Intracellular Insulin-Receptor Dissociation and Segregation in a Rat Fibroblast Cell Line Transfected with a Human Insulin Receptor Gene*

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The initial cellular processing of insulin and the insulin receptor includes binding of the ligand to the receptor and endocytosis of the ligand-receptor complex (1−3). Although the initial events are similar, the ultimate itinerary of insulin and the insulin receptor are much different. Most of the internalized insulin is targeted into a pathway where the ligand is degraded to low molecular weight products which are then released from the cell. A smaller fraction of internalized insulin traverses a non-degradative, retroendocytotic (4,5) pathway and is released intact. In contrast, only a small fraction of internalized receptors are degraded and most are recycled back to the plasma membrane to be reutilized (6,7).

To explain the differences in the intracellular fates of ligand and receptor, it has been proposed that following endocytosis insulin dissociates from the receptor within an endocytotic vesicle (1). This “endosome” is then processed and the ligand and receptor are segregated into distinct vesicles, each with different intracellular destinations. Morphologic studies (1,8) support these concepts, but no biochemical assay has been developed to study these proposed events in insulin-receptor processing. To approach this issue, we have developed a method to study intracellular insulin-receptor dissociation and segregation in a rat fibroblast cell line that has been transfected with a normal human insulin receptor gene (HIRC).1 Due to gene amplification, this cell line expresses about 500 times the normal number of native fibroblast insulin receptors (9) producing a large number of internalized insulin-receptor complexes, allowing simplified methods for assay of this intracellular fate. These receptors have been shown to internalize, recycle, and mediate insulin degradation normally (9). In the current study, we have further characterized the above processes in these fibroblasts and have studied the mechanisms of intracellular dissociation and segregation of insulin-receptor complexes.

MATERIALS AND METHODS

Cell Culture—Rat fibroblasts were transfected with a plasmid containing the human insulin receptor gene as well as a gene coding for dihydrofolate reductase as described previously (10). Cells were grown in T75 flasks (Costar) in Dulbecco’s modified Eagle’s medium with 10% fetal calf serum and 500 μM methotrexate to select fibroblasts not containing the transfected plasmid. 2−3 days before an experiment, 90-mm culture dishes were seeded with 2−3 × 10⁶ cells per dish with the above medium.

Measurements of Insulin Binding, Internalization, and Intracellular Ligand Degradation—Insulin binding was performed in the cultured cells according to a modification of the method of Caro et al. (11).

1 The abbreviations used are: HIRC, human insulin receptor cells; HEPES, 4-(2-hydroxyethyl)−1-piperazineethanesulfonic acid; Tricine, N-[2-hydroxy-1,1-bis(hydroxyethyl)methyl]glycine; PBS, phosphate-buffered saline; PEG, polyethylene glycol; DSS, diacinnimidyl suberate; CAR, cell associated radioactivity.

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After washing three times with 2 ml of binding buffer (minimal essential medium, 25 mM HEPES, 25 mM Tricine, 1% bovine serum albumin), 0.4 ng/ml [125I]iodoinsulin, in addition to varying concentrations of unlabeled pork insulin, was added to a final volume of 2 ml of binding buffer at pH 7.6, 4-35°C. Following the desired incubation period, the cells were washed five times with 2 ml of ice-cold PBS, pH 7.8 at 4°C. One ml of 0.5% Triton X-100 in PBS was added to each dish at 4°C; the dishes were shaken for 10 min to solubilize cells and displace them from the dish. The total cell associated radioactivity was quantitated by counting the solubilized cell solution in a γ-counter. The specific cell associated radioactivity was calculated by subtracting the cell associated radioactivity found in cells incubated in the presence of tracer and cold insulin. HIRC cells were incubated at 37°C, with an anti-insulin receptor antibody at a dilution of 1:100. Protein A was then used to precipitate antibody-receptor complexes as described previously (16). After resuspending in Laemmli’s solution and 100 mM dithiothreitol, the solution was boiled, and 180-μl aliquots were analyzed by polyacrylamide gel electrophoresis according to the method of Laemmli (17). Molecular weight standards included thyroglobulin (330,000), ferritin (220,000), myosin (200,000), β-galactosidase (116,500), phosphorylase b (94,000), bovine serum albumin (67,000), catalase (60,000), ovalbumin (45,000), and lactic dehydrogenase (36,000).

RESULTS

Solubilization of Insulin and Insulin-Receptor Complexes—The quantitative study of the intracellular itinerary of insulin-receptor complexes requires solubilizing plasma membrane and intracellular complexes under conditions that maintain the association of ligand and receptor. In other ligand-receptor systems, such as human chorionic gonadotropin and asialoglycoproteins, the nonionic detergent Triton X-100 had been shown to be an effective solubilizing agent (18, 19). Furthermore, Triton stabilized these complexes and did not promote dissociation of the ligand from the receptor (20, 21). The efficiency of Triton to solubilize insulin-receptor complexes was examined in two ways. After adding radiolabeled insulin to monolayers of HIRC cells for 60 min at 4°C, various concentrations of detergent were added for increasing lengths of time as shown in Fig. 1. When incubated for less than 8 min with 0.25% Triton X-100, less than 90% of the cell associated radioactivity was recovered from the culture dishes, suggesting that cells, incompletely solubilized, remained attached to the dish. Greater than 95% of the cell associated radioactivity was recovered from the dishes when cells were incubated with the higher concentrations (0.5-1%) of Triton for 4 min or longer.

To measure the extent of solubilization of the recovered radioactive material, the extraction medium was diluted 100-

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**Fig. 1. The effect of varying Triton X-100 concentration on extracting cell associated radioactivity.** HIRC cells were incubated with [125I]insulin for 1 h at 4°C. Control cells were solubilized with 1% Triton for 30 min and the total cell associated radioactivity (CAR) was measured (specific CAR = 35%). Various concentrations of detergent were then added to parallel dishes of HIRC cells for the indicated times, and the extraction medium was pipetted from the dish. The amount of radioactivity recovered from the dish was expressed as a percentage of the total cell associated radioactivity. Each data point is the mean of duplicate samples from two experiments. ⋄, 1.0% Triton; ○, 0.5% Triton; Δ, 0.25% Triton.
fold in PBS (final Triton X-100 concentration < 0.01%) and spun at 200,000 x g for 30 min in an ultracentrifuge. These centrifugation conditions precipitate cellular debris and membrane fragments (14, 16). 95% of the recovered radioactivity, extracted with 0.25—1% Triton X-100, was contained in the supernatant. In contrast, in cells that were exposed to no detergent and scraped off the dish with a rubber policeman, only 30% of the radioactivity was found in the supernatant.

**Precipitation of Insulin-Receptor Complexes**—The selective precipitation of insulin-receptor complexes, but not of free insulin, with the use of PEG, was first described by Cuatrecasas (16). This agent has been used extensively in soluble insulin receptor binding assays. The ability of PEG to precipitate soluble insulin-receptor complexes derived from plasma membranes is shown in Fig. 2. HIRC cells were incubated with radiolabeled insulin for the indicated times at 4 °C, a temperature where internalization of insulin and insulin receptors are completely inhibited (22). After solubilizing the cells, total cell associated radioactivity (CAR) and the ability of the radioactivity in an aliquot of extraction medium to precipitate in 12.5% PEG were determined. The total amount of insulin, bound to surface receptors (as measured by the total CAR), rose progressively over 3 h and did not reach a steady state. The increase in the radioactivity in the PEG precipitate paralleled the increase in total CAR, suggesting that PEG precipitation of cellular radioactivity is an accurate quantitative measure of the amount of insulin bound to surface receptors.

To determine if PEG precipitation of intracellular radioactivity is a quantitative measure of insulin receptor complexes, a competition curve for insulin binding was compared to a competition curve for intracellular PEG-precipitable radioactivity. The latter experiment was performed by incubating HIRC cells with tracer 125I-insulin and various concentrations of unlabeled insulin for 2 h at 4 °C. Internalization was initiated by replacing the cold buffer with 37 °C insulin-free binding buffer. After 6 min, the cells were incubated with ice-cold acidic (pH 3.5) binding buffer to remove insulin bound to surface receptors and then solubilized with 0.5% Triton. The radioactivity in an aliquot of extraction medium precipitated in 12.5% PEG was then quantitated. As shown in Fig. 3, the competition curve for the PEG-precipitated internalized radioactivity had a similar shape and ED60 as the competition curve for total cell associated radioactivity, indicating that the PEG method quantitatively precipitates the intracellular insulin-receptor complexes derived from the cell surface. The stability of insulin-receptor complexes following solubilization is shown in Table I. HIRC cells were incubated with tracer concentrations of radiolabeled insulin for 2 h at 4 °C before solubilization with various concentrations of detergent for increasing periods of time. The maximum percent of the total radioactivity in the extraction medium that precipitated in 12.5% PEG was 85—90%. The decrease in precipitable radioactivity when cells were solubilized with either 0.25 or 0.5% Triton from 2 to 16 min was negligible. However, when cells were solubilized with 1% Triton, the PEG-precipitable radioactivity fell from 87 to 70%

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**Table I**

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>0.25% Triton X-100</th>
<th>0.5% Triton X-100</th>
<th>1% Triton X-100</th>
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**FIG. 2.** Time course of binding of radiolabeled insulin to surface receptors and the ability of radioactivity to precipitate in 12.5% PEG. HIRC cells were incubated with 0.4 ng/ml 125I-insulin at 4 °C and at the above times, the cells were solubilized with 0.5% Triton X-100, and the total cell associated radioactivity (total binding) was measured. A 200-μl aliquot of the solubilized cell solution was added to 200 μl of 0.45% γ-globulin and 400 μl of 25% PEG, vortexed, and centrifuged. The radioactivity in the precipitate was measured. The data are expressed as a percent of the radioactivity that is cell associated (©, total binding) or PEG-precipitable (C) at 180 min and are the mean ± S.E. of three separate experiments.
after 8 min, suggesting that the higher concentration of Triton caused a significant amount of dissociation of insulin from the insulin receptor. Integrating the previous five experiments, the optimal conditions for solubilizing monolayers of HIRC cells without significantly dissociating insulin from the insulin receptor were determined. For all further experiments, 0.5% Triton X-100 for 5–10 min at 4 °C was used to solubilize plasma membrane and intracellular insulin-receptor complexes. Trapping the unbound free insulin in the PEG precipitate was determined by solubilizing HIRC cells with Triton and adding radiolabeled insulin immediately before PEG precipitation. Only 4–5% of the total radioactivity was recovered in the precipitate.

**Insulin Binding, Internalization, and Intracellular Degradation**—HIRC cells bind, internalize, and degrade insulin similarly to other insulin target tissues such as adipocytes and hepatocytes (9). The time courses of these processes are shown in Fig. 4. Total specific CAR rose rapidly and reached steady state by 20 min at 37 °C. The total CAR was composed of radiolabeled insulin bound to surface receptors and internalized by the cells. Since incubating cells with an acidic medium dissociates insulin from surface receptors, the time course of nonacid-extractable CAR represents the rate of internalization of insulin. Only a small fraction of insulin was internalized by HIRC cells at 2 min; however, after 2 min, the rate of internalization of insulin increased and reached a steady state by approximately 10 min. At the steady state, 60% of the total CAR was intracellular radioactivity. Cells containing only intracellular radioactivity (acid-washed cells) were then solubilized, and the ability of the radioactivity to precipitate in 7.5% trichloroacetic acid was then measured. Trichloroacetic acid precipitates insulin and large insulin intermediates but low molecular weight degradation products remain soluble (5). As shown in Fig. 4, 4 min after adding insulin to cells, all of the intracellular radioactivity was trichloroacetic acid-precipitable. By 6 min, a small amount of trichloroacetic acid-soluble material appeared, indicating the time needed for insulin to first reach the intracellular degradation compartment. At 20 and 60 min, the percent of intracellular radioactivity which was trichloroacetic acid-soluble was 10 and 40%, respectively.

**Dissociation of Insulin-Receptor Complexes**—Since most intracellular insulin receptors recycle back to the plasma membrane, one would predict that the divergent pathways of insulin and receptor processing would occur before the intracellular degradation of ligand (13). To quantitate the time course of insulin-receptor complex dissociation, radiolabeled insulin was incubated with cells for 2 h at 4 °C. At time zero, the cells were reincubated with insulin-free 37 °C buffer to initiate internalization in a relatively synchronous manner. At the indicated times, the cells were cooled to 4 °C, insulin bound to surface receptors was removed with the acid extraction technique, then the cells were solubilized, and the remaining radioactivity was analyzed for its ability to precipitate in 12.5% PEG. As shown in Fig. 5, the total intracellular radioactivity increased rapidly after warming the cells, reached a peak by 10 min, and then decreased as the cells released their radioactivity into the medium. The total intracellular radioactivity was composed of both PEG-precipitable (insulin-receptor complexes) and PEG-soluble (free insulin and insulin degradation products) material. 2 min after initiating internalization, 90% of the total intracellular radioactivity was composed of insulin-receptor complexes. The number of complexes reached a maximum by 5 min and then decreased rapidly with a t½ of approximately 10 min. There was a distinct and measurable delay in the appearance, rate of rise, and peak of intracellular PEG-soluble material compared with PEG-precipitable material. Integrating Figs. 4 and 5, the PEG-soluble material was composed of intact ligand for the first 6 min after internalization, resulting from the dissociation of the insulin-receptor complexes.
tion of insulin-receptor complexes. By 10 min the amount of PEG-soluble material, now composed of both intact and degraded insulin, reached a peak and then declined as PEG-soluble material was released from the cell.

The inset of Fig. 5 demonstrates the percentage of total intracellular radioactivity which was precipitable in 12.5% PEG with time. Using the present technique, the maximal percent of cell associated radioactivity which was PEG-precipitable was between 85 and 90% (see Table I). Two minutes after the initiation of internalization 77% of the intracellular radioactivity was PEG-precipitable, indicating that even at this early time point some intracellular dissociation had already occurred. The initial rate of decline of PEG-precipitable material was rapid, reaching a minimum by 10–20 min and remaining relatively constant thereafter. The % of the initial rapid dissociation phase of insulin-receptor complexes was approximately 7 min.

**Effect of Monensin and Chloroquine on Intracellular Processing of Insulin-Receptor Complexes**—Many insulin-sensitive target tissues have been shown to accumulate radiolabeled insulin intracellularly in the presence of monensin or chloroquine (23–28). To determine the effect of these agents in HIRC cells, the cells were incubated with 0.4 ng/ml 125I-insulin in the absence or presence of 50 μM monensin or 100 μM chloroquine at 37 °C. As shown in Table II, by 60 min, the total intracellular radioactivity was approximately 5- to 6-fold and 3-fold greater in cells incubated with monensin and chloroquine, respectively, as compared with control cells.

We (5) have previously shown that the accumulated intracellular radioactivity is intact insulin, suggesting that these agents block processing at a step before degradation of ligand to low molecular weight products (5, 13). To investigate whether these agents blocked dissociation of insulin-receptor complexes, HIRC cells were incubated with radiolabeled insulin for various times in the presence or absence of monensin, and the intracellular radioactivity was analyzed for its ability to precipitate in PEG. As shown in Fig. 6, monensin completely inhibited the dissociation of insulin-receptor complexes. At 2 min, 73% versus 85% of the intracellular radioactivity was PEG-precipitable in control and monensin-treated cells, respectively, again showing that in the absence of monensin intracellular dissociation occurred very soon after internalization. As the initial insulin-receptor complexes dissociated, the percent of PEG-precipitable material declined rapidly and reached a steady state by approximately 10 min, accounting for 35% of the total intracellular radioactivity. In contrast, 80–85% of the total intracellular radioactivity remained PEG-precipitable in the presence of monensin from 2 to 60 min. Addition of 100 μM chloroquine also markedly inhibited dissociation of insulin-receptor complexes. The percent of intracellular radioactivity that was PEG-precipitable in chloroquine-treated cells at 2, 20, and 60 min was 80, 70 and 65%, respectively.

To demonstrate further chloroquine’s effect to inhibit dissociation and thereby promote intracellular accumulation of insulin-receptor complexes, intracellular ligand and receptor were covalently cross-linked with DSS. DSS penetrates plasma membranes and has the ability to cross-link intracytosolic proteins (15). HIRC cells were incubated with radiolabeled insulin and, at the indicated times, were cooled to 4 °C, and surface receptor-bound insulin was removed with acid. The cells were then incubated with 5 μM DSS for 20 min at 4 °C before solubilization. An aliquot was boiled in Læmmli’s solution and 100 mM dithiothreitol and analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. The autoradiogram is shown in Fig. 7A. Discrete bands were seen at a relative molecular weight of 130,000, consistent with the α-subunit of the insulin receptor. The time course of appearance of intracellular insulin-receptor complexes in the absence and presence of chloroquine is demonstrated in the odd- and even-numbered lanes, respectively. There was a time-dependent increase in the intensity of the bands which was further enhanced by chloroquine. The dried gel was sliced and the insulin-receptor associated radioactivity determined; the results are shown in Fig. 7B. The time course of appearance of cross-linkable intracellular insulin-receptor complexes reached a steady state by 20 min with a % of 7 min. Chloroquine increased the number of insulin-receptor complexes by 5–10 min; after this time the number of insulin-receptor complexes increased gradually and did not reach a steady state within 30 min.

**Intracellular Segregation of Insulin and Insulin Receptors**—Insulin-receptor complexes dissociate presumably when the internalized complex encounters an acidic vesicular compartment and monensin prevents dissociation of insulin-receptor complexes by neutralizing these acidic vesicles. With this line of thinking, if the dissociated insulin and receptor remain in the same vesicular compartment (i.e. not segregated) then monensin-induced vesicle neutralization should lead to reas-

![Fig. 6. Time course of the effect of monensin and chloroquine on the fraction of intracellular radioactivity that is PEG-precipitable.](image-url)
Fig. 7. Autoradiogram of covalently bound intracellular insulin-receptor complexes in the presence and absence of chloroquine. A, HIRC cells were incubated with 1 ng/ml 125I-insulin for the indicated times in the absence (odd-numbered lanes) or presence (even-numbered lanes) of 100 μM chloroquine. The cells were then washed and exposed to acid to remove surface receptor-bound insulin. The cells were then incubated with the cross-linking agent disuccinimidyl suberate (1 mM) in PBS for 20 min at 4 °C. After washing, the cells were solubilized in 0.5% Triton, 1 mM N-ethylmaleimide, and 2 mM phenylmethylsulfonyl fluoride. The receptor complexes were immunoprecipitated with an anti-insulin receptor antibody and protein A. After boiling in Laemmli’s solution and 100 mM dithiothreitol, equal volumes were applied to the sodium dodecyl sulfate-polyacrylamide gel. The autoradiogram from one of two experiments is shown after electrophoresis and autoradiography. B, the bands, migrating at the relative molecular weight of 130,000, were sliced from the dried polyacrylamide gel and counted in a γ-counter. Closed circles represent the results for control cells and open circles for the chloroquine-treated cells.

Fig. 8. Time course of dissociation of intracellular insulin-receptor complexes and the effect of monensin on rebinding. HIRC cells were incubated with 0.4 ng/ml 125I-insulin and 50 μM monensin for 90 min at 37 °C. The cells were washed in 4 °C acidic binding buffer to remove surface receptor-bound insulin and reincubated at the indicated times in fresh, monensin-free, binding buffer at 37 °C. At 2, 5, 10, 20, 40, and 60 min, monensin (50 μM) was re-added to parallel dishes of cells. Control cells (●) or cells retreated with monensin (○) were solubilized, and the ability of the radioactivity to precipitate (A) or remain soluble (B) in 12.5% PEG was determined as described under “Materials and Methods.”

Association of intact insulin to the receptor. After physical segregation occurs, monensin would no longer promote reassociation, and this approach can be used to determine the point of physical segregation of the ligand from receptor following intracellular dissociation (27). To examine this step, HIRC cells were incubated with radiolabeled insulin and 50 μM monensin for 3 h at 37 °C. With this protocol, large numbers of insulin-receptor complexes accumulate intracellularly. Monensin was then removed by washing the cells with 4 °C binding buffer, and the insulin bound to surface receptors was extracted with acid. The cells, containing only internalized insulin-receptor complexes, were reincubated in fresh monensin-free buffer at 37 °C and, over time, the ability of the solubilized radioactivity to precipitate in 12.5% PEG was measured. The time course of the absolute amounts of intracellular precipitable and soluble radioactivity is shown in Fig. 8, A and B, respectively. At time 0, 85% of the total intracellular radioactivity (precipitable plus soluble counts/min) was precipitable, indicating that before reincubation essentially all the intracellular radioactivity represented intact insulin-receptor complexes. After the addition of the 37 °C buffer, there was a rapid decline in precipitable material as insulin-receptor complexes dissociated, proceeding with an apparent t½ of approximately 5 min. A concomitant marked increase in PEG-soluble material was observed for the first 5 min, followed by a rapid decline due to subsequent release of radioactivity from the cells.

At 2, 5, 10, 20, 40, and 80 min, 50 μM monensin was added back to the cells as noted by the dotted lines in Fig. 8, A and B. When monensin was added at 2 and 5 min, there was a marked increase in PEG-soluble radioactivity (Fig. 8A) accompanied by a corresponding decrease in PEG-soluble radioactivity (Fig. 8B), indicating that the dissociated insulin was able to reassociate with its receptor. However, after 5 min,
the addition of monensin inhibited further insulin-receptor dissociation but did not lead to any reassociation and had no effect on the time course of PEG-soluble material. These findings suggest that after 5 min, the dissociated insulin and receptor become physically segregated so that reassociation cannot occur despite neutralization with monensin.

**DISCUSSION**

This study of insulin and receptor processing was performed on rat fibroblasts, transfected with cDNA encoding the normal human insulin receptor gene, that express approximately 500 times the normal number of insulin receptors per cell. These high expression cells allowed us to perform experiments with a minimal number of cells but with high percentages of specific binding of radiolabeled $^{125}$I-insulin to its receptor. Thus, we were able to solubilize the cells with low concentrations of detergent and analyze the intracellular compartment with a sufficient amount of radioactive label. The insulin receptors of HIRC cells display similar structure and binding characteristics as receptors of other insulin target tissues (9). Furthermore, the insulin receptors in HIRC cells internalize, recycle, and mediate insulin degradation in a normal manner (9). Therefore, in view of the high level of insulin receptor expression achieved in these cells, they represent an ideal model to study intracellular insulin and receptor processing.

The method of selective polyethylene glycol precipitation of solubilized insulin receptors has added to our understanding of the intracellular processing of insulin and receptors. In agreement with electron microscopic and autoradiographic (1, 28) techniques, it was demonstrated that after binding insulin and its receptor are internalized as a complex. Dissociation begins very soon after internalization and, by 2 min, 10% of the intracellular ligand has already dissociated from the receptor. The rate of dissociation is rapid thereafter, and by approximately 7.5 min, 50% of the intracellular complexes have dissociated. The time course of dissociation of insulin-receptor complexes parallels the dissociation of asialoerythropoietin from its receptor in isolated hepatocytes (21) but is much faster than the prolonged dissociation of human chorionic gonadotropin from its receptor in MA-10 cells (29).

The mechanism of dissociation of insulin-receptor complexes was investigated using the carboxylic ionophore, monensin (30). This agent has been shown to mediate the transfer of $\text{H}^+$ across cell membranes, usually involving exchange with $\text{Na}^+$ (31). Studies in other ligand-receptor systems have demonstrated that dissociation is linked to the acidification of specific endosomal vesicles, perhaps generated by proton pumps (32). Tycko and Maxfield (33, 34) and Wieland et al. (35) have shown that $\alpha_2$-macroglobulin and mannose-glycoprotein receptor complexes enter an acid environment (as measured by the fluorescein-dextran method) very soon after internalization of receptor-ligand complexes has occurred, but before the ligand is transported by lysosomes. Furthermore, monensin reversibly raises the pH of these prelysosomal acid vesicles (34, 35). The inhibition of dissociation of ligand-receptor complexes by monensin in prelysosomal compartments has also been demonstrated for asialoerythropoietins (21) and human chorionic gonadotropin (29). With the current technique of PEG precipitation of solubilized receptors extracted from HIRC cells, we have been able to add to the above findings and show that monensin inhibits intracellular dissociation of insulin-receptor complexes and that this most likely occurs by preventing acidification of prelysosomal vesicles. The effect of chloroquine to inhibit insulin-receptor dissociation was identical to that seen with monensin. Although chloroquine is a lysosomotropic agent which inhibits lysosomal degradative function even in cell free systems (36), chloroquine also has major effects on prelysosomal dissociation of ligand-receptor complexes (21). These findings are consistent with the subcellular fractionation studies of Posner et al. (37) who demonstrated that insulin accumulates in a prelysosomal Golgi-like vesicle in the presence of chloroquine.

Following dissociation, morphologic (38, 39) as well as subcellular fractionation techniques (40, 41) have demonstrated that receptor and ligand physically separate and move into separate vesicular structures. In the present investigation, a biochemical assay, modified from Wolkoff et al. (27) in their studies of asialoglycoproteins, was used to study insulin-receptor segregation. Five min after the internalized insulin-receptor complexes begin to dissociate, most of the ligand and receptor reside within the same compartment as evidenced by their ability to reassociate upon neutralization of the compartment by the proton ionophore monensin. This compartment most likely represents acidic endocytotic vesicles. Five min after dissociation begins neutralization of vesicles inhibits further complex dissociation, but reassociation no longer occurs. This indicates that the insulin and receptor are now physically segregated and are no longer in the same compartment. An alternative possibility is that the receptor or ligand have been altered, or degraded, thereby decreasing their ability to rebind. However, receptors rapidly recycle back to the plasma membrane, which are structurally intact and bind insulin normally (6, 7). Furthermore, the bulk of the intracellular insulin, under these conditions, is intact (5, 13, 22). Therefore, degradation of ligand and/or receptor seems an unlikely explanation for the lack of reassociation, and we favor the view that this denotes the point of physical segregation of insulin and its receptor.

In summary, discrete steps in the pathway of insulin-receptor processing have been studied. After binding, insulin and its receptor are internalized as a complex. Shortly after internalization, dissociation occurs within an acidic endosomal vesicle which is sensitive to monensin and chloroquine. Five min after dissociation begins, the ligand and receptor are segregated into distinct compartments followed by degradation of ligand and recycling of the receptor. The process of dissociation is a necessary step in the preparation of receptor function and may be important in deactivating the cellular response to insulin.

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Insulin Receptor Itinerary