/Ed_{50}p$ of about 2 has been reported (10, 14). This discrepancy appears to be due to unknown variables introduced when using different lots of horse serum to maintain the cells in culture.

Table 2. hCG-induced loss of steroidogenic responses

HCG present during incubation, ng/ml	Progesterone produced, ng/μg DNA			
	Basal	hCG	Cholera toxin	8-Br cyclic AMP
0	0.055 ± 0.015 (100)	65.9 ± 1.4 (100)	45.4 ± 0.45 (100)	69.4 ± 0.6 (100)
1	0.54 ± 0.26 (982)	45.0 ± 9.2 (68)	38.0 ± 3.1 (84)	42.8 ± 6.2 (62)
40	0.65 ± 0.36 (1182)	5.2 ± 0.1 (7.9)	28.4 ± 4.1 (63)	36.9 ± 1.9 (53)

Cells were preincubated in 4 ml of growth medium containing the indicated concentration of hCG for 14 hr at 37°C. After washing, the cells received 4 ml of assay medium containing buffer only (basal) or one of the compounds indicated. Progesterone was then measured in triplicate dishes after a 4-hr incubation at 37°C. Each number represents the mean and range of two independent experiments. The numbers in parentheses represent percentages relative to controls.

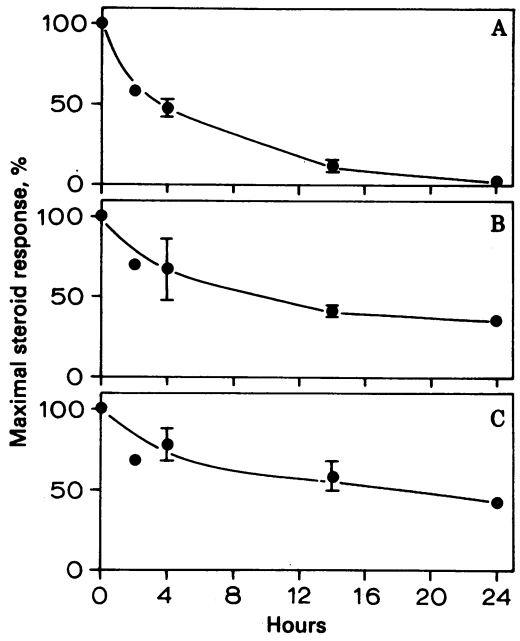

FIG. 3. Time course of hCG-induced desensitization of steroidogenic responses. Cells were incubated (37°C) in 4 ml of growth medium containing hCG at 40 ng/ml. At the times indicated, the cells were washed and then exposed to 2 ml of assay medium containing hCG (*A*), cholera toxin (*B*), or 8-Br cyclic AMP (*C*). Progesterone was then measured after a 4-hr incubation at 37°C. Results are expressed as percentage of the steroidogenic response that each stimuli produced in matched controls. The 4- and 14-hr points represent the mean ± SEM of three independent experiments; the 2- and 24-hr points represent results of a single experiment.

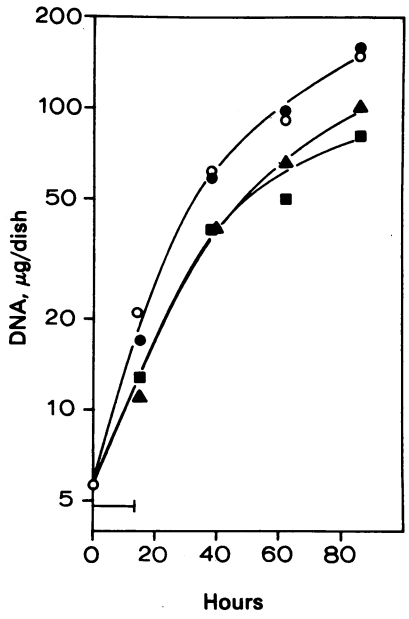

FIG. 4. Growth curves for the MA-10 cells. Cells were plated in 6-cm dishes containing 5 ml of growth medium and incubated for 24 hr at 37°C. At this time ($t = 0$), the medium was replaced by medium containing buffer only (○), 1 nM hCG (●), 0.6 nM cholera toxin (■), or 1 mM 8-Br cyclic AMP (▲), and the cells were incubated for 14 hr at 37°C (indicated by the bar at the bottom); the stimuli were then removed by aspirating and washing the dishes twice with warm growth medium. Each dish then received 5 ml of growth medium. At the times indicated, the DNA content was measured in duplicate dishes. The medium was replaced daily.

percentage of control, ^{125}I-hCG binding was 100% and 95% in the cells exposed to cholera toxin and 8-Br cyclic AMP, respectively.

Steroidogenic Stimuli Are Not Toxic to the MA-10 Cells. In all the experiments described above, we noted that sometimes dishes that had been exposed to cholera toxin or particularly 8-Br cyclic AMP for 14 hr had about 20% less cells (i.e., less DNA) than did control dishes. Exposure to hCG, however, did not produce this effect.

To establish that whatever toxicity that might be caused by the 14-hr exposure to cholera toxin or 8-Br cyclic AMP was reversible, the following experiment was done. Cells were exposed for 14 hr to growth medium containing buffer, hCG, cholera toxin, or 8-Br cyclic AMP. At this time the medium was removed and the cells were washed and incubated further in growth medium containing no additions. hCG had no effect on the amount of DNA present in the dishes at the end of 14 hr (Fig. 4). On the other hand, dishes exposed to 0.6 nM cholera toxin or 1 mM 8-Br cyclic AMP had about 60% as much DNA as the controls. (Note that these concentrations are higher than those used in the desensitization experiments.) When the steroidogenic stimuli were removed, however, the growth rate in all groups was comparable to that of the control cells (doubling time, 14–16 hr).

DISCUSSION

The results presented herein show that exposure of cultured Leydig tumor cells to hCG leads to desensitization of steroidogenic responses. Upon binding to the MA-10 cells (at 37°C), most of the bound hCG is internalized and degraded within 12 hr (18). During this process, the cells lose ^{125}I-hCG binding activity. This loss of ^{125}I-hCG binding (*i*) is due to a decrease in the maximal binding capacity rather than to changes in affinity; and (*ii*) is not due to residual occupancy of the receptor.

The loss of gonadotropin receptors is accompanied by a reduction in the ability of the MA-10 cells to respond, with increased progesterone production, to hCG, cholera toxin, and 8-Br cyclic AMP.

Table 3. Cholera toxin and 8-Br cyclic AMP induce loss of steroidogenic responses

Pretreatment	Progesterone, ng/μg DNA			
	Basal	hCG	Cholera toxin	8-Br cyclic AMP
None	0.016 ± 0.008 (100)	48.5 ± 2.7 (100)	27.5 ± 1.4 (100)	42.5 ± 6.2 (100)
Cholera toxin at 24 pM	10.9 ± 4.3 (681)	26.4 ± 0.2 (54)	13.8 ± 2.5 (50)	25.9 ± 1.7 (61)
8-Br cyclic AMP at 70 μM	4.9 ± 2.3 (306)	29.3 ± 6.6 (60)	17.2 ± 4.8 (63)	29.3 ± 9.3 (69)

Cells were preincubated in 4 ml of growth medium containing the compounds indicated for 14 hr at 37°C. After washing, the cells received 4 ml of assay medium containing buffer only (basal) or one of the compounds indicated. Progesterone was then measured in triplicate dishes after a 4-hr incubation at 37°C. Each number represents the mean and range of two independent experiments. The numbers in parentheses represent percentages relative to controls. During the pretreatment period, the cells exposed to cholera toxin or 8-Br cyclic AMP produced 173 and 140 times more progesterone, respectively, than did the controls.

The dose–response curves for the stimulation of progesterone production by hCG show that the maximal amount of progesterone produced by the cells previously exposed to hCG is decreased. The magnitude of this change is similar to the decrease in gonadotropin receptors. The sensitivity of the cells to hCG was also found to change. The amount of hCG required to produce half-maximal stimulation of progesterone production (ED_{50}) increased 2- to 3-fold in the hCG-exposed cells. As a result of these changes, the degree of coupling between hormone binding and the stimulation of progesterone production (as measured by ED_{50}) increased. The concentration of hormone required to give maximal stimulation of progesterone production, however, was similar in control and hCG-exposed cells.

That the heterologous down-regulation of gonadotropin receptors in the MA-10 cells by mEGF also results in similar changes in the progesterone responses to hCG was recently reported (10). When hCG receptors were reduced with mEGF, the maximal amount of progesterone produced in response to hCG was reduced accordingly, and the ED_{50} for progesterone production increased 2-fold (10).

These results show that the reduction of hCG receptors in the MA-10 cells by homologous (hCG) or heterologous (mEGF) hormones leads to the same changes in the subsequent steroidogenic response of the cells to hCG. In contrast, the *in vivo* down-regulation of gonadotropin receptors by homologous hormone in normal Leydig or luteal cells leads to variable changes in the steroidogenic responses to hCG. In these systems, changes in the maximal amount of steroid produced or sensitivity to hCG depends on the amount of hormone injected, the route of administration, the tissue used, and the methods used to prepare the cells (4–7, 11, 19, 20).

Similar to the results of others with normal rat luteal or Leydig cells (1, 2, 4, 6, 7, 11, 13), we have found that exposure of Leydig tumor cells to hCG also results in a reduction of the subsequent steroidogenic response of the cells to cholera toxin or 8-Br cyclic AMP. This loss of responsiveness (desensitization) to other steriodogenic stimuli is not as great as the loss of responsiveness to gonadotropin. A prolonged (14 hr) exposure to a saturating concentration (40 ng/ml) of hCG resulted in a 90–95% reduction in the number of hCG receptors, an 80–90% reduction in the maximal progesterone response to hCG, and a 40–50% reduction in the maximal progesterone response to cholera toxin or 8-Br cyclic AMP. Thus, hCG affects the MA-10 cells at two loci: (*a*) the hCG receptor; and (*b*) the "effector system."

The reduction in gonadotropin receptors seems to be due (at least partially) to the binding and metabolism of the bound hCG (see above). The lesion(s) in the effector system seem to be due to the stimulation of steroidogenesis (by hCG) rather than to the reduction of receptors. This hypothesis is supported by the following findings: (*i*) similar lesions can be induced by exposing the cells to steroidogenic factors (cholera toxin and 8-Br cyclic AMP) under conditions such that ^{125}I-hCG binding is not changed (Table 3); and (*ii*) the reduction of gonadotropin receptors by mEGF (a hormone that has little or no effect on steroid production) does not result in a loss of responsiveness to 8-Br cyclic AMP or cholera toxin (10).

The results presented herein and previously (10, 21) clearly show that homologous or heterologous reduction of gonadotropin receptors in the MA-10 cells leads to a reduction in the ability of hCG to stimulate progesterone production. This reduction is observed mainly in the maximal amount of progesterone produced, but changes in sensitivity also occur. The main difference observed so far between homologous and heterologous reduction of gonadotropin receptors is that homologous down-regulation, but not heterologous regulation, is accompanied by lesion(s) in the effector system. The nature of such lesion(s) must be different than the principal lesion described in normal Leydig cells (11, 12) because the MA-10 cells produce progesterone rather than testosterone.

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1. Catt, K. J., Harwood, J. P., Aguilera, G. & Dufau, M. L. (1979) *Nature (London)* **280**, 109–116.
2. Haour, F. & Saez, M. (1978) in *Structure and Function of the Gonadotropins*, ed. McKerns, K. W. (Plenum, New York), pp. 497–516.
3. Sharpe, R. M. (1976) *Nature (London)* **264**, 644–646.
4. Tsuruhara, T., Dufau, M. L., Cigorraga, S. & Catt, K. J. (1977) *J. Biol. Chem.* **252**, 9002–9009.
5. Payne, A. H., Wong, K.-L. & Vega, M. (1980) *J. Biol. Chem.* **255**, 7118–7122.
6. Conti, M., Harwood, J. P., Dufau, M. L. & Catt, K. J. (1977) *J. Biol. Chem.* **252**, 8869–8874.
7. Sen, K. K., Azhar, S. & Menon, K. M. J. (1979) *J. Biol. Chem.* **254**, 5664–5671.
8. Erickson, G. F., Wang, C. & Hsueh, A. J. W. (1979) *Nature (London)* **279**, 336–338.
9. Ascoli, M. & Puett, D. (1978) *J. Biol. Chem.* **253**, 4892–4899.
10. Ascoli, M. (1981) *J. Biol. Chem.* **256**, 179–183.
11. Cigorraga, S. B., Dufau, M. L. & Catt, K. J. (1978) *J. Biol. Chem.* **253**, 4297–4304.
12. Chasalow, F., Marr, H., Haour, F. & Saez, J. (1979) *J. Biol. Chem.* **254**, 5613–5617.
13. Harwood, J. P., Conti, M., Conn, P. M., Dufau, M. L. & Catt, K. J. (1978) *Mol. Cell. Endocrinol.* **11**, 121–135.
14. Ascoli, M. (1981) *Endocrinology* **108**, 88–95.
15. Ascoli, M. & Puett, D. (1978) *Proc. Natl. Acad. Sci. USA* **75**, 99–102.
16. Ascoli, M. & Puett, D. (1978) *J. Biol. Chem.* **253**, 7832–7838.
17. Ascoli, M. (1978) *J. Biol. Chem.* **253**, 7839–7843.
18. Ascoli, M. (1981) *Ann. N.Y. Acad. Sci.*, in press.
19. Dufau, M. L., Cigorraga, S., Baukal, A. J., Sorrell, S., Bator, J. M., Neubauer, J. F. & Catt, K. J. (1979) *Endocrinology* **105**, 1314–1321.
20. Sharpe, R. M. & NcNeilly, A. S. (1980) *Mol. Cell. Endocrinol.* **18**, 75–86.
21. Segaloff, D. L., Ascoli, M. & Puett, D. (1981) *Biochim. Biophys. Acta* **675**, 351–358.