We have previously reported that incubation of rat hepatoma cells with insulin causes a complete and reversible loss of responsiveness to insulin. In order to determine the role of the insulin receptor in desensitization, we have examined the effect of insulin on insulin binding. Exposure of rat hepatoma cells to insulin causes a time-dependent decrease in insulin binding capacity which is detectable at 30 min and maximal at 4-6 h, after which time insulin binding remains 40–50% that of untreated cells. Scatchard analysis indicates that insulin causes a decrease in the number of receptors with little change in the binding affinity. Insulin-induced down regulation of receptors, observable at insulin concentrations as low as 3 ng/ml, is half-maximal at 10–20 ng/ml and is maximal at 100 ng of insulin/ml. When insulin is removed from the culture medium, the cells slowly recover insulin binding capacity; recovery is minimal at 2–4 h but nearly complete after 24 h. Recovery of insulin responsiveness, in contrast, is complete as early as 2 h after insulin is removed.

The extent of down regulation of receptors (50–60%) is not sufficient to account for the complete insulin desensitization. In addition, recovery of maximal responsiveness to insulin occurs long before recovery of insulin binding. Therefore, insulin-induced desensitization to insulin is not caused by down regulation of receptors but must involve a post-receptor mechanism.

The biological effects of insulin are mediated by its binding to specific insulin receptors on the plasma membrane. The number of insulin receptors on cells can be regulated by the concentration of insulin in their environment (1–3). In humans and rodents in vivo, hyperinsulinemia has been associated with reduced insulin receptor concentrations (1, 2, 4, 5). Insulin-induced down regulation of receptors in vitro was first described by Gavin et al. in IM-9 lymphocytes (6) and has since been reported in human fibroblasts (7, 8), rat adipocytes (9, 10), and hepatocytes (11–13). In all systems studied thus far, the decrease in binding capacity has been approximately 50–60%. Decreased responsiveness to insulin, defined as a decrease in the maximal biological effect, has been described in adipocytes (10), and a decreased sensitivity, defined as a shift in the dose-response curve, has been described in fibroblasts (14). However, the role of receptor regulation in the overall regulation of insulin responsiveness and in the pathophysiology of insulin resistance remains to be elucidated (1, 2, 4).

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Desensitization of Hepatoma Cells to Insulin Action

EVIDENCE FOR A POST-RECEPTOR MECHANISM*

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Hepatoma tissue culture cells, an established line of rat hepatoma cells, display typical insulin receptors (15) and have two well defined responses to this hormone, induction of tyrosine aminotransferase (16) and stimulation of amino acid transport (16, 17). We have previously reported that incubation of HTC cells with insulin causes a complete but reversible desensitization to the effects of this hormone (18). HTC cells therefore provide a favorable system in which to investigate both receptor regulation and its role in the regulation of insulin responsiveness.

We report here that insulin causes a near-complete desensitization to the further action of insulin, but only a partial decrease in the binding capacity for this hormone. The magnitude of the reduction in insulin binding and the rate of its recovery when insulin is removed are incompatible with receptor regulation being the mechanism for regulation of insulin responsiveness. We conclude that desensitization is mediated by a post-receptor mechanism.

MATERIALS AND METHODS

Cells—HTC cells were maintained in either spinner or monolayer culture in antibiotic-free Eagle’s minimal essential medium supplemented with 5% calf serum and 5% fetal calf serum as described previously (19).

Reagents—Dexamethasone was a gift from Dr. Walter Gall, Merck and Co., and crystalline porcine zinc insulin (Lot 615-D63-10, 25.4 units/mg) was a gift from Dr. R. E. Chance, Lilly Research Laboratories. Bovine serum albumin and Hepes were purchased from Sigma Chemical Co. Amino [1-14C]isobutyric acid (52 mCi/mM) and [3H]-insulin (80–100 μCi/μg) were purchased from New England Nuclear and [G-3H]julin (900 μCi/mmol) from Amersham.

Amino Acid Transport—Amino acid transport was studied by measuring the initial rate of uptake of the nonmetabolizable amino acid a-aminoisobutyric acid. HTC cells in spinner culture were collected in the logarithmic phase of growth by centrifugation and resuspended to 0.8 X 10⁷ cells/ml in serum-free medium containing 50 μg/ml of neomycin and 0.1% bovine serum albumin (induction medium). Dexamethasone was added to a final concentration of 0.1 μM and the cells were incubated for 16–18 h in a gyrotory shaker water bath at 37 °C. Insulin addition or other manipulations were performed as described in the legends to the figures. Cell viability, as judged by trypan blue exclusion, remained 90–95% for as long as 65 h under these conditions. The initial rate of AIB transport was measured as described by Heaton and Gelehrter (19), except that the assay volume was 2 ml and the AIB concentration was 0.51 mM. Radioactivity contributed by trapped water was determined by the addition of [3H]julin to each incubation. Velocity of transport is expressed as nanomoles of substrate taken up per min per mg of protein. Protein was measured by the method of Lowry et al. (20).

Tyrosine Aminotransferase Assay—Tyrosine aminotransferase was assayed as described previously (15, 18). Transaminase specific activity is reported as milliunits/mg of soluble protein. 1 Milliunit catalyzes the formation of 1 nmol of p-hydroxyphenylpyruvate per min at 37 °C.

1 The abbreviations used are: HTC, hepatoma tissue culture; AIB, a-aminoisobutyric acid; P/NaCl, phosphate-buffered saline; Hepes, 4-(2-hydroxyethyl)1-piperazineethanesulfonic acid.
Insulin Binding Assay—Suspensions of HTC cells in induction medium, containing additions as described in the figure legends, were incubated in induction medium at 37 °C, centrifuged, and washed with 2 volumes of P./NaCl. The cell pellets were then resuspended at 2 to 5 × 10^6 cells/ml in binding buffer, consisting of 0.1 M Hepes, pH 7.4, 0.1 M NaCl, 1.2 mM MgSO4, 2.5 mM KCl, 10 mM glucose, 1 mM EDTA, and 1% bovine serum albumin. 0.52 ml were used for each 0.6-ml binding assay. ^125I-labeled insulin was used at 0.04 μCi/ml (0.4-0.6 ng/ml) with or without 10 μg/ml of unlabeled insulin. At the end of a 3-h incubation at 15 °C, duplicate 0.2-ml portions of the assay mixture were layered on 0.15 ml of binding buffer at 4 °C and centrifuged for 1 min at 8000 × g. The supernatant fraction was removed and the cell pellets were counted in a Beckman 8000 Autoradiography spectrometer. Specific binding represents binding which can be prevented by 10 μg/ml of unlabeled insulin.

Insulin-binding was also determined on cells in monolayer. Confluent monolayer cultures of HTC cells in 35-mm tissue culture dishes were incubated in induction medium as described in the legends to figures. The medium was removed and the cells were washed twice with 2 ml of P./NaCl, pH 7.4, at 37 °C and once at 4 °C. Binding buffer (0.75 ml) containing ^125I-insulin (0.04 μCi/ml) with or without unlabeled insulin was added and the monolayers were incubated at 15 °C for 3 h. The binding buffer was then removed and the monolayers were washed twice with P./NaCl at 4 °C. The cells were dissolved in 0.1 N NaOH and the radioactivity was determined.

RESULTS

Insulin-induced Desensitization to Insulin Action—The synthetic glucocorticoid dexamethasone (0.1 μM) induces a 5- to 10-fold increase in tyrosine aminotransferase activity and a 90% inhibition in the initial rate of AIB transport in HTC cells (17, 21, 22). Addition of insulin to dexamethasone-treated cells results in a further 2- to 3-fold increase in transaminase activity and a 5- to 10-fold increase in the rate of AIB transport (16, 17, 23). These effects of insulin are half-maximal at approximately 35 ng/ml and are maximal at concentrations of 200 ng/ml and greater (15). Maximal transport velocity and transaminase activity occur 2 and 4 h after insulin addition, respectively (18).

In the experiment shown in Fig. 1A, insulin (4 μg/ml) increased the rate of AIB transport 10-fold within 2 h. After 2 h, the rate of transport decreased despite the continued presence of insulin until, by 24 h, the transport rate in insulin-treated cells was similar to that in cells incubated with dexamethasone alone. Since HTC cells are known to degrade this hormone, additional insulin was added to portions of the culture at 4, 6, and 11.5 h in this experiment. The amount of insulin added was sufficient to replace that which was degraded, as determined by radioimmunoassay of medium from previous experiments. The readdition of insulin did not prevent the decrease in AIB transport velocity (Fig. 1A). Furthermore, addition of 4 μg/ml of insulin to cultures which had been incubated for 24 h with this hormone failed to stimulate AIB transport (Fig. 1B), indicating that the cells had become insensitive to insulin action.

Insulin induces a similar loss of responsiveness to the insulin induction of tyrosine aminotransferase (19). In contrast to the effect of insulin on AIB transport, the induction of transaminase activity by insulin is additive to that by dexamethasone. In addition, we have demonstrated that insulin can desensitize HTC cells to further insulin action in the absence of dexamethasone. In these experiments, monolayer cultures of HTC cells were incubated for 18 h in serum-free medium with or without 0.1 μM dexamethasone and then with or without 4 μg/ml of insulin. After 4 h of incubation with insulin, tyrosine aminotransferase activity was increased from 55 milliunits/mg of protein to 112 milliunits/mg in the presence of dexamethasone, and from 4 milliunits/mg to 10.5 milliunits/mg in the absence of steroid. After 24 h of incubation with insulin, at which time tyrosine aminotransferase activity had fallen to basal levels (58 milliunits/mg and 4.5 milliunits/mg, respectively), fresh insulin (4 μg/ml) was added to some of the cultures previously incubated with or without insulin. Addition of insulin to cells previously exposed to dexamethasone alone or to no hormones caused the expected 2- to 3-fold increase in transaminase activity (68 milliunits/mg to 140 milliunits/mg and 4 milliunits/mg to 9.8 milliunits/mg, respectively). Readdition of insulin to cells previously incubated with insulin, with or without dexamethasone, caused no induction of tyrosine aminotransferase. Thus, desensitization to insulin action occurs whether or not the cells have been previously incubated with dexamethasone.

Characterization of Insulin Binding in HTC Cells—The time course of ^125I-insulin binding to monolayer cultures of HTC cells at 15 °C is shown in Fig. 2. A similar time course is observed when the binding assay is performed on cells in induction culture. Steady state binding is achieved after 3 h of incubation and subsequent assays were carried out for this length of time. Nonspecific binding, as determined in the presence of 10 μg/ml of unlabeled insulin was typically 10-20% of total binding. After 3 h of incubation with cells at 15 °C, 90-95% of the ^125I-insulin in the medium was trichloroacetic acid-precipitable, indicating that there was little degradation of insulin at this temperature. In addition, 75-95% of the labeled insulin remained intact as determined by rebinding experiments, in which ^125I-insulin previously incubated with cells for 3 h at 15 °C was used in binding to fresh cells.

Binding of ^125I-insulin could be prevented by the addition of increasing concentrations of unlabeled insulin; half-maximal displacement of tracer binding occurred at 40-50 ng/ml of unlabeled insulin (Fig. 3, upper curve). Scatchard analysis of binding data from a saturation binding experiment is shown in Fig. 4. In this experiment, insulin binding was assessed at concentrations of 80 pm-17 nm (500 pg/ml-100 ng/ml); in-
classes of insulin binding sites, the Scatchard plot can be resolved into two linear components: a high affinity binding site with a $K_d$ of approximately 1.7 nM with approximately 3,000 sites/cell and a low affinity binding site with a $K_d$ of approximately 16 nM with approximately 150,000 sites/cell.

**Down Regulation of Insulin Binding**—In order to determine the role of insulin receptor regulation in the regulation of insulin responsiveness, we have studied the effect on insulin binding of incubation of HTC cells with insulin. In the experiments shown in Fig. 5, dexamethasone-treated HTC cells in monolayer culture were incubated at 37 °C with 4 μg/ml of

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**Fig. 2. Time course of insulin binding to HTC cells.** Confluent monolayer cultures of HTC cells in 35-mm tissue culture dishes were incubated for 20 h at 37 °C in induction medium containing 0.02% CaCl₂ and 0.1 μM dexamethasone. The cells were washed as described under "Materials and Methods" and binding buffer containing $^{125}$I-insulin (0.04 μCi/ml), with (●) or without (□) 10 μg/ml of unlabeled insulin, was added to each. The cultures were incubated at 15 °C for the indicated times and the binding assay was terminated as described under "Materials and Methods." Each symbol represents an individual culture dish. □—□, specific binding (total less nonspecific).

**Fig. 3. Competition for binding of $^{125}$I-insulin by unlabeled insulin.** HTC cells in suspension culture were incubated for 18 h at 37 °C in induction medium containing 0.1 μM dexamethasone. The control culture (●) and incubation at 37 °C continued for 24 h. The control culture (○) was incubated with 4 μg/ml of insulin for 20 min before the cells were washed in order to correct for any insulin which might not be removed in the wash. The cells were then washed and $^{125}$I-insulin binding was measured as described under "Materials and Methods." Each symbol represents the average of duplicate samples.

**Fig. 4. Scatchard analysis of insulin binding to HTC cells.** HTC cells in suspension culture were incubated in induction medium containing 0.1 μM dexamethasone for 42 h at 37 °C and the cells were washed as described under "Materials and Methods." The binding assay contained total insulin concentrations in the range of 0.5-100 ng/ml (80 pm-17 nm) with $^{125}$I-insulin added so that the specific activity ranged from 388 μCi/nmol at low insulin concentrations to 15 μCi/nmol at high insulin concentrations. After 3 h of incubation at 15 °C, the assays were terminated as described under "Materials and Methods." Each sample was corrected for nonspecific binding.

**Fig. 5. Time course of insulin-induced down regulation of insulin receptors.** Confluent monolayers of HTC cells in 35-mm tissue culture dishes were incubated in induction medium containing 0.02% CaCl₂ and 0.1 μM dexamethasone for 18 h at 37 °C. The medium was then removed and fresh induction medium containing dexamethasone and 4 μg/ml of insulin was added. Incubation at 37 °C continued until the times indicated, when the medium was removed, the cells were washed, and $^{125}$I-insulin binding was measured at 15 °C as described under "Materials and Methods." Corrections have been made for nonspecific binding. Control refers to insulin binding observed in cells treated with insulin and washed immediately. Two experiments are represented here: in one (●) control binding was 30.8 pg of insulin bound/mg of protein and in the other (▲) control was 29.9 pg/mg. Each point represents the average of triplicate cultures.
insulin. At the indicated times, the cells were washed to remove insulin and 125I-insulin binding was measured at 15 °C. Incubation with insulin caused a time-dependent decrease in insulin binding which was detectable at 30-60 min and maximal by 4-6 h. In the continued presence of insulin, binding remained decreased for at least 24 h. Typically, a 50-60% decrease in insulin binding was observed. Dexamethasone did not affect either insulin binding or its down regulation by insulin.

When HTCC cells were incubated with 4 μg/ml of insulin for 3.5 h at 15 °C rather than 37 °C and washed to remove insulin, there was no decrease in insulin binding activity (Table I). Therefore, the decreased insulin binding does not simply reflect occupancy of receptor by previously bound insulin (which should be maximal after 3 h at 15 °C) but rather it represents a temperature-dependent regulation of insulin binding activity.

Down regulation of binding is achieved by a decrease in the capacity of binding rather than a change in the affinity of binding. Fig. 3 shows a competition binding experiment performed on cells which have been incubated with insulin at 37 °C for either 20 min (upper curve) or 24 h (lower curve). The concentration of unlabelled insulin required to displace one-half of the labeled insulin is the same for both conditions (40-50 ng/ml), indicating that the binding affinity has not been significantly altered, even though the total specific binding at 0.5 ng/ml of 125I-insulin is reduced by 60%. Furthermore, this insulin treatment does not change the shape of the Scatchard plot or of the average affinity profile (28).

Although the experiments described above were performed with very high concentrations of insulin (4 μg/ml), incubation of HTCC cells with much lower concentrations of hormone also decreases insulin binding capacity. In the experiment shown in Fig. 6, monolayer cultures of HTC cells were incubated for 24 h at 37 °C with insulin at concentrations ranging from 1-4000 ng/ml. The monolayers were incubated with insulin for 30 min at 37 °C, washed to remove insulin, and assayed for 125I-insulin binding capacity. Insulin concentrations as low as 3 ng/ml caused a measurable decrease in binding. Half-maximal and maximal down regulation of insulin binding were attained at concentrations of 10-20 ng/ml and 100 ng/ml, respectively.

**Recovery of Insulin Binding**—The reversibility of the insulin-induced down regulation of insulin binding is shown in Fig. 7A. Suspension cultures of dexamethasone-treated HTC cells were incubated with insulin for 24 h (at which time insulin binding was reduced by approximately 50%) and were then washed, resuspended in insulin-free medium, and incubated at 37 °C. Cells incubated in insulin-free medium show little recovery of insulin binding capacity for approximately 2 h after which there is a slow, time-dependent recovery of binding which was not complete until at least 24 h. Cultures maintained in insulin-containing medium showed little change.

**TABLE I**

**Temperature dependence of insulin-induced down regulation of binding**

Monolayer cultures of HTC cells were incubated in induction medium containing 0.02% CaCl2 and 0.1 μM dexamethasone for 17 h at 37 °C. The medium was then removed and replaced with fresh induction medium containing 10% fetal calf serum but not insulin. Incubation was continued at 37 °C. At the times indicated in the figure, samples were removed and 125I-insulin binding was determined as described under "Materials and Methods." The average of triplicate cultures.

| Time of incubation | Temperature of incubation |
|--------------------|---------------------------|
| h                  | 15 °C  | 37 °C  |
| 0                  | 18.3 (100%) |
| 2                  | 18.0 (98%)  | 15.3 (83%)  |
| 3.5                | 19.0 (104%)  | 10.8 (59%)  |
in insulin binding capacity. Similar results were obtained when these experiments were performed in monolayer culture. Thus, the recovery of insulin binding activity is much slower than is the down regulation of binding by insulin, a phenomenon also described in other cell types (3, 12, 25).

Evidence for Post-receptor Regulation of Insulin Responsiveness—In the same experiment described in the previous section (Fig. 7A), we also compared the time course of recovery of insulin responsiveness (Fig. 7B) with that of insulin binding. Two hours after insulin was removed from the culture, fresh insulin (4 μg/ml) was again added to a portion of the culture and amino acid transport was measured 1, 2, and 3 h later. Dexamethasone-treated cells which had not previously been incubated with insulin were also tested for insulin responsiveness. Within 2 h of incubation in insulin-free medium, HTC cells had regained full responsiveness to 4 μg/ml of insulin, as assessed by the stimulation of AIB transport. In contrast, there was little, if any, recovery of insulin binding activity at this time. Thus, the cells recovered the ability to respond maximally to high concentrations of insulin long before they recovered full insulin binding capacity. Therefore, the lack of response of 4 μg/ml of insulin, which is observed after 24 h of incubation with this hormone, cannot be caused by the 50% decrease in insulin binding capacity.

In order to assess further the role of receptor down regulation in insulin-induced desensitization, we examined the dose-response relationship for insulin stimulation of AIB transport 2 h after removing insulin from cells previously incubated for 24 h with this hormone. We compared it with that of cells which had been incubated for the same period of time without insulin (Fig. 8). In this experiment, the insulin binding activity in insulin-treated cultures was 56% of that in control cultures. This decrease in insulin binding caused a rightward shift in the dose-response curve; half-maximal stimulation of AIB transport was achieved at 120 ng/ml in insulin-treated cells compared to 35 ng/ml in control cells. Basal levels of transport and maximal stimulation by insulin were the same in both conditions. Thus, the maximal responsiveness to insulin concentrations of 1000 ng/ml or higher was the same in cells with 56% of control receptor activity as in control cells. Identical results were observed in two similar experiments. These results are consistent with the presence of spare receptors for insulin stimulation of AIB transport and show the expected rightward shift in concentration dependence caused by decreasing the number of receptors. The results indicate that down regulation of insulin receptors can alter the dose-response relationship for insulin but cannot account for the total unresponsiveness to high concentrations of insulin shown in Figs. 1 and 7. We conclude that the complete desensitization to insulin action is the result of a post-receptor regulatory phenomenon.

**DISCUSSION**

Cellular resistance to the actions of physiologic concentrations of insulin may reflect either decreased sensitivity or decreased responsiveness to insulin, or both. Decreased insulin sensitivity generally refers to a rightward shift in the insulin dose-response curve without a change in the magnitude of the insulin effect and is consistent with a decrease in the number or affinity of insulin receptors. Decreased insulin responsiveness, on the other hand, refers to a decrease in the maximal response to insulin and is consistent with a post-receptor defect in insulin action. The combination of both a decreased maximal response to insulin and a shift to the right in the dose-response curve suggests effects at both the receptor and post-receptor levels (29).

Insulin regulation of cellular sensitivity to its own actions has now been described in a number of different experimental systems and the characteristics of this regulation are generally similar in all systems examined (1–3, 8–13). The decreased sensitivity is the result of a decreased number of insulin receptors rather than a change in their apparent affinity. The down regulation of receptors is time- and concentration-dependent and is specific for insulin in that the ability of various insulin analogues to decrease receptor number correlates with their relative binding affinity for the insulin receptor (3). It is noteworthy, however, that agents which mimic insulin action (spermine, H₂O₂, wheat germ agglutinin, and antiinsulin antibodies) can apparently down-regulate insulin receptors in rat hepatocytes (13). Finally, the down regulation is partial (50–70%), rather than complete, and is reversible, although the time course of recovery of insulin binding is much slower than that of down regulation. The mechanism of down regulation appears to involve endocytosis and internalization (3, 7, 30) and either sequestration (31) or degradation (32) of the insulin-receptor complex. Unfortunately, in most of these studies, the down regulation of receptors has not been correlated with any alteration in biological responsiveness to the hormone.

In contrast, the incubation of 3T3-L1 fatty fibroblasts with antibodies directed against the insulin receptor causes a time-dependent decrease in insulin responsiveness, measured by the insulin stimulation of glucose uptake and oxidation, during a time in which there is no further change in insulin binding. These observations were interpreted to indicate an action at a post-receptor step. The glucose transport system did not appear to be the site of action since transport remained normally responsive to both spermine and vitamin K₃ (25).

Incubation of isolated rat adipocytes with insulin causes a time- and concentration-dependent decrease in both insulin receptors and in the maximal insulin stimulation of glucose transport. The latter effect was thought to reflect an action on the glucose transport system itself, because spermine and anti-insulin receptor antibodies also had a reduced ability to
stimulate glucose transport in insulin-treated cells (10).

We report here that incubation of HTC cells with insulin causes a temperature-, time-, and concentration-dependent decrease in insulin binding. The down regulation of insulin receptor activity is maximal within 4–6 h and half-maximal within 2–3 h. Half-maximal down regulation is achieved at insulin concentrations of 10–20 ng/ml (<2–3 nm), a figure comparable to the estimated $K_d$ of the high affinity binding site. Insulin binding is decreased by 50–60%, reflecting a decreased number of insulin receptors without apparent change in their affinity of binding. Concomitantly, insulin causes a complete desensitization to insulin action, assessed either as the stimulation of amino acid transport or induction of tyrosine aminotransferase activity. The magnitude of the down regulation of binding is clearly not sufficient to account for the near complete loss of cellular responsiveness to insulin. The insulin-induced decrease in receptor activity is slowly reversible upon washing away insulin. However, as documented in Fig. 7, the time course of recovery of hormonal responsiveness and binding are clearly dissociable. Two hours after removing insulin from cells incubated with this hormone for the previous 24 h, full responsiveness to maximally effective concentrations of insulin has been restored whereas there is little or no recovery of insulin binding. The decreased number of insulin receptors does produce a rightward shift in the dose-response relationships for insulin stimulation of AIB transport but does not affect maximal responsiveness to insulin (Fig. 8). We conclude that down regulation of receptors cannot be the sole mechanism for regulation of insulin responsiveness and that desensitization is mediated primarily if not exclusively by post-receptor mechanisms.

HTC cells provide a useful model for studying the regulation of hormone responsiveness. They are an established cell line with well defined insulin receptors and insulin responses, both of which can be regulated by insulin. In addition, HTC cells display receptors for the insulin-like growth factor multiplicity stimulating activity with which insulin does not interact (15). We have reported that multiplication stimulating activity induces tyrosine aminotransferase activity and stimulates AIB transport to the same extent and with the same time course as does insulin. Simultaneous addition of maximally effective concentrations of insulin and multiplication stimulating activity have no additive effect on either enzyme induction or stimulation of transport, suggesting a common step in their mechanisms of action (15). Of particular interest is the observation that insulin-induced desensitization of HTC cells to insulin action is accompanied by unresponsiveness to multiplication stimulating activity action.2 This preliminary observation suggests that insulin acts at a post-receptor step that is common to the action of both insulin and multiplication stimulating activity.

We have observed a similar desensitization to insulin action in the H-35 rat hepatoma cell line (33). A complete loss of inducibility of tyrosine aminotransferase by insulin was found in cells previously incubated for 24 h with insulin in the presence or absence of dexamethasone.3

There is increasing evidence that insulin resistance may play an important role in states of glucose intolerance such as obesity and non-insulin-dependent diabetes mellitus (4). Changes in insulin receptor activity have now been described in a number of pathologic conditions, but the etiologic or pathophysiologic significance of these changes in explaining the insulin resistance associated with these states remains controversial (2, 4). Therefore, experimental systems which allow detailed study of regulation of both receptor and post-receptor events in the overall regulation of insulin responsiveness are important for defining the pathophysiology of insulin-resistant states in man.

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