REGULATION OF LUTEINIZING HORMONE RECEPTORS IN TESTICULAR INTERSTITIAL CELLS BY GONADOTROPIN

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Summary

The concentration of rat testicular receptors for luteinizing hormone, measured by specific binding of [^{125}I] chorionic gonadotropin, was reduced for several days after a single injection of gonadotropin. Rapid and prolonged loss of receptors was observed after high doses of chorionic gonadotropin, and a delayed but equally prolonged loss of receptors occurred after low doses of chorionic gonadotropin, and the rapidly metabolized gonadotropin, ovine luteinizing hormone. These findings demonstrate that luteinizing hormone receptors are subject to regulation by elevated gonadotropin concentrations, and suggest that receptor loss is initiated by occupancy of a small proportion of the available receptor sites.

The actions of gonadotropins upon testicular interstitial cells are initiated by binding to specific, high-affinity receptors located upon the plasma membrane. Occupancy of such gonadotropin receptors by LH* or hCG* has been shown to stimulate steroidogenesis and cyclic AMP* formation in dispersed interstitial cells (1). The binding properties of testicular LH receptors have been extensively analyzed *in vitro* (2,3), and the receptor sites have been solubilized, characterized and partially purified (4,5). However, the regulation of gonadotropin receptor concentration in the interstitial cell has not been examined in detail. The present studies were performed to determine whether the testicular concentration of LH receptors is regulated by exposure to elevated gonadotropin levels.

Materials and Methods

Immature intact and hypophysectomized male rats of 90-100 g body weight were obtained from Hormone Assay Co., Chicago, Ill., and adult male rats

* Abbreviations: Luteinizing Hormone, LH; human chorionic gonadotropin, hCG; adenosine-3',5'-monophosphate, cyclic AMP.

(250-300 g) from Charles River Labs., Wilmington, Mass. The hypophysectomized animals were used 10 days after operation. Ovine LH (NIH S-19) or hCG (Pregnyl, Organon; 3000 IU/mg) were dissolved prior to subcutaneous injection in 0.5 ml of 0.01 M phosphate buffer pH 7.4 containing 0.15 M NaCl and 0.1% bovine serum albumin. Animals were then killed at various intervals, serum was collected for hCG or testosterone assay, and testes were decapsulated and assayed for LH receptor content. All assay solutions and particulate binding fractions were prepared in the phosphate-buffered medium described above.

Testicular concentrations of LH receptors were determined by in vitro binding assays, employing [^{125}I]labeled hCG prepared by radioiodination with lactoperoxidase as previously described (8). Decapsulated testes were homogenized in buffer in a tissue blender at 13,000 rpm for one minute. After centrifugation at 20,000 g for 15 minutes, the pellet was resuspended in 40 ml buffer, centrifuged again and rehomogenized to give a final tissue concentration of 100-200 mg/ml. LH receptor concentrations were determined by incubation of serial dilutions of homogenate with a saturating concentration of labeled hormone. The following reagents were sequentially added to 12 x 75 mm glass tubes: 100 µl of buffer, with or without 100 IU hCG; 50 µl of [^{125}I]hCG (200,000 cpm, 5 to 10 ng); and 100 µl of tissue suspension. Three serial 1:1 dilutions of the tissue suspension were analyzed in triplicate by incubation at room temperature for 16-18 hours. The incubation mixtures were then diluted with 5 ml of ice-cold buffer and centrifuged at 1500 x g for 15 minutes. The sediments were washed once and bound radioactivity was determined in a γ-spectrometer. The amount of specificially bound radioactivity at saturation was expressed as picomoles of bound hormone on the basis of the measured specific activities of the tracer preparations. Serum hCG was assayed by a double antibody radioimmunoassay procedure, and serum testosterone was measured by radioimmunoassay following extraction and LH-20 column chromatography (9,10).

Results

Administration of 50 and 200 IU of hCG to immature male rats was followed by a marked reduction in gonadotropin binding capacity between 3 and 24 hours after injection (Fig. 1). The available binding sites remained low for at least 2 days, and returned to the control level at 6 days. In contrast, the injection of 5 IU hCG was followed by a slight increase in receptor binding after 8 hr., with a subsequent inhibition of receptor binding between 8 and 48 hours, and returned to the control level after 6 days (Fig. 1).

Similar experiments were performed in hypophysectomized immature rats to eliminate interference by endogenous gonadotropins. As shown in Fig. 2, injection of 500 IU hCG caused a drop in [^{125}I]hCG binding within 3 hours. The receptor content remained low for at least 4 days, and returned to the control level at 7 days. Injection of 5 IU hCG initially caused a slight increase in hCG binding for several hours, and the number of available receptors remained near the control level for 24 hours. However, the concentration of binding

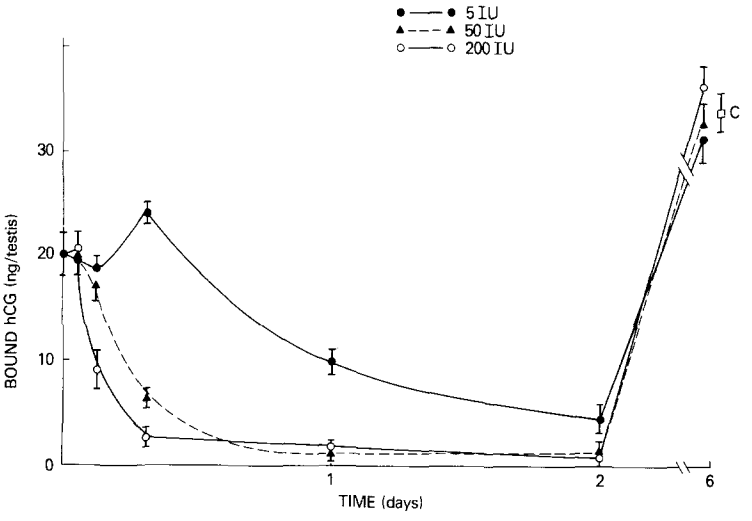

FIGURE 1. Binding of [^{125}I]hCG to testicular LH receptors of immature intact rats after single injections of 5, 50 and 200 IU hCG. C = control level.

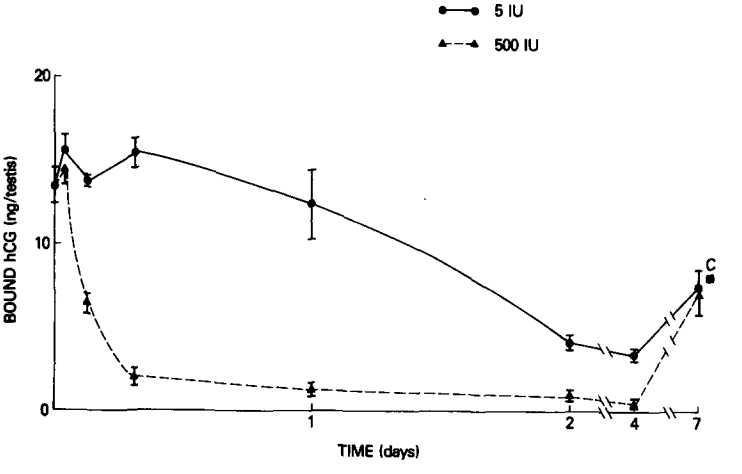

FIGURE 2. Binding of [^{125}I]hCG to testicular LH receptors of immature hypophysectomized rats after single injections of 5 and 500 IU hCG. C = control level.

sites then decreased over the next 24 hours and remained low until the 4th day. By the 7th day, the receptor content had returned to the control level.

In animals that received 5 and 500 IU hCG, serum hCG peaked after 8 hr.

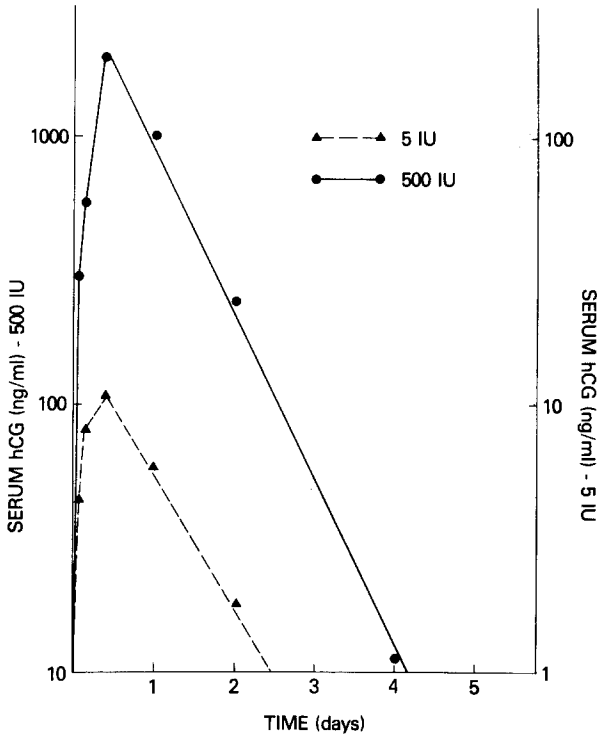

FIGURE 3. Serum hCG concentration in immature hypophysectomized rats after single injections of 5 and 500 IU hCG.

at 11 and 2000 ng/ml respectively and fell with a half-life of about 24 hours (Fig. 3). After 5 IU hCG, serum hCG decreased from 6 to 2 ng/ml between 1 and 2 days after injection. Thus, the decrease in hCG binding capacity in animals receiving 5 IU hCG did not reflect the circulating hCG concentrations, and the sustained loss of receptor binding capacity between 2 and 4 days occurred in the absence of significant circulating gonadotropin levels.

To demonstrate further that the prolonged inhibition of testicular hCG binding was not related to the presence of circulating gonadotropin, intact adult animals were injected with 500 μg of ovine LH, which has an extremely short plasma half-life (15 min.) in rats (11). The blood level of testosterone rose sharply one hour after hormone injection and returned to the control level 12 hrs. later (Fig. 4). In contrast to the rapid rise and fall of serum

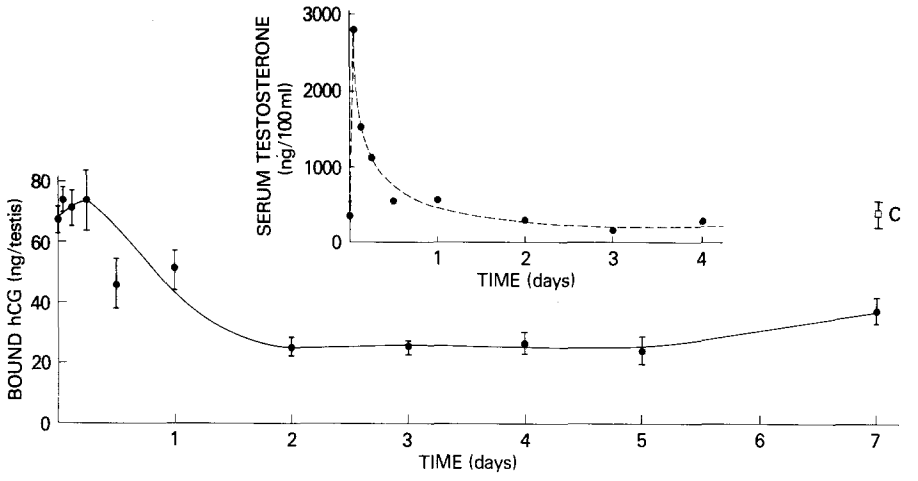

FIGURE 4. Testicular binding of [^{125}I]hCG and serum testosterone (insert) in adult male rats after administration of 500 µg ovine LH. C = control level.

testosterone, testicular LH receptors rose slightly over 6 hrs., then decreased to one-third of the control level at 48 hrs. and remained low for up to 5 days after injection. Thus, the sustained loss of binding sites induced by hCG was also apparent after ovine LH, indicating that regulation of LH receptors is a property of both gonadotropins.

Discussion

A prolonged decrease in available LH/hCG binding sites after a single injection of exogenous gonadotropin has been demonstrated in the testes of immature, hypophysectomized and adult rats. Although the initial rapid fall in gonadotropin receptors after high doses of hCG (200 and 500 IU) could be due to occupancy by exogenous hormone, the onset of receptor loss was delayed in animals that received a low dose (5 IU) of hCG, or the rapidly metabolized gonadotropin, ovine LH. These observations indicate that negative regulation of gonadotropin receptors by exogenous gonadotropins occurs in a manner analogous to the hormone-dependent regulation of insulin and growth hormone receptors by elevated ligand concentrations (6,7).

The sustained inhibition of hCG binding in testes after exogenous gonado-

tropin could result from several mechanisms. First, occupancy of receptor sites by exogenous gonadotropin could obviously inhibit subsequent binding in vitro, particularly in animals that received the higher dose (50 to 500 IU) of hCG. Second, the receptors could be temporarily inactivated after hormone binding, and the binding sites may only slowly be reactivated. Third, the receptor molecules may be completely degraded after occupancy, rather than recycled for future use. Finally, in addition to degradation or inactivation of occupied receptors, the interaction of receptors with gonadotropin could initiate a further regulatory process which leads to loss of unoccupied receptors. Such a process could operate via increased degradation of existing receptors, or by inhibition of synthesis of new receptors. Negative regulation of receptors is particularly likely to have occurred in animals that received 5 IU hCG or 500 μg ovine LH, since the decrease of available binding sites was delayed (Figs. 1, 2 and 4) and did not reflect the blood levels of gonadotropin (Fig. 3) and testosterone (Fig. 4).

Measurement of LH receptors in animals treated with ovine LH also revealed that inhibition of receptor binding was delayed until 24 hours to 48 hours after injection. However, serum testosterone levels indicated that ovine LH evoked maximum testosterone production within 1 hour, with a subsequent rapid decline to control values (Fig. 4). The association of a marked testosterone response with no decrease in available receptor sites indicates that only a small fraction of the receptor population needs to be occupied to evoke a major steroidogenic response of the testis. This finding is consistent with the existence of "spare" receptors for LH in the rat testis as previously demonstrated in vitro (1,12). These results establish the existence of negative regulation of testicular LH receptors by LH and hCG, and suggest that an active process of receptor loss is initiated by occupancy of a relatively small proportion of the available receptor sites.

The negative regulation of LH receptors by increased gonadotropin levels in vivo could provide an explanation for the clinical observation that men with

hCG-producing tumors do not show the expected elevation of testosterone secretion (13). While the absence of a testosterone response in such patients may be related to the associated decrease in secretion of follicle-stimulating hormone (14), the present observations provide support for the proposal (6) that gonadotropin-induced loss of LH receptors in the gonads could be responsible for this phenomenon. Furthermore, recent studies in this laboratory have shown that hormone-induced loss of testicular LH receptors, as demonstrated in this paper, is also characterized by loss of cyclic AMP and testosterone responses to gonadotropin (Hsueh, Dufau and Catt, in preparation). Similarly, we have demonstrated the occurrence of gonadotropin-induced loss of LH/hCG receptors and adenylate cyclase in the ovary (Conti et al., submitted for publication). These observations and others (6,7,15,16) are consistent with the view (6) that hormone-dependent receptor regulation is an important and general mechanism *in vivo*.

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