Regulation of Receptor Concentration by Homologous Hormone

EFFECT OF HUMAN GROWTH HORMONE ON ITS RECEPTOR IN IM-9 LYMPHOCYTES*

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When cultured human lymphocytes of the IM-9 line were exposed to human growth hormone (hGH) at 37°C, washed for 2 hours, and incubated with 125I-hGH, the binding of 125I-hGH was reduced. The magnitude of the reduction in binding was dependent on the concentration of growth hormone present as well as the duration of the exposure. As little as 2 \times 10^{-11} M (0.5 ng/ml) growth hormone had a discernible effect. Growth hormone at 2 \times 10^{-10} M (5.0 ng/ml), which is a low resting concentration of hormone in vivo and occupies about 20% of the receptors at steady state at 30°C, produced a 50% reduction in binding while 20 ng/ml, which occupies about 50% of the receptors under steady state conditions, produced an 80% loss of receptors. Further increases in growth hormone concentration produced little further effect on receptor loss. Thus, the loss of receptors at a given concentration of growth hormone (up to 20 ng/ml) in the preincubation at 37°C was greater than the occupancy produced by that concentration of growth hormone receptors under steady state conditions at 30°C. Analysis of the data indicated that the decrease in binding of 125I-hGH was due to a loss of receptors per cell without any change in affinity of receptor for hormone or in cell number.

The concentration of insulin receptors on these cells was affected by the insulin concentration in the medium, and the concentration of growth hormone receptors was affected by growth hormone, but neither hormone had any effect on the heterologous receptors. Exposure of the cells to cycloheximide (0.1 mM) produced a progressive but smaller loss of growth hormone receptors, and the effect of cycloheximide was additive to the receptor loss induced by growth hormone, suggesting that cycloheximide inhibited synthesis of receptors while growth hormone accelerated loss of receptors.

When growth hormone was removed from the medium, receptor concentrations were restored rapidly; half of the loss was restored by 6 to 8 hours and the full complement of receptors was restored by 24 hours following removal of the hormone. If the growth hormone was removed and replaced with cycloheximide, the return of the receptors was delayed until the cycloheximide was removed. Thus restoration of the receptors appeared to require the synthesis of new proteins. These data indicate that in the IM-9 lymphocytes the concentration of growth hormone receptors is very sensitive to regulation by growth hormone and also add further support to the suggestion that hormones in general actively regulate the concentration of their own receptors.

Obesity, hyperinsulinemia, and insulin resistance are associated with a decrease in insulin receptor concentrations per cell or per unit of cell surface. Correction of the hyperinsulinemia is associated with amelioration of the metabolic abnormalities and a restoration of insulin binding to normal. Extensive studies in vivo in both rodents and man have demonstrated under many circumstances a remarkable inverse correlation between the chronic (but not acute) concentration of circulating insulin and the concentration of insulin receptors on target cells (1–6). This relationship has been demonstrated directly in cell culture. When cultured lymphocytes of the IM-9 line were exposed to insulin, washed, and incubated with 125I-insulin, there was a loss of insulin receptors that was time-, temperature-, and insulin concentration-dependent (7). The decrease in 125I-insulin binding was totally attributable to a decrease in the number of insulin receptors per cell rather than to any alterations in affinity of the receptors for insulin.

The IM-9 lymphocytes also have cell-surface receptors specific for human growth hormone (8, 9) and in the present study we show that the growth hormone receptors on these cells are even more sensitive to regulation by the ambient concentration of growth hormone than was the insulin receptor upon exposure to insulin.

* Portions of this study were presented at the annual meeting of the American Society for Clinical Investigation and American Federation for Clinical Research, May, 1974 (14).
EXPERIMENTAL PROCEDURE

Materials

In these studies we used immunochemical grade human growth hormone preparations (1601 and 1623D), and porcine and bovine (NIH-B16) growth hormone, generously supplied by Dr. A. E. Wihelm and the National Pituitary Agency. Na<sup>125</sup> (IMS > 300) was purchased from Amersham/Searle Company; [H<sup>1</sup>]leucine from New England Nuclear; chloramine-T method at specific activities of 50 pCi/ng, or an average 80% of the labeled hormone was bound (Fig. 1, upper curve); unlabeled hGH competed with <sup>125</sup>I-hGH for binding so that unlabeled IGH at 2.0 ng/ml reduced specific binding of <sup>125</sup>I-hGH by about 10%, and at 20 ng/ml produced a 50% reduction in specific binding of labeled hGH (Fig. 1, upper curve).

When the cells were preincubated under identical conditions except in the presence of unlabeled hGH at 10<sup>-11</sup> M (2 ng/ml), washed, and reacted with <sup>125</sup>I-hGH in the presence of unlabeled hGH, the binding of <sup>125</sup>I-hGH was reduced by 80% (Fig. 1, lower curve). Each concentration of unlabeled growth hormone in the binding assay always produced the same percentage reduction in the specific binding of the <sup>125</sup>I-hGH to receptors with all sets of cells irrespective of what concentration of hGH had been present during the preincubation, indicating that the reduced binding of <sup>125</sup>I-hGH was due to a reduction in the number of receptor sites per cell without any change in affinity; this conclusion was confirmed by

<sup>1</sup> The abbreviations used are: PBS, phosphate-buffered saline; hGH, human growth hormone; FSH, follicle stimulating hormone; LH, luteinizing hormone; Hesper, N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid.
Scatchard analysis of the data\(^\text{a}\) (13). It should be noted that 2 ng/ml (10\(^{-16}\) M) of growth hormone is a low resting physiological concentration of the hormone \textit{in vivo} and that when present in the assay produces only about a 10% fall in the binding of labeled hormone, \textit{i.e.} occupies only 10% of the receptor sites at steady state, whereas this hormone concentration when present in the preincubation medium for 16 to 18 hours produced a 30 to 50% loss of receptor sites (14).

**Time Course of Receptor Loss**—The loss of growth hormone receptors upon exposure of these cells to unlabeled growth hormone at 37\(^\circ\) (\textit{i.e.} during "preincubation") increased as a function of the duration of the preincubation (Fig. 2). With unlabeled hGH at 2 \(\times\) 10\(^{-10}\) M (5 ng/ml) preincubation of 45 to 120 min produced a 20% decrease in concentration of hGH receptors per cell; by 4 hours of preincubation there was a 35% decrease in receptors and by 24 hours, a 55% decrease. As noted earlier, the shapes of the competition curves in the assay were identical; each concentration of unlabeled hormone in the assay produced an identical percentage fall in the binding of the labeled hormone, \textit{e.g.} 20 to 30 ng/ml of unlabeled hGH in assay produced a 50% reduction in specific binding in nearly all experiments. Thus these data again confirmed that the reduction in binding was due entirely to a decrease in number of receptor sites without any alteration in affinity, and Scatchard analyses were consistent with that conclusion.

Acute exposure of the cells to 5 ng/ml of hGH at the end of the preincubation but before the wash produced no alteration in the binding of \(^125\)I-hGH (Fig. 1, \textit{upper curve}). This was similar to the results obtained with insulin receptors where chronic exposure of the cells to insulin at 37\(^\circ\) both \textit{in vivo} and \textit{in vitro} produced a loss of insulin receptors while acute exposure was without effect (1, 2, 7, 15, 16). The temperature over the range of 15-37\(^\circ\) during the preincubation had little effect on the binding of \(^125\)I-hGH, this is in contrast to insulin effects on the insulin receptor which were significantly diminished with preincubation at 15-22\(^\circ\) (7).

**Effect of Concentration of hGH on Receptor Loss**—The rate and extent of receptor loss was dependent upon the concentration of growth hormone (Fig. 3, \textit{left}). With unlabeled growth hormone in the preincubation medium at 2 \(\times\) 10\(^{-9}\) M (400 ng/ml), a very high concentration that is occasionally observed in vivo, there was an 85% loss of receptors by 4 hours with no further change up to 24 hours of preincubation. A 10-fold lower concentration of growth hormone in the preincubation medium produced less of a loss of receptors with a maximum effect observed by 4 hours and a steady state up to 24 hours. With growth hormone at 2 \(\times\) 10\(^{-10}\) M there was a 40% receptor loss by 4 hours which persisted at 8 hours, followed by a restoration of about one-fourth of the lost receptors by 24 hours. With growth hormone at 2 \(\times\) 10\(^{-11}\) M there was a 25% loss of receptors by 8 hours followed by restoration of about half of the lost receptors by 24 hours. \textit{Initially we had attributed the secondary rise in growth hormone receptors at late time points to partial depletion of hormone from the preincubation medium. However, when aliquots of the preincubation medium were removed and hormone content was measured by radioimmunoassay...***

\(^*\)These experiments were performed over several years using two different lots of hGH and multiple passages of different batches of lymphocytes of the IM-9 line. We think that modest changes in the potency of the hormone with time of storage or responsiveness of the cells, or both, accounts for the fact that 2 \(\times\) 10\(^{-11}\) M hormone had no effect in Fig. 1, whereas it had a definite effect in Fig. 3; 2 \(\times\) 10\(^{-10}\) M from 4 to 24 hours in Fig. 2 had an increase in receptor loss but had a smaller recovery of receptors in Fig. 3; 2 \(\times\) 10\(^{-9}\) M hGH had an effect equal to 2 \(\times\) 10\(^{-8}\) M hormone in Fig. 1, but a smaller effect in Fig. 3; the total binding capacity per cell in Fig. 2 is almost twice that in Fig. 1: this brackets the range of observed differences.

\(^a\)F. C. Kosmakos, J. Roth, and J. R. Gavin. III. manuscript in preparation.

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Effect of Human Growth Hormone on Its Receptors

Fig. 3. Left, time course of growth hormone-induced loss of growth hormone receptors. All cells were preincubated for 24 hours. At intervals growth hormone was added. After 24 hours at 37° the cells were sedimented, supernatants discarded, and the cells were washed for 2 hours and incubated with 123I-hGH for measurements of specific binding of 123I-hGH to the cells. The specific binding of 123I-hGH is plotted as a function of the duration of time during which the growth hormone was present in the preincubation and is expressed as a per cent of specific binding of 125I-hGH in the controls. The controls were cells that were preincubated under identical conditions except in the absence of hGH. Right, radioimmunoassay of hGH in medium of cells preincubated with hGH. Cells were preincubated with hGH as described above. At intervals the cells were sedimented and an aliquot of the cell-free medium was removed, and the concentration of hGH was measured by radioimmunoassay. The concentration of hGH in nanograms/ml in the supernatant as determined by radioimmunoassay is plotted as a function of duration of preincubation of cells with hGH. The solid symbols are the concentrations of hGH in the supernatant of cells described for the left panel. The open symbols represent data from another similar experiment.

say, we detected little or no loss of hormone during 24 to 48 hours of preincubation (Fig. 3, right). We have not excluded the remote possibility that during the preincubation, reactivity of the hormone for receptor was markedly reduced with no effect at all on its immunoreactivity.

Receptor Loss Versus Receptor Occupancy—We compared the effect of a given concentration of hGH on loss of receptors when preincubated with cells for 4 to 24 hours at 37° with the occupancy of receptor sites at steady state when incubated with 123I-hGH for 90 min at 30° in the binding assay. With hGH at 10^{-11} M to 10^{-8} M (0.2 to 200 ng/ml), loss with preincubation exceeded that of occupancy under steady state conditions (Fig. 4). Thus, 2 ng/ml (10^{-10} M) of growth hormone occupied only about 10% of the receptor sites under steady state conditions of the binding assay but when present in the growth medium for 24 hours at 37° produced a 30 to 50% decrease in receptor sites (Fig. 4). Growth hormone at 20 to 30 ng/ml (10^{-9} M), which occupied 50% of the receptors, produced a 75% loss in receptor sites. With hGH at 50 ng/ml loss reached 80 to 85% of the receptors while occupancy was 60%; at higher hormone concentrations, no further loss was observed while occupancy increased to 100%.

Both theoretical and experimental data indicate that the cells that had been preincubated with hGH did not contribute significant amounts of unlabelled hGH to the medium during the binding assay. In the preincubation studies, growth hormone at 5 ng/ml had been incubated with 10^6 cells/ml. From our studies of growth hormone binding at steady state at 30° we calculate that at these concentrations of cells and hormone, only about 1% of the growth hormone was bound to the cells (of which about 90% was specific binding and 10% nonspecific), which would be equivalent to 50 pg of growth hormone on 10^6 cells. Thus, in the binding assay, where we measure the receptor concentration, we used 10^6 cells/ml and we expect that the cells contained at most a total of 500 pg of growth hormone/ml. Even if all of this growth hormone had been released into the medium during the binding assay at 30°, no effect would have been observed on the binding of 125I-hGH.

Alternatively, we calculate what contamination of the incubation medium by growth hormone during the binding assay would have been necessary to yield a 50% reduction in binding of 125I-hGH. For this result (per ml of solution), the growth hormone would have to have been about 20 ng, which would have to have been carried by the 10^6 cells. This would be equivalent to 2 ng on 10^6 cells during the preincubation. Thus, cells that had been preincubated with 5 ng/ml of growth hormone needed to have taken up and held 40% of the total growth hormone in the medium despite the fact that the cells occupy an infinitesimal fraction of the total volume of the preincubation medium. They would then have retained all of the growth hormone (2 ng) through the washes (2 hours) and then released all of it into the incubation medium early during the 90-min binding assay. First, by immunoassay of the cell-free supernatant of preincubation media (see above and Fig. 3, right) it is clear that half of the hGH was not taken up by the cells during the preincubation. Second, cells were preincubated with hGH at 2 x 10^{-10} M and 2 x 10^{-9} M (5 and 50 ng/ml), washed, and incubated for 90 min at 30° under conditions of the binding assay except that 125I-hGH was omitted. Aliquots of this cell-free supernatant, when added to fresh cells in the presence of 125I-hGH for 90 min at 30°, had no effect on 125I-hGH binding (Table I). Thus, cells that had been preincubated with hGH and washed released undetectably low amounts of hGH into the medium during the binding assay.
that had been exposed for 24 hours to this concentration of
for 24 or 48 hours lost about 75% of their receptors. When cells
concentration of insulin receptors on the cells was determined
effect from added growth hormone (Table II).

The following experiment was performed. We preincubated the
cells with hGH (5 or 50 rig/ml) or insulin (10 or 100 rig/ml), or
been exposed to growth hormone at concentration of 50 rig/ml
recovery by 24 hours (Fig. 7, left). If the growth hormone was
removal of growth hormone is due to exposure to growth hormone
accounts for the greater scatter of data in these experiments.

Effect of hGH and Insulin on hGH and Insulin
Receptors—Since the concentration of hGH receptors on the
IM-9 lymphocytes can be modulated by exposure to hGH and
the concentration of insulin receptors in the same cell line can
be modulated by insulin, we wanted to determine if there was
an effect of one hormone on the receptors of the other hormone.
The following experiment was performed. We preincubated the
cells with hGH (5 or 50 ng/ml) or insulin (10 or 100 ng/ml), or
both. The concentration of growth hormone receptors was
determined by the concentration of growth hormone to which
the cells had been exposed during the preincubation without
any effect of the added insulin (Fig. 5). Similarly, the
concentration of insulin receptors on the cells was determined
by the concentration of insulin in the preincubation with no
effect from added growth hormone (Table II).

Recovery of Receptors—Cells that had lost their receptors
due to exposure to growth hormone recover their full comple-
ment of receptors within 24 hours after removal of growth
hormone from the medium. Fig. 6 shows that cells that had
been exposed to growth hormone at concentration of 50 ng/ml
for 24 or 48 hours lost about 75% of their receptors. When cells
that had been exposed for 24 hours to this concentration of
Period under the same conditions. When cells that had been exposed to cycloheximide or to hGH for 24 hours were transferred to fresh medium for 24 hours both sets of cells recovered nearly all of their receptors (Fig. 8). In another experiment when the cultured human lymphocytes were exposed to cycloheximide (10^{-3} M) for 24 hours at 37° about half of the receptor sites were lost. Cycloheximide at 10^{-2} M concentration appeared to produce a modestly greater effect than 10^{-3} M or 10^{-4} M but also caused a much greater loss of cell viability (Fig. 9).

The effect of cycloheximide on the concentration of growth hormone receptors per cell was time- and concentration-dependent. With cycloheximide at 10^{-1} M, a fall in receptor content was noted by 2 to 5 hours of exposure with a maximum effect observed by 8 hours. Further incubation to 18 hours caused a further increase in nonviable cells but no further loss of growth hormone receptors per cell. With cycloheximide at 10^{-4} M the loss of growth receptors was slower but by 10 hours the same magnitude of effect was achieved (Fig. 10). Note that with both concentrations of cycloheximide there was a time-dependent increase in the fraction of nonviable cells. Because with prolonged exposure to cycloheximide, there was an increase in the fraction of nonviable cells, further experiments were performed with only short exposures to cycloheximide.

With cells exposed to cycloheximide at 10^{-4} M for 2 to 6 hours there was only a small reduction in receptor number. When these cells were exposed for 6 hours to growth hormone at 5 ng/ml they had a 60% loss of receptors; if hGH had been left in for 24 hours, it would not have produced any further loss than that which had been observed at 6 hours. Thus the effect of cycloheximide appears to be slower than that observed with growth hormone. When both cycloheximide and growth hormone were added together to the cells for the 6-hour period, an additive loss of receptor content was observed (Fig. 11). The results of these experiments suggested that the effect of growth hormone appeared to reach a steady state within a few hours, whereas the effect of cycloheximide appeared to continue for longer time periods. Further, when the two agents were present together, their effects appeared to be additive, suggesting that cycloheximide inhibited the synthesis of new receptors while growth hormone accelerated the degradation of receptors.

Table III shows that in our system cycloheximide at 10^{-4} M concentration inhibited protein synthesis, reducing the incorporation of radioactive leucine into trichloroacetic acid-precipitable material by 90% or more; insulin and hGH had only a modest effect on the incorporation of [3H]leucine.

**DISCUSSION**

The present study shows that the concentration of growth hormone receptors on the IM-9 lymphocytes is directly related to the concentration of growth hormone to which the cells had been exposed. The magnitude of this effect was dependent both on the concentration of growth hormone and on the duration of the exposure. The observed effect appears to be exclusively one of changing the concentration or number of receptors per cell rather than any effect on the affinity of the receptor for hormone. The effect was limited to the growth hormone receptors; the insulin receptors on these same cells were totally unaffected, and conversely, insulin, while it affected the insulin receptor, had no effect on the growth hormone receptors. The studies with cycloheximide and growth hormone suggest to us that growth hormone acts by accelerating the loss of receptors while cycloheximide inhibits the synthesis of the receptors. There is also a suggestion that growth hormone in addition to markedly accelerating the loss of receptors may produce a modest stimulation of receptor synthesis. This may account for the observation that the maximum effect of 10^{-11} to 10^{-10} M growth hormone on receptor concentration may occur early and that prolongation of the exposure to hormone at that concentration may result in a level of receptor that is somewhat above the level observed early after exposure (Fig. 3); studies will be needed to clarify this point.

The loss of receptors does not appear to require rapid protein synthesis since in the presence of both cycloheximide and growth hormone the effect of growth hormone was additive to...
Fig. 7. Reappearance of receptors after removal of hGH. Cells were preincubated with and without hGH, \(2 \times 10^{-10} \text{M} \) (5 ng/ml), for 24 hours and washed for 2 hours (see "Methods"). The washed cells were resuspended in growth medium at 37°C. Cells that had been preincubated in the absence of growth hormone were resuspended in growth medium without growth hormone ("controls"). The data shown on the left are from cells that had been preincubated with growth hormone and resuspended in the presence of cycloheximide, \(10^{-4} \text{M} \), (dashed line, solid circle). The data on the right are from cells that had been preincubated with growth hormone and resuspended in the presence of cycloheximide, \(10^{-1} \text{M} \), (solid line, solid squares). The cells that had been resuspended in growth medium with cycloheximide were transferred after 6 hours to fresh growth medium that contained neither cycloheximide nor growth hormone (right, dashed lines, open squares). Incubation in the absence of both agents (solid lines, open circles) is shown in both panels. The specific binding of \(^{125}\text{I}-\text{hGH}\) is expressed as a per cent of the specific binding of \(^{125}\text{I}-\text{hGH}\) to controls. The control cells specifically bound 12% of the \(^{125}\text{I}-\text{hGH}\); cells that had been preincubated for 24 hours in growth hormone had reduced their receptors to 42% of that level (this is indicated as time zero). The horizontal axis is the time in hours following the completion of the wash step.

Fig. 8. Effect of preincubation with cycloheximide or with hGH on \(^{125}\text{I}-\text{hGH}\) binding. Cells were preincubated for two consecutive 24-hour periods in the presence and absence of cycloheximide or hGH, or both (in all cases the medium was changed after the first 24 hours of the preincubation), washed and incubated with \(^{125}\text{I}-\text{hGH}\) for measurements of \(^{125}\text{I}-\text{hGH}\) binding in the presence and absence of unlabeled hGH. The percentage of the total \(^{125}\text{I}-\text{hGH}\) in the system that was bound to lymphocytes is plotted as a function of the growth hormone concentration in the binding assay.

Fig. 9. Effect of preincubation with cycloheximide on \(^{125}\text{I}-\text{hGH}\) binding. Cells were preincubated for 24 hours (without hGH) in the absence or presence of cycloheximide, washed, and incubated with \(^{125}\text{I}-\text{hGH}\) in the presence and absence of unlabeled hGH (without cycloheximide). The per cent of the total \(^{125}\text{I}-\text{hGH}\) in the assay that is bound to the lymphocytes is plotted as a function of the growth hormone concentration in the assay. Cell exclusion of trypan blue dye was determined after the wash step.

The fate of the lost receptors is not known. They may be released into the medium, inactivated, or degraded, either in situ or at intracellular sites following endocytosis. We have no data to favor any of these alternatives. It is also not clear how the cell knows to degrade receptors. It may be that the hormone combines with the receptor, activates the normal...
Interestingly, occupancy of only a small per cent of the receptors produces significant receptor loss, e.g. occupancy of only 10% of the receptors produced a 35% loss. However, at the other end of the dose-response curve, with very high hormone concentration, even those that occupy essentially all the receptors, there is only a loss of 80 to 85% of the receptors. Thus, it would appear that as receptor sites are lost the capacity of the cells to further reduce the concentration of receptors is impaired, i.e. the cells with fewer receptors are now resistant to a biological effect of the hormone. Interestingly,
given dose of growth hormone has a markedly different effect, depending upon the prior exposure to the hormone.

We have commented previously that there are numerous examples both in vivo and in vitro where chronic exposure of target cells to high levels of hormone results in a decrease in sensitivity of the target cell to the hormone and conversely the chronic deprivation of the stimulatory agent may result in an increased sensitivity to the introduction of the stimulatory agent. We suggested from our studies with insulin receptors that this effect is mediated at the level of the target cell receptor and that this mechanism may be widespread (7). In Table IV we have listed other examples in which chronic exposure to a hormone appears to produce a change in the concentration of the hormone’s own receptors. It should be noted that with the three hormones which produced an increase in the concentrations of their own receptors, the hormone was injected in vivo. Therefore it is not possible at the moment to exclude the possibility that the hormone did not increase its own receptor concentration directly at the target cell but did so via some intermediary agent. Also, reports of receptor loss with the steroid hormones are difficult to interpret precisely, because receptors are present in both cytosol and nucleus, and loss of cytosol binding sites may reflect translocation to the nucleus, as one step in the action of the hormone, or a net loss of total receptors (30, 31).

In addition to the effects that hormones have on the concentrations of their own receptors, we and others have shown that insulin and several other hormones also regulate the affinity of their own receptors (32-35). A hormone can regulate its own receptors, and also can act to alter the receptors of other hormones. Ranke and Parks reported that when IM-9 human lymphocytes were grown in the presence of estradiol, the binding of 125I-hGH decreased due to a fall in the affinity of hormone for its receptor without any change in the receptor concentration (36). Funder has shown that the affinity of the glucocorticoid receptor for its hormones is profoundly affected by progesterone; the latter steroid does not bind to the glucocorticoid binding sites but presumably acts by binding at another site on or near the receptor (37). Olefsky and co-workers showed that in rats treatment with glucocorticoids produced a fall in insulin binding to hepatocytes and adipocytes that appeared to be due to reductions in both receptor affinity and receptor concentration (38). Zeleznik and colleagues had shown that follicle stimulating hormone injected in vivo

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Hydrocortisone (40). Milgram and colleagues have shown that rats have an increase in thyrocalcitonin receptors in the kidney stimulated the appearance of LH receptors in the ovary (39).

Major site for regulation of target cell sensitivity. Clearly, the responsiveness of target cells to hormone, irrespective of the presence of "spare receptors." In addition, a hormone may alter the receptors of another hormone may actually be a homologous effect. Similarly insulin binding to its receptors was inversely related to chronic circulating levels of glucocorticoids, but we suspect that the circulating insulin may have been the actual mediator of this effect.

In summary, many hormones have rapid direct effects on the concentration as well as the affinity of their own receptors. In addition, a hormone may alter the receptors of another hormone. Changes in affinity or concentration of the receptors will of necessity alter the sensitivity of the target cell to stimulation by hormone, irrespective of the presence of "spare receptors." Clearly, the responsiveness of target cells to hormone can be altered by changes in any step in the pathway within the target cell. The initial locus of interaction of hormone with target cell, the receptor itself, appears to be a major site for regulation of target cell sensitivity.

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Note Added in Proof—Additional studies demonstrating the effect of a hormone on hormone receptors are as follows. Chevillotte and co-workers (42) have shown that in rats, treatment with angiotensin is followed by a decrease in the concentration of angiotensin receptors in membrane fractions of the uterus. Soloff (43) has shown that in ovariectomized rats treatment with estrogens caused an increase in both the affinity and number of the receptor sites for oxytocin.

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