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Regulation of Luteinizing Hormone Receptors and Adenylate Cyclase Activity by Gonadotrophin in the Rat Ovary

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SUMMARY

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Administration of human chorionic gonadotrophin (hCG) to immature rats with luteinized ovaries caused a time- and dose-related decrease in ovarian content of receptors for luteinizing hormone (LH). The marked reduction in LH/hCG receptor concentration was not accompanied by a change in binding affinity for hCG. Subcutaneous injection of 2 μg of hCG produced a maximum serum hCG concentrations of 10 ng/ml, and was followed by a marked decrease in ovarian binding capacity. Measurement of receptor-bound hCG by radioimmunoassay after acid elution showed that the initial loss of binding capacity was due to occupancy of receptors by the administered hormone. After several hours occupancy no longer accounted for the decrease in binding capacity, and by 24 hr there was a 90% loss of total receptor sites. The ovarian content of receptors remained low for several days after the desensitizing dose of hCG had been cleared from the circulation. The lowest dose of hCG to cause a measurable loss of receptors was 20 ng/100 g of body weight, suggesting that regulation of receptors may occur with physiological concentrations of gonadotrophins. Small degrees of receptor occupancy were followed by major losses of binding capacity, indicating that an active or cooperative process might be involved in the mechanism of receptor loss. Solubilization of control and desensitized ovaries with a nonionic detergent showed the same degree of receptor loss observed in particulate ovarian fractions, with no change in the sedimentation properties of the residual binding sites. Gonadotrophin-induced loss of LH/hCG receptors was associated with rapid, substantial loss of the ability of LH to stimulate adenylate cyclase activity in ovarian homogenates. This effect was hormone-specific, since the responses of adenylate cyclase to sodium fluoride and prostaglandin E_1 were not impaired when the LH response was completely abolished. Furthermore, the response of adenylate cyclase to LH was not restored by the GTP analogue 5'-guanylylimidodiphosphate. Administration of actinomycin D and cycloheximide before and during desensitization did not affect the extent or duration of receptor loss or the LH responsiveness of adenylate cyclase. These studies provide further evidence for the regulation of ovarian gonadotrophin receptors by the homologous hormone, and demonstrate that receptor loss is a major factor in the prolonged desensitization of adenylate cyclase by elevated gonadotrophin concentrations.

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INTRODUCTION

Several hormone-sensitive tissues have recently been shown to undergo changes in target cell receptor content in the presence of the homologous hormone. Studies on the insulin receptor showed an inverse relationship between receptor concentration and the circulating levels of insulin, providing a basis for the phenomenon of insulin resistance in hyperinsulinemic states (1). Similar changes in concentration have since been observed for a number of receptors exposed to elevated hormone concentrations, including those for growth hormone (2), catecholamines (3), and thyrotrophin-releasing hormone (4). In some systems, hormone-induced loss of receptors was accompanied by loss of the ability of the hormone to stimulate adenylate cyclase (5, 6). Since many of the effects of peptide hormones are mediated by cyclic 3′,5′-AMP, these changes could account for the refractory or desensitized states observed in hormone-sensitive tissues. It has recently been reported that the testes (7, 8) and ovaries (9) of rats treated with LH[1] or hCG exhibited marked loss of gonadotrophin receptors. In the ovary, such receptor loss was associated with loss of the ability of LH to stimulate adenylate cyclase (9, 10). We now report further studies on gonadotrophin-induced changes in LH/hCG receptors[2] and adenylate cyclase of the desensitized rat ovary, and a detailed analysis of the relationship between receptor occupancy and receptor loss during hCG-induced desensitization.

METHODS

Animal treatment and hormone preparations. Twenty-five-day-old female rats were injected with 50 IU of pregnant mare serum gonadotrophin (Gestyl, Organon), followed 65 hr later by 25 IU of hCG (Pregnyl, Organon). Eight days after the first injection, the animals were given a single desensitizing dose of hCG by subcutaneous injection or via the jugular vein under light ether anesthesia. In some experiments cycloheximide (100 μg/100 g of body weight) or actinomycin D (100 μg/100 g of body weight) was administered intraperitoneally before and with the desensitizing doses of hCG. Groups of three to five rats were killed at various times by decapitation, and blood was collected for assay of serum hCG concentration. The ovaries were rapidly removed and frozen in liquid nitrogen until assayed for LH receptor content and adenylate cyclase activity. The quantities of hCG administered as Pregnyl to the test animals were converted from international units to micrograms of purified hCG, taking a preparation of 10,000 IU/mg as standard. For radioiodination, we employed a purified preparation of hCG (biological activity, 10,000 IU/mg) donated by Dr. R. Canfield, Department of Medicine, Columbia University.

Determination of LH/hCG binding capacity. The binding of [^{125}I]hCG as a measure of the LH receptor content of the tissue was studied in washed, 27,000 × *g* fractions of the ovarian homogenate as previously described (9). The binding inhibition data were converted into saturation curves and Scatchard plots by a computer curve-fitting procedure as described by Ketelslegers *et al.* (11). Binding capacity in control and desensitized ovaries was also determined in preparations solubilized by treatment with Triton X-100 (12). The solubilized preparation was analyzed by sucrose density gradient centrifugation (12) to determine whether there were any changes in the sedimentation characteristics of the residual receptors in the desensitized tissue.

Measurement of tissue-bound hCG. The 27,000 × *g* pellet was resuspended in phosphate-buffered saline (Dulbecco's phosphate buffer). An aliquot was used for the binding assay as indicated above, and another aliquot was recentrifuged at 27,000 × *g*, washed twice to remove unbound hormone, and resuspended in cold 0.025 M formic acid. The suspension was shaken at 4° for 2 hr to release tissue-bound hCG (13), and centrifuged at 27,000 × *g* for 15

[1] The abbreviations used are: LH, luteinizing hormone; hCG, human chorionic gonadotrophin; Gpp(NH)p, 5′-guanylylimidodiphosphate.

[2] LH and hCG interact with identical receptors in testis and ovary, described here as LH/hCG receptors.

min. The supernatant was buffered to pH 7.4 with NaOH and stored at −70° until assayed. The eluted hCG was determined by radioimmunoassay, using a double-antibody technique (14). The efficiency of the extraction procedure (90–95%) was determined by recovery of [^{125}I]hCG bound to ovarian particles prior to acid treatment. The results were expressed as picomoles of hCG bound per milligram of protein in the 27,000 × *g* pellet. In some experiments in which [^{125}I]hCG was injected, occupancy was determined by counting tissue-bound radioactivity in the ovary at various times of removal from the rat, or by counting an aliquot of the washed 27,000 × *g* pellet. The amount of hormone bound was calculated from the known specific activity of the tracer, and expressed as picomoles per milligram of protein.

Adenylate cyclase activity. Adenylate cyclase activity was determined in ovarian homogenates prepared in a buffer consisting of 27% sucrose, 25 mM Tris-HCl (pH 7.5), and 1 mM EDTA at 4° (15). One ovary was homogenized with 2 ml of the medium in a Dounce homogenizer, using two strokes with a loose pestle and two strokes with a tight pestle. The homogenate was filtered once through nylon mesh and assayed within 20 min of preparation. The assays were conducted kinetically, by serial withdrawal of four 100-μl samples from an incubation mixture of 500 μl, usually at 3- or 4-min intervals. The rate of activity, as picomoles of cyclic AMP formed per milligram of protein per minute at 30°, was calculated from the line of fit for the four points. The final assay mixture contained 25 mM Tris-HCl (pH 7.5), 5 mM $MgCl_2$, 1 mM EDTA, 2.5 mM ATP, [α-^{32}P]ATP to give a final specific activity of 10–20 cpm/pmole, and an ATP-regenerating system of creatine phosphate and creatine phosphokinase (9). Responses to gonadotrophin were measured by addition of ovine LH (NIH S18) to a final concentration of 10 μg/ml. The reaction was stopped by addition of 1 N perchloric acid, and cyclic [^{32}P]AMP was separated by the Dowex-alumina method (16) prior to liquid scintillation counting.

RESULTS

LH-hCG receptor binding. The decrease in binding capacity measured in washed, 27,000 × *g* particulate fractions from homogenized ovaries of hCG-treated rats is shown in Fig. 1. The hCG binding capacity in untreated rats was about 1.3 pmoles/mg of protein, and it decreased by 50% and 85% in animals killed 24 hr following desensitizing doses of 0.2 and 2.0 μg of hCG. From the saturation curves shown, or by Scatchard analysis, there was no change in the binding affinity of LH/hCG receptors in the desensitized tissues. The observed K_a of 10^{10} M^{-1} is similar to that determined in previous studies in the luteinized rat ovary (12). The same degree of receptor loss was observed in solubilized preparations from desensitized tissue, and there were no changes in sedimentation characteristics of the residual receptor when analyzed by sucrose density gradient centrifugation (data not shown).

The temporal and quantitative relationships between occupancy and loss of LH/hCG receptor sites are shown in Fig. 2. The binding capacity of the control rats

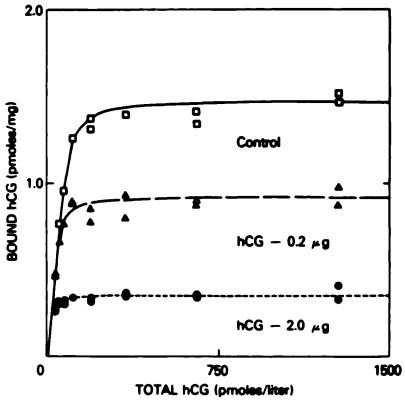

FIG. 1. *Saturation curves for [^{125}I]hCG binding to particulate fractions from control and desensitized rat ovaries*

Primed rats were treated as described in METHODS, desensitized with a single injection of 0.2 or 2.0 μg of hCG, and killed 24 hr later. Binding was studied as described in METHODS and analyzed as shown above and by Scatchard plots to determine the binding capacity and affinity constant of the LH/hCG receptors.

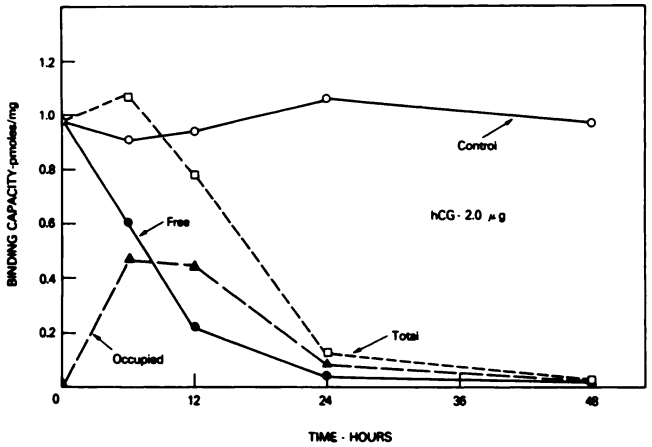

FIG. 2. *Temporal relationship between free and occupied binding sites for hCG during desensitization in rat ovary*

Rats were given 2.0 μg of hCG and killed at intervals up to 48 hr. Free binding sites were determined by [^{125}I]hCG binding to particulate receptors, and occupancy was measured by elution and radioimmunoassay of tissue-bound hCG as described in METHODS. By summation of free and occupied sites, the total receptor capacity was determined.

remained constant over the 48-hr period, at about 1 pmole/mg of protein. In animals treated with 2.0 μg of hCG, the free receptor capacity fell by about 50% at 6 hr and by 90% at 24 hr. On the other hand, receptor occupancy due to the administered hCG initially increased, remained elevated for 6–12 hr, then declined to low levels at 24 hr. By summation of the free and occupied receptors, the total receptor capacity could be derived (Fig. 2). Thus the fall in free receptors to about 50% at 6 hr was completely attributable to occupancy, since the total receptor content remained unchanged. At 24 hr, however, there was a marked fall in total receptors, which could not be accounted for by occupancy. There are, therefore, two phases associated with hCG-induced loss of receptors, followed by a third phase during which the receptor population returns to normal in 6–7 days as previously described (9).

The duration of the occupancy phase corresponded closely with the elevated serum levels of hCG which followed administration of the desensitizing dose (Fig. 3). After the 2.0-μg dose of hCG, serum levels reached a maximum in 6 hr and rapidly declined to zero in 24–30 hr. These and other studies using [^{125}I]hCG (7, 9) suggest

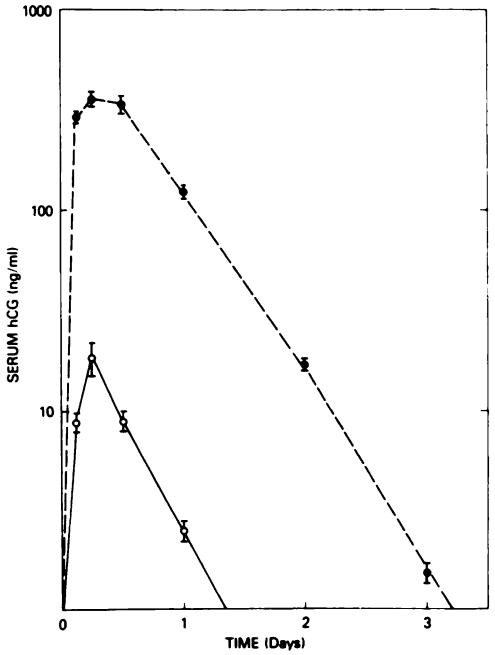

FIG. 3. *Serum concentrations of hCG at various times following subcutaneous injection of 2* (○——○) *or* (●- - -●) *20 μg of hCG*

that the subsequent prolonged receptor loss is neither due to nor maintained by high circulating levels of hCG. When hCG

was given by intravenous injection, maximal occupancy occurred in 3-6 hr, while the time course of total receptor loss was unchanged (Fig. 4). As in the earlier experiment (Fig. 2), there was no net loss of total receptors at 6 hr, and the maximal decrease in receptors was seen at 24 hr. The extent of receptor loss (75-80%) was less following the lower dose of hCG.

The different characteristics of receptor loss observed at 6 and 24 hr are clearly illustrated in Fig. 5, together with the dose-response relationship. At 6 hr, as shown in the upper panel, a dose of 0.1-1.0 μg of hCG was required to effect any decrease in free receptors, and there was no diminution in total receptors. At 24 hr, hCG doses in the range of 0.01-0.1 μg caused a decrease in free receptors which was not due to occupancy, since the total number of receptor sites was also decreased. The remarkable sensitivity of ovarian receptor concentration to hCG, at doses as low as 10-20 ng/100 g of body weight, indicates that these changes may reflect normally occurring physiological events, such as the LH surge, during which serum LH levels reach several nanograms per milliliter (17).

From the studies shown in Figs. 2, 3, and 5, in which tissue-bound hCG was determined as a measure of receptor occupancy, it is apparent that only a small degree of initial occupancy is needed to produce a much larger loss of the receptor

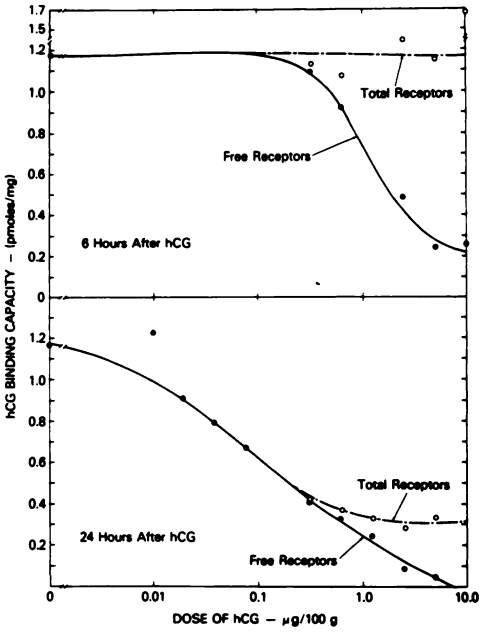

FIG. 5. *Effect of various desensitizing doses of hCG upon ovarian binding capacity at 6 and 24 hr*

Primed rats were treated with doses of hCG from 0.01 to 20.0 μg by subcutaneous injection and killed 6 or 24 hr later. Both free and occupied receptors were determined, but only free and total receptor number are shown in the graphs.

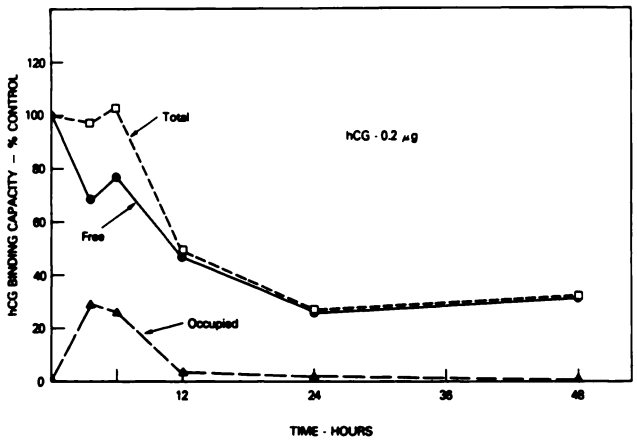

FIG. 4. *Time course of hCG binding capacity following desensitization by intravenous injection of 0.2 μg of [^{125}I]hCG*

Free binding capacity was determined as described in METHODS, and occupancy was determined from the counts present in the ovary on removal from the animal. Total binding capacity was calculated by summation of free and occupied receptors.

population at 24 hr. This relationship is shown more clearly in Fig. 6, in which the extent of receptor loss is plotted as a function of the degree of occupancy. About 1% occupancy led to a 50% loss of total receptors, and 20–30% occupancy caused almost complete loss of the receptor population.

Cycloheximide and actinomycin D were administered to rats either before or during desensitization with hCG, to examine the effect of impaired synthesis of RNA and protein on the process of receptor loss. As shown in Figs. 7 and 8, both agents caused a small degree of receptor loss in the control animals but did not alter the rate or extent of receptor loss in the desensitized animals. Other studies have shown that in some systems these agents affect desensitization (4, 18, 19), while in other cases they do not (3, 6).

Adenylate cyclase. As reported previously (9), gonadotrophin-induced loss of LH/hCG receptors was associated with a decrease in the ability of LH to stimulate adenylate cyclase activity in homogenates of desensitized ovaries. The loss was found to be hormone-specific (Fig. 9), since the response to prostaglandin E_1 was not affected in the desensitized tissue at a time when the effect of LH was completely abolished. After 48 hr, as shown in this experiment, there was no change in basal or fluoride-stimulated enzyme activity. In the frog red blood cell, *beta* adrenergic desensitization of receptor sites has been reported to be reversed by the GTP analogue Gpp(NH)p (20). In the luteinized rat ovary, Gpp(NH)p produced a time-dependent stimulation of adenylate cyclase activity in both the absence and presence of LH. In desensitized ovarian tissue LH did not stimulate adenylate cyclase in the presence or absence of Gpp(NH)p (Fig. 10). Treatment of rats with cycloheximide did

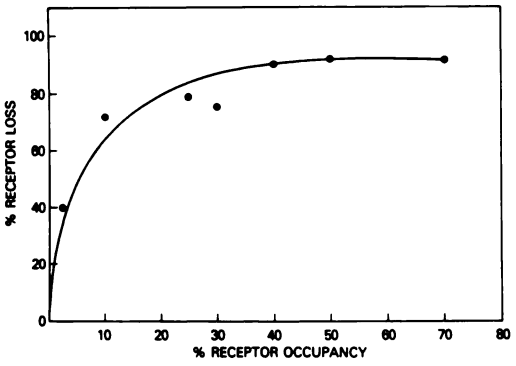

FIG. 6. *Relationship between occupancy of receptor sites and extent of receptor loss*

Free receptor sites were determined at 24 hr, while the maximum degree of occupancy was determined at 5 hr, as described in METHODS, from a number of experiments using various doses of hCG. Receptor occupancy and receptor loss were each calculated as a percentage of total receptor capacity.

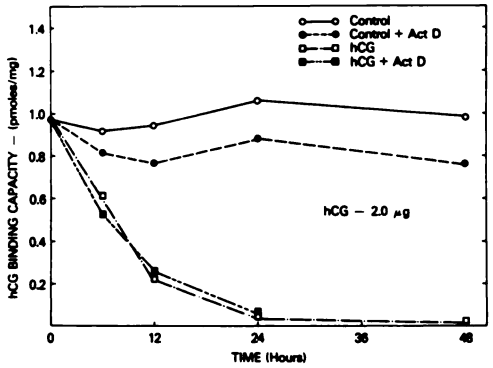

FIG. 7. *Effect of actinomycin D on hCG-induced receptor loss in luteinized rat ovary*

Actinomycin D (100 μg/100 g of body weight) was given 3 hr before and at the time of desensitization by hCG.

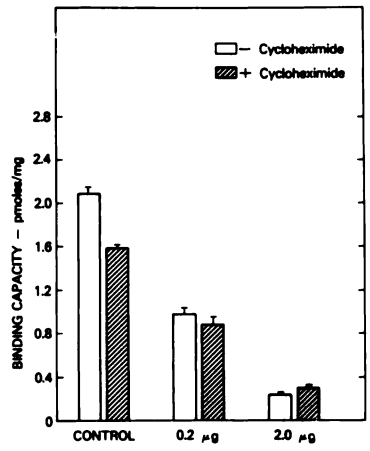

FIG. 8. *Effect of cycloheximide on hCG-induced receptor loss in luteinized rat ovary*

Cycloheximide (100 μg/100 g of body weight) was given intraperitoneally 0.5 hr before and at the time of hCG desensitization, and rats were killed 24 hr later.

not alter the LH stimulation of adenylate cyclase in control or desensitized ovarian tissue (data not shown).

DISCUSSION

Administration of hCG to female rats leads to a loss of LH/hCG receptors and a corresponding decrease in the ability of LH to stimulate adenylate cyclase *in vitro*. The present report describes several of the qualitative and quantitative aspects of this phenomenon, and indicates possible mechanisms for the receptor loss. The process of receptor loss is very sensitive to gonadotrophic hormone, with detectable losses at desensitizing doses of hCG as low as 20 ng/100 g of body weight. This dose of hCG produces serum concentrations of hCG that are within physiological levels of LH in the rat. These observations can account for the report of Marsh *et al.* (21) that rat ovarian tissue becomes refractory to LH stimulation of cyclic AMP formation following hCG treatment. In both ovary and testis (22) it is clear that the initial loss of free binding capacity is due to occupancy of receptors by the hCG used to induce the refractory state. In the ovary, this condition persists for about 6 hr (Fig. 2) and is followed by a second phase in which the total receptor number declines rapidly, reaching a nadir at 24 hr. The time course of receptor loss was not altered when hCG was given by intravenous injection, suggesting that receptor occupancy initiates processes that cause the loss of receptors and are independent of the presence of hCG in the circulation. The persistence of decreased tissue receptor content after gonadotrophin treatment is also unrelated to circulating hCG concentrations. In attempts to recover receptors during the process of receptor loss by treating the preparation with nonionic detergents, no increase in receptors was observed after solubilization (data not shown). Therefore the first stage of receptor loss may be associated with irreversible inactivation of the receptor, rather than with sequestration or occlusion within the cell membrane.

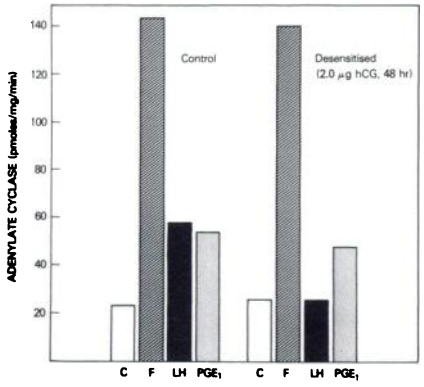

FIG. 9. *Specific loss of LH stimulation of adenylate cyclase in desensitized rat ovary*

Primed rats were desensitized with 2.0 μg of hCG and killed 48 hr later. Adenylate cyclase was assayed kinetically as described in METHODS, and the rate was determined from the line of fit. The drugs were present at the following concentrations; LH, 10 μg/ml; prostaglandin E_1 (PGE_1), 1 μg/ml; NaF, 10 mM.

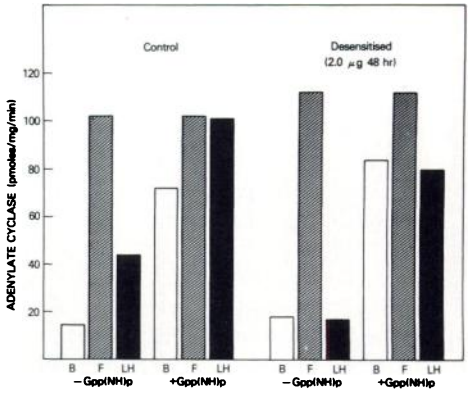

FIG. 10. *Effect of Gpp(NH)p on adenylate cyclase activity in control and desensitized rat ovaries*

Primed rats were treated with 2 μg of hCG subcutaneously and killed 24 hr later. Adenylate cyclase was assayed in the absence and presence of 0.1 mM Gpp(NH)p, without and with addition of 10 mM NaF and 10 μg/ml of LH.

In studies in the testis, the desensitizing doses of hCG caused a transient increase in cyclic AMP and testosterone production before the development of the refractory state (22). Thus it appears that effective hormone-receptor coupling is required in order to achieve the refractory state. Other evidence for this hypothesis is the finding that occupation of catecholamine receptors by adrenergic antagonists will not lead to desensitization (3). Also, a mutant lymphoma cell lacking hormone-sensitive ade-

nylate cyclase, but with apparently normal catecholamine receptors, does not show receptor loss in desensitizing experiments (23). Normal hormonal action therefore may require the formation of a hormone-receptor-adenylate cyclase complex which is only transiently active in producing cyclic AMP. It is further postulated that the active complex functions for a relatively short time, perhaps 30–60 min, after which it becomes inactive and refractory to further hormonal stimulation. This situation is seen during the early phase of desensitization in the ovary, when there is no loss in total receptor number (Figs. 2 and 4) and yet the adenylate cyclase response to LH *in vitro* can no longer be evoked (9). During this period the complex may be undergoing changes in the cell membrane, or it may be internalized by the cell in a manner analogous to that described for epidermal growth factor (24). From 6 to 24 hr a second phase occurs, in which receptor occupancy, measured either directly (Fig. 2) or by radioactive tracer (Fig. 4), declines in association with a marked fall in total receptor sites. The extensive desensitization seen with low doses of hCG is illustrated in Fig. 6. It is clear that only a small degree of occupancy causes a much greater loss of receptor number; for example, 1% occupancy caused about a 50% loss in 24 hr. These observations, as well as similar findings for the growth hormone receptor (2), strongly suggest that there may be an active or cooperative process involved in the mechanism of receptor loss. If indeed receptors do have a one-time function with respect to activation of adenylate cyclase, the existence of reserve receptors could serve as a mechanism to maintain the capacity to respond when a fraction of the receptor population has already interacted with the hormone. As the hormone concentration is increased, receptor loss is amplified and additional occupancy leads to even greater degrees of receptor loss. Such a process could function as a limiting mechanism to prevent overstimulation of the target cell and the biological consequences of excess hormone production.

A critical question with respect to the desensitization phenomenon is whether changes in receptors and adenylate cyclase are reflected in altered biological effects of these hormones in their respective target cells. In interstitial cells prepared from desensitized testes (22), receptor loss is accompanied by a failure to respond to hCG with cyclic AMP and testosterone production. In luteal cells prepared from desensitized ovaries, progesterone production in response to hCG is also impaired.[3] In both systems there is a slow recovery of the steroid response to gonadotrophic hormone, which corresponds approximately to recovery of the receptor population. Thus the hormone-induced changes in receptors and adenylate cyclase are reflected in altered biological effects of these hormones. The observations reported here, and other studies on the desensitization phenomenon, strongly suggest that these changes are an important aspect of the mechanism by which hormones alter cellular processes.

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