Effects of Insulin on Adrenoceptor Binding and the Rate of Catecholamine-induced Lipolysis in Isolated Human Fat Cells*

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The mechanisms by which insulin inhibits catecholamine-induced lipolysis in fat cells are unknown. In this study the possible role of an interaction between insulin and the adrenoceptors on human fat cells was investigated. Insulin inhibited, in a dose-dependent fashion, the specific binding of hydrophobic as well as hydrophilic nonselective β-receptor radioligands but had no effect on the binding of α2-selective radioligands. The results of saturation experiments and competition-inhibition experiments under both equilibrium conditions and nonequilibrium conditions revealed that insulin reduced the total number of β-adrenergic binding sites (maximum effect 25%) without changing the β-adrenoceptor affinity. This insulin effect was rapid and reversible; one-third of the effect occurred within 1 min of incubation and it was completely reversed within 30 min after withdrawal of insulin. It could be mimicked by a polyclonal rabbit insulin receptor antibody but not by insulin mimickers acting distal to the initial interaction between the hormone and its specific insulin-receptor binding site. The β-adrenoceptor binding to a plasma membrane-enriched fraction decreased at the same time as it increased to a microsomal enriched fraction after insulin treatment, indicating a redistribution of β-adrenoceptors in the cell. In lipolysis experiments performed under conditions like those in the binding experiments, insulin inhibited the rate of lipolysis with a lag period of 3 min. Furthermore, the hormone caused a dose-dependent maximum 10-fold shift to the right of the dose-response curve for isoprenaline-induced lipolysis without changing the amplitude of the curve. This effect of insulin was specific for the β-adrenergic receptors system, since insulin markedly decreased the amplitude of the dose-response curve for parathyroid hormone-induced lipolysis. In addition, the effect of insulin on isoprenaline-induced lipolysis could be mimicked by long-lasting fractional inactivation of the β-adrenoceptors. The dose-response relationships for the inhibitory effects of insulin on β-adrenoceptor binding and the lipolytic sensitivity to isoprenaline were almost identical. Half-maximum and maximum effects occurred at about 5 and 100 microunits/ml of insulin, respectively. In conclusion, the exposure of human fat cells to physiological insulin doses is followed by a rapid and dose-dependent translocation of β-adrenoceptors from the exterior to the interior of the cell and a subsequent dose-dependent decrease in the lipolytic sensitivity to β-adrenergic agonists, without a change in maximum lipolysis. This interaction between insulin and the β-adrenoceptor seems to require a complete insulin signal from the receptor to the effector and it may constitute one mechanism by which insulin acutely inhibits catecholamine-induced lipolysis in human fat cells.

Insulin and catecholamines are of major importance for the control of lipid mobilization from fat cells. Only these hormones have pronounced acute effects on lipolysis in human adipose tissue (1). Catecholamines stimulate the rate of lipolysis by interactions with β-adrenergic receptors. However, in human and hamster adipocytes, catecholamines may also inhibit the rate of lipolysis by interaction with the α2-adrenergic receptors (2). The mechanisms by which these effects of catecholamines on fat cell lipolysis are mediated are largely unknown (2). It is well established that insulin inhibits catecholamine-stimulated lipolysis. However, the mechanisms by which insulin exerts this acute antilipolytic effect remain unknown. Several models of action have been suggested, such as the inhibition of adenylate cyclase activity, stimulation of high affinity cyclic AMP phosphodiesterase, inhibition of the cyclic AMP-dependent protein kinase activity and a direct inhibitory effect on the hormone-sensitive lipase (3). An additional mechanism of insulin action which, it seems, remains unexplored is the inhibition of catecholamine-induced lipolysis by specific interactions with fat cell adrenoceptors. It has become increasingly evident that hormones may produce their effects by heterologous hormone-receptor interactions in addition to actions via their own receptor-effector pathways (see Ref. 4 for detailed discussion). This may include interplay between catecholamines and insulin as well. It has recently been reported that catecholamines reduce the adipocyte insulin receptor number (4-7) and that this may partly explain some of the inhibitory effects of catecholamines on the insulin-stimulated hexose transport in adipocytes (4, 6). Chronic insulin exposure has also been shown to inhibit the lipolytic response to β-adrenergic agonists in cultured 3T3-L1 cells (8). Although these hormone effects may be of importance for pathologic conditions such as catecholamine-induced insulin resistance, it is unknown whether heterologous hormone-receptor interactions between insulin and catecholamines play a physiologic and regulatory role. The aim of the present study was to determine whether insulin has acute effects on adrenoceptor binding and whether such effects are of importance for the ability of insulin to inhibit the catecholamine-induced rate of lipolysis. This was done by using recently developed methods which allow a direct com-

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parison of lipolysis and adrenergic binding in intact human adipocytes (9, 10).

**MATERIALS AND METHODS**

**Preparation of Isolated Fat Cells**—Subcutaneous adipose tissue was obtained from operations on patients undergoing elective abdominal surgery. None of the patients had jaundice, metabolic disorders, or malignant diseases. No attempt was made to select the patients on the basis of age, sex, or body weight. General anesthesia was induced with a short-acting barbiturate and maintained with phenylbutin in combination with N₂O and O₂. The patients fasted overnight and saline alone was infused intravenously before removal of the adipose tissue, which was done at the beginning of surgery. The specimens of adipose tissue were transported to the laboratory in saline. The study was approved by the Ethics Committee at Karolinska Institute. Fat cells were isolated as described in detail previously (9, 10). In brief, adipose tissue fragments were incubated for 60 min with 0.3 mg/ml collagenase in Krebs-Ringer phosphate (KRP) buffer containing 40 mg of bovine serum albumin per milliliter. Fat cells were isolated from the digested stroma by filtration and repeated washing. Subcellular fractions were prepared from the isolated fat cells by re-suspension of 4 ml of packed fat cells in 30 ml of 0.25 M sucrose, 4 °C. The cells were then homogenized by six up-and-down strokes in a glass Potter-Elvehjem homogenizer fitted with a Teflon pestle driven at 1200 rpm. The crude extract was defatted by centrifugation (1000 × g for 60 s), and the infranatant was aspirated with a syringe from below the fat plug. This solution was centrifuged in the manner described by Jarett (11) to obtain three fractions: a plasma membrane-enriched fraction, a microsomal-enriched fraction, and a cytosolic fraction. The protein content was estimated by the method of Lowry et al. (12).

**Lipolysis Experiments**—The assay has been described in detail elsewhere (9, 10). Briefly, diluted suspensions (1–2 × 10⁶ cells/ml) of isolated adipocytes were incubated for 2 h unless otherwise stated in KRP buffer (pH 7.4, 37 °C). The medium contained 40 g/l bovine serum albumin, 2 g/l glucose, and 0.1 g/l ascorbic acid as well as various concentrations of isoprenaline and/or insulin. An aliquot of the medium was taken for glycerol determinations. Each incubation included various concentrations of isoprenaline and/or insulin. An aliquot of the medium was taken for glycerol determinations. Each incubation was run in duplicate and air was used as the gas phase. The isoprenaline responsiveness in the lipolysis experiments was calculated as the difference between basal glycerol release and glycerol release at the maximum effective agonist concentration. The concentration of agonist that produced a half-maximum effect (ED₅₀) was calculated either manually or from logistic transformation of the dose-response curves, as described elsewhere (9). In some experiments the adipocytes were preincubated for 60 min in the same buffer, as described above, before the lipolysis experiments were performed. We have repeatedly demonstrated (10, 13) that when human fat cells are isolated and incubated in this dilute manner, there is no influence of endogenous adenosine contamination on the rate of catecholamine-induced lipolysis. There was no change in the lipid weight of fat cells during these incubation conditions.

**Radioligand Binding Experiments**—These experimental procedures have also been described in detail previously (9, 10). In brief, 3 × 10⁶ isolated adipocytes were incubated at 37 °C in 1 ml of the same buffer as in the lipolysis experiments, except that the albumin content was reduced to 10 g/l. The radioligands were used as [H]yohimbine in the α₂-adrenergic receptor assay, and [H]CGP (nonselective hydrophilic β-adrenergic ligand) or [¹²⁵I]CYP (nonselective hydrophobic β-adrenergic ligand) in the β-adrenergic receptor assay. Non-specific binding was determined in three fractions: a plasma membrane-enriched fraction, a microsomal-enriched fraction, and a cytosolic fraction. The protein content was estimated by the method of Lowry et al. (12).

The pooled results of a large number of adrenergic binding experiments on different individuals are shown in Fig. 1. A preincubation of human adipocytes during 60 min with 1000 micromolars/ml insulin resulted in a reduction of about 25% (range 14–34%) in the subsequent binding of the hydrophobic β-adrenergic receptor ligand [¹²⁵I]CYP to the adipocytes, as compared with control cells incubated in buffer without insulin (p < 0.01). Much the same results as with [¹²⁵I]CYP were obtained with the hydrophilic β-adrenergic receptor ligand [H]CGP. The binding of the α₂-adrenergic receptor ligand [H]yohimbine to the adipocytes was unchanged after preincubation with insulin. The exclusion of glucose from the incubation binding assay were washed twice with the incubation buffer and thereafter the same agent that was used during the preincubation was added to the buffer during the binding assay.

**Preparations Used**—Bovine serum albumin (fraction V) (lot No. 63F-0748) was obtained from Sigma. Collagenase prepared from *Clostridium histolyticum* was of Sigma type I. [H]Yohimbine (specific activity, 82.6 Ci/mmol) and [¹²⁵I]CYP (specific activity, 2280 Ci/ mmol) were purchased from Du Pont/New England Nuclear. [¹²⁵I]CYP (specific activity, 41 Ci/mmol) was obtained from Amersham Corp. Phentolamine was a gift from Ciba-Geigy, Gothenburg, Sweden. (±)-Propranolol and (±)-isoprenaline came from Sigma. Crystalline, guacon-free porcine insulin was a gift from Vitrum AB, Stockholm, Sweden. Rabbit polyclonal insulin receptor antibody serum was a generous gift from Dr. James N. Livingston, Rochester, NY. All other chemical were commercially available and of the highest degree of purity.

**Statistical Methods**—The values presented are the means and, when appropriate, the standard error of the mean. The Student's t test, linear regression analysis, and the F distribution test (14) were used for the statistical analyses. When displacement curves were compared in the binding experiments a computer-based calculation program (SCAPIT) was used (15). In the kinetic experiments it was not suitable to pool data from experiments with different individuals. The latter data are instead presented as one representative experiment out of 3–4 experiments.

**RESULTS**

Fig. 1. Effects of insulin on binding of [¹²⁵I]yohimbine (5 nmol/l), [¹²⁵I]CYP (250 pmol/l), and [¹²⁵I]CGP (1 nmol/l) to human adipocytes. The adipocytes were preincubated for 60 min with (hatched bars) or without (open bars) 100 micromolars/ml insulin. The cells were then washed with KRP buffer containing 40 mg/ml albumin and incubated, using the binding assays described under "Materials and Methods." The values are the mean ± S.E. of nine experiments. The data with and without insulin were evaluated statistically with the Student's paired t test.
buffer did not change the results (data not shown).

The binding experiments mentioned above were performed with only one high concentration of the radioligand. To determine more exactly the insulin-induced changes in the β-adrenergic receptor-binding isotherms, saturation experiments were performed (Fig. 2). Adipocytes were preincubated for 60 min with or without 1000 microunits/ml insulin and then incubated with increasing concentrations of \(^{[125]I}\)CYP from 8 to 1000 pmol/l. Scatchard analysis of a typical experiment showed two almost parallel lines. When these were compared using the F distribution test (14) it was found that the position but not the slope differed significantly (p < 0.01), indicating that the maximum receptor number was reduced in insulin-treated cells, whereas the receptor affinity was not altered.

The possibility that insulin may influence the β-adrenergic receptor affinity was further explored in the experiments shown in Fig. 3. In a previous study (10) we observed that in intact human cells labeled with a β-antagonist, such as \(^{[125]I}\)CYP, the addition of a β-agonist to the medium was followed by a rapid (i.e., within 1 min) agonist-induced decrease in the affinity of the β-adrenoceptor for the agonist. This may depend on a sequestration of the receptors into the cells, the existence of two independent receptors, or that the β-adrenoreceptors may have different affinity states for catecholamines, i.e., a high affinity state during the initial ligand-receptor interaction which is altered to a low affinity state as equilibrium binding is approached. To determine whether insulin could influence this process displacement, experiments with isoprenaline were performed. The effect of isoprenaline on \(^{[125]I}\)CYP binding to adipocytes was determined in cells incubated for 1 or 60 min which had been preincubated for 60 min with or without insulin (1000 microunits/ml). As can be seen, there was a decrease in the affinity for isoprenaline when the incubation was terminated after 60 min, as compared with the 1-min incubation. However, there was no difference between the displacement curves of control or insulin-treated cells. This was also true when in other experiments binding data obtained after 60 min of incubation (equilibrium conditions) were compared using a computer-based calculation program (15). In these experiments, 20 different concentrations of isoprenaline were used to obtain an optimal fit of the displacement curve to a multisite model (15). About 30% of the receptors were found to be in a high affinity state in both control and insulin-treated cells and the Kd values for low and high affinity binding sites were not altered by insulin (data not shown).

Fig. 4, upper panel, shows the dose-response relationship for the insulin inhibition of β-adrenoceptor binding. In these experiments the effect of a 60-min preincubation with increasing concentrations of insulin on the subsequent binding of \(^{[125]I}\)CYP was examined. A clear dose-response effect of insulin was observed; ED50 was obtained at about 5 microunits/ml insulin and maximal effect was obtained with 190 microunits/ml insulin.

To determine the time course for the effect of insulin (1000 and 100 microunits/ml) on the reduction of adipocyte binding of \(^{[125]I}\)CYP the experiments shown in the lower panel of Fig. 4 were performed. A rapid effect of insulin when added at both concentrations was observed, although the high hormone dose was somewhat more effective. That is, half-maximal effect occurred at an earlier time point with the high insulin concentration than with the low insulin concentration. This probably depends on the fact that adipocyte insulin receptors are occupied by insulin at faster rates in the presence of high as compared with low hormone concentrations. Already after 1 min of preincubation there was a marked reduction in the binding of \(^{[125]I}\)CYP to the adipocytes (one fourth to one-third of the maximal effect). Thereafter, there was a further and gradual decrease in \(^{[125]I}\)CYP binding until, after 40–60 min, the effect of insulin on binding reached a steady state. The effect of insulin was also fully reversible since the reduced
**Insulin and Adrenoceptors**

**FIG. 4.** Insulin and $[^{125}\text{I}]$CYP binding (250 pmol/l) to human adipocytes. Upper panel, dose-response relationship. The adipocytes were preincubated for 60 min with various concentrations of insulin, and then washed and submitted to the binding assay with $[^{125}\text{I}]$CYP, as described in the legend to Fig. 1. The binding of $[^{125}\text{I}]$CYP to the cells was expressed as a percentage of control cells; that is, cells not exposed to insulin. The values are the mean ± S.E. of two experiments. From the insulin concentration of 10 microunits/ml and higher the effect on the $[^{125}\text{I}]$CYP binding was significant ($p < 0.05$). Arrow indicates the ED$_{50}$ value. Lower panel, time course for the effect of insulin on $[^{125}\text{I}]$CYP binding to human adipocytes. The adipocytes were preincubated for different periods, with 1000 microunits/ml (panel A) and 100 microunits/ml (panel B) insulin. They were then washed and subjected to binding with $[^{125}\text{I}]$CYP, as described in the legend to Fig. 1. The binding of $[^{125}\text{I}]$CYP to the cells was expressed as a percentage of control cells; that is, cells not exposed to insulin (the specific binding in these fat cells did not change with time and was 180 fmol/g of lipid). Arrows indicate the time points when half-maximal effect of insulin occurred. The values are the mean ± S.E. of three experiments. In panel A, the effect of insulin on $[^{125}\text{I}]$CYP binding was significant from 40 min and onward ($p < 0.05$) and in panel B the effect of insulin on $[^{125}\text{I}]$CYP binding was significant from 60 min and onward ($p < 0.05$).

$\beta$-adrenoceptor binding after insulin exposition had returned to initial levels within 30 min when the cells were reincubated in insulin-free buffer (Table I).

In an attempt to determine whether the interaction between

**TABLE I**

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<th>Time course of recovery of insulin-induced down-regulation of $\beta$-adrenoceptor binding</th>
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<td>Fat cells were preincubated with 100 microunits/ml insulin for 60 min and replaced in insulin-free buffer. After 0, 10, 30, and 60 min, fat cells were removed for quadruplicate determination of $[^{125}\text{I}]$CYP binding (250 pmol/l) or $[^{3}\text{H}]$CGP binding (1 nmol/l). Values are percent of specific binding of control cells (i.e., cell not exposed to insulin). One representative experiment out of three is shown. Each incubation was done in triplicate.</td>
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<th>Radioligand</th>
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<tr>
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<td>0 min</td>
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<tr>
<td>$[^{125}\text{I}]$CYP</td>
<td>79</td>
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<tr>
<td>$[^{3}\text{H}]$CGP</td>
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insulin and $[^{125}\text{I}]$CYP binding to adipocytes was specific for the hormone, fat cells were preincubated with different insulin mimickers (Fig. 5). It was found that only insulin and rabbit insulin receptor antibody serum (but not control serum) reduced the subsequent binding of $[^{125}\text{I}]$CYP to the fat cells. The insulin mimickers concanavalin A (which has interaction with the insulin receptor that differ from insulin) or spermine and hydrogen peroxide (which have their main action at steps subsequent to the insulin receptor) did not produce an effect. The non-insulin-like antilipolytic agent nicotinic acid also had no effect (data not shown).

To determine whether insulin treatment leads to redistribution of $\beta$-adrenergic receptors within the adipocyte, subcellular-fractions were prepared from fat cells incubated with or without 1000 microunits/ml insulin. It was found that insulin treatment reduced the $[^{125}\text{I}]$CYP binding in the plasma membrane-enriched fraction and increased the binding in the microsomal-enriched fraction (Fig. 6). In the cytosolic frac-
The time course for the antilipolytic effect of 1000 microunits/ml insulin on isoprenaline-induced lipolysis was also studied. The initial effect of insulin was observed after a lag period of 3 min. Before that time the insulin and control curves were parallel (data not shown). The high insulin concentration was used to let a large number of insulin receptors be occupied per unit of time, so that the time course experiment was not influenced by the rate of insulin receptor occupancy.

The interaction between insulin and lipolysis was examined in detail in the experiments depicted in Fig. 7. Fat cells were incubated with or without increasing concentrations of isoprenaline in the absence or presence of different concentrations of insulin. The dose-response curves for isoprenaline at various insulin concentrations showed two different characteristics (Fig. 7A). First, with increasing concentrations of insulin, there occurred a gradual and marked horizontal shift of the curves to the right. Second, with increasing insulin doses there was a gradual but less marked vertical lowering of the curves. This suggests that insulin had at least two different effects on lipolysis.

First, there was a small inhibition of the maximal isoprenaline-induced lipolysis which may be due only to the inhibition of basal lipolysis. Second, there was a more marked inhibition of isoprenaline sensitivity. These two effects of insulin were further explored by plotting the dose-response curves in different ways. In Fig. 7B, the ability of insulin to inhibit basal lipolysis was compared with the effect of insulin on the maximal isoprenaline-induced rate of lipolysis. It was observed that both of these insulin effects were clearly dose-dependent, but small and almost identical. At the highest hormone concentration (100 microunits/ml), insulin inhibited basal lipolysis and the maximal isoprenaline-induced rate of lipolysis in the same order of magnitude (0.6 pmol of glycerol/g of lipid/h). Furthermore, when basal glycerol release was subtracted in the isoprenaline dose-response curves, it was found that insulin only shifted the curves to the right without changing their amplitude (graph not shown). Thus, these data strongly indicate that insulin inhibits basal lipolysis but does not change isoprenaline responsiveness (maximum effect). The effect of insulin on isoprenaline sensitivity is depicted in Fig. 7C. A clear insulin-induced, dose-dependent inhibition of isoprenaline sensitivity was observed. The ED50 values for isoprenaline were 5 x 10^-10, 1 x 10^-9, 2 x 10^-9, 3 x 10^-9, and 5 x 10^-9 for the insulin concentrations, 0, 0.1, 1, 10, and 100 microunits/ml, respectively. Thus, a half-maximum effect on isoprenaline sensitivity occurred at about 5 microunits/ml insulin. In uncharted experiments, higher concentrations of insulin up to 10,000 microunits/ml had no additional effects on basal lipolysis, insulin responsiveness, or insulin sensitivity. It should be noted that the dose-response relationship for insulin inhibition of ED50 for isoprenaline was in close harmony with that for insulin inhibition of [125I]CYP binding in Fig. 4.

In most cell systems only a fraction of the β-adrenergceptors have to be occupied to obtain a full biological effect after catecholamine stimulation, which is due to the existence of functional spare receptors. We have recently observed that a receptor reserve also exists for β-adrenergic induced lipolysis in human fat cells (10). According to the spare receptor hypothesis, a small reduction in receptor number is accompanied by a shift to the right without a change in the amplitude of the lipolytic dose-response curve (16). The results with binding and lipolysis in Figs. 4 and 7 best fit a receptor model in which insulin reduces the number of available spare β-adrenergceptors. To examine further which effects a reduction of β-adrenergic receptor number had on the shape of the dose-response curve for isoprenaline-induced lipolysis, experiments were performed in which different fractions of the...
receptors were blocked. $[^{125}I]$CYP was used as a pseudo-
irreversible blocking agent, since the ligand has a slow disso-
ciation rate from the $\beta$-adrenoceptors ($t_{1/2}, 90$ min) (9). Fat
cells were incubated with different concentrations of $[^{125}I]$CYP
to block different fractions of the $\beta$-adrenergic receptors.
Thereafter, the cells were washed and incubated for 30 min
with increasing concentrations of isoprenaline, and glycerol
release was measured. As can be seen from Fig. 8, increasing
fractional inactivation of available binding sites up to 46% of
the receptors was accompanied by a successive shift to the
right of the isoprenaline dose-response curve without a change
in the amplitude of the curve. When a larger receptor fraction
was inactivated there was an additional reduction of isoprena-
line responsiveness (data not shown). This results suggest
that spare receptors are involved in isoprenaline-induced li-
polysis in human fat cells. Fig. 8 also shows that a reduction of
the receptor number with 15% receptor occupancy, a 65% receptor occupancy of the dose-
response curve for isoprenaline about 10-fold to the right
without any reduction of the amplitude. These data are in
close harmony with those observed for insulin in Figs. 4 and
7.

The specificity of the interaction between insulin, cate-
cholamines, and lipolysis was also examined. The insulin
mimicker concanavalin A, which did not alter $\beta$-adrenergic
receptor binding as shown above, decreased the amplitude of
the isoprenaline dose-response curve, whereas insulin shifted
the dose-response curve to the right without changing the
amplitude of isoprenaline-induced rate of lipolysis (data not shown).

Another insulin mimicker, spermine, had effects similar to
those of concana valin A on isoprenaline-induced lipolysis.
Furthermore, the effect of insulin on parathyroid hormone-
induced lipolysis was clearly different from that on isoprena-
line-induced lipolysis. The main effect of insulin was an
inhibition of the parathyroid hormone responsiveness (data
not shown).

\begin{figure}
\centering
\includegraphics[width=\textwidth]{figure7}
\caption{Lipolytic dose-response curves for adipocytes incubated with or without different concentra-
tions of isoprenaline together with different concentrations of insulin. The adipocytes were incubated
for 60 min and at the end of the incubation an aliquot of the medium was taken for glycerol estimation. A shows
the complete isoprenaline dose-response curves. $B$ shows the effect of insulin on basal and maximal isoprenaline-
induced rates of glycerol release. The isoprenaline curve was generated by subtracting the glycerol release at the
maximum effective isoprenaline concentration for each insulin concentration from the corresponding maximal
value for cells not exposed to insulin. $C$ shows the effect of various isoprenaline concentrations on glycerol release,
expressed as a function of the maximum effect of isoprenaline on each insulin concentration. The symbols for cells
exposed to different insulin concentrations are as in A. The results are the mean of experiments on nine different
individuals.}
\end{figure}

\begin{figure}
\centering
\includegraphics[width=\textwidth]{figure8}
\caption{Effect of pseudo-irreversible receptor blockade on the relationship between lipolysis and $\beta$-adrenergic receptor
occupancy. Isolated human fat cells were preincubated for 60 min
in the absence or presence of two different concentrations of $[^{125}I]$CYP (8 and 31 pmol/l). This $\beta$-receptor antagonist has a slow disso-
ciation rate from human fat cells ($t_{1/2}, 90$ min) (9). After washing, the
cells were incubated in radioactivity free buffer with or without
various concentrations of isoprenaline in the absence or presence of $10^{-5}$ mol/l propranolol (to determine nonspecific binding). Glycerol
release over basal levels and specific cell-associated radioactivity were
determined after 30 min of incubation. The total fractions of total
binding sites were estimated from Scatchard analysis of specific
binding of $[^{125}I]$CYP at the end of the preincubation period. The
results of one representative experiment out of three are given. The
percentage values represent the fraction of total radioligand
binding sites occupied at the end of the preincubation period. Arrows indicate the concentrations at which isoprenaline gives half-maximal effect.}
\end{figure}
In human fat cells, unlike adipocytes in most other species, catecholamines have both stimulatory and inhibitory effects on lipolysis, which are mediated by $\beta$- and $\alpha_2$-adrenoceptors, respectively (2). The present study shows that insulin exposure causes a specific interaction with $\beta$- but not with $\alpha_2$-adrenoceptors in human fat cells. Thus, insulin acutely inhibits the specific binding of $\beta$-adrenoceptor radioligands to these cells. The inhibition was observed with a hydrophilic radioligand which labeled cell surface binding as well as with a hydrophobic radioligand which also labeled sequestered binding sites (17). This indicates that insulin induces a true reduction in cell surface $\beta$-adrenoceptor binding. The results of the saturation and competition-inhibition experiments strongly suggest that insulin reduces the $\beta$-adrenoceptor binding without changing the affinity of the $\beta$-adrenoceptor. The findings with subcellular fractions indicate that insulin acutely translocates human adipocyte $\beta$-adrenoceptors from the plasma membrane to an intracellular particulate compartment.

The inhibitory effect of insulin on $\beta$-adrenoceptor number occurred within the physiologic range of insulin concentrations and it was both rapid and reversible. After only 1 min of exposure with a high insulin concentration (1000 microunits/ml) a significant portion of the $\beta$-adrenoceptor binding sites were decreased and the maximum effect occurred at 40 min. A somewhat slower effect was observed with lower concentrations of insulin, since, in that case, it takes a longer time to occupy the amount of insulin receptors necessary to elicit an effect. As much as one-fourth of the total $\beta$-adrenoceptor population could be inhibited by physiologic insulin doses. So far as we know, this is the first study in which an acute effect of insulin on $\beta$-adrenoceptor binding has been observed. However, in an earlier preliminary study, prolonged incubation (24 hours) with pharmacologic insulin concentrations caused a reduction in $\beta$-adrenoceptor number in cultured 3T3-L1 adipocytes to the same extent as in our study (18).

It is well established that $\beta$-adrenoceptors undergo homologous down-regulation (and desensitization) after exposure to catecholamines (for review, see Ref. 19). The present results indicate that they undergo heterologous down-regulation also through the interaction with insulin. The molecular mechanisms underlying this effect of insulin are unknown but so also are the detailed mechanisms underlying all the other biologic effects of insulin. The insulin $\beta$-adrenoceptor interaction may be due to mechanisms that resemble or differ from those responsible for the homologous down-regulation of the $\beta$-adrenoceptor. Both catecholamine binding to $\beta$-adrenoceptrons (20) and insulin binding to insulin receptors (21) are associated with phosphorylation of the homologous receptor, which may mean that there is a common mechanism involving phosphorylation-dephosphorylation reactions. These reactions may be responsible for the process that leads to internalization of the insulin (22) and $\beta$-adrenergic receptors (23) after interaction of the hormones with its receptors. In the present study, an internalization of $\beta$-adrenergic receptors was also observed after insulin treatment, supporting the idea of a common mechanism for the regulation of this process for insulin and $\beta$-adrenergic receptors.

The effect of insulin on the $\beta$-adrenoceptor seems to require a complete insulin signal—i.e. it is initiated by the binding of insulin to the specific hormone binding site on the insulin receptor molecule. This theory is supported by our findings that only insulin and a polyclonal insulin receptor antibody had inhibitory effects, whereas other insulin mimickers such as spermine and hydrogen peroxide (acting at postreceptor binding levels) or concanavalin A (which has a different receptor interaction than insulin) had no effects on $\beta$-adrenoceptor binding. The effect of insulin on the $\beta$-adrenoceptor was not due to a decrease in the rate of lipolysis per se, since nicotinic acid (a potent non-insulin-like antilipolytic agent) did not alter the $\beta$-adrenoceptor.

A significant question is whether the interaction between insulin and the $\beta$-adrenoceptor plays any physiologic role in hormone action. The results of the lipolysis experiments suggest that the interaction described is of importance for the ability of insulin to inhibit catecholamine-induced lipolysis. First, there was a close correlation between the decrease in $\beta$-adrenergic receptor binding and the increase in the $ED_{50}$ of isoprenaline-induced lipolysis by insulin. Half-maximum effect on binding and $ED_{50}$ occurred at about 5 microunits/ml insulin and the maximum effect on binding and $ED_{50}$ occurred at 100 microunits/ml insulin. Second, the time course for the reduction of $\beta$-adrenergic receptors and the antilipolytic effect of isoprenaline-stimulated lipolysis were similar, that is, effects could be seen within a few minutes.

The maximum effect of insulin on $\beta$-adrenergic receptor binding observed in this study may seem small in relation to the effect of the hormone on $\beta$-adrenergic induced lipolysis. However, the results of the binding studies reflect the net sum of continuous internalization and recycling of $\beta$-adrenoceptors. Therefore, the possible separate effects of insulin on $\beta$-adrenoceptor internalization or re-cycling may be greater than the measured effect on steady-state $\beta$-adrenoceptor binding. Unfortunately, there are no methods available to study separately internalization or recycling of $\beta$-adrenoceptors in fat cells. Furthermore, according to the spare receptor hypothesis (16), a moderate decrease in $\beta$-adrenergic receptor number is accompanied by a more marked shift to the right in the isoprenaline dose-response curve without a change in the amplitude of the curve (that is a decrease in sensitivity and unaltered responsiveness). In a recent study, we found a nonlinear relationship between isoprenaline-induced rate of lipolysis and $\beta$-adrenoceptor binding in human fat cells, which could be partly explained by spare receptors (10). From the present results with fractional receptor blockade it was observed that a relatively small reduction of the $\beta$-adrenergic receptor number (15%) was associated with a rather great shift (10-fold) to the right of the lipolytic dose-response curve for isoprenaline. These experiments confirm our previous results with fat cells (10) but differ somewhat from studies with other cell types, which show a less marked effect of adrenoceptor blockade on the cellular response to catecholamines. The discrepancies may have a methodologic basis. Most earlier investigators have not, in contrast to us, compared receptor binding and biologic action under identical conditions (see Ref. 10 for detailed discussion). Furthermore, it is possible that the relationship between adrenoceptor binding and catecholamine action differs between various cell types. In addition, the present study results show that a small insulin-induced reduction of the $\beta$-adrenergic receptors also gives a marked shift to the right of the lipolytic dose-response curve for isoprenaline without altering isoprenaline responsiveness. The effects of adrenoceptor blockade and insulin on isoprenaline sensitivity were similar. In earlier experiments with rat adipocytes, insulin shifted the adrenaline lipolysis dose-response curve to the right without altering adrenaline responsiveness (24). This suggests that interactions between insulin and $\beta$-adrenoceptors may take place in rat fat cells as well.

Although some of the present results suggest that insulin may inhibit catecholamine-induced lipolysis by translocating
β-adrenoceptors, other findings in this study suggest additional mechanisms for the antilipolytic effect of the hormone. The inhibition of basal and parathyroid hormone-induced lipolysis cannot be explained by an insulin β-adrenoceptor interaction. The same is true for the observed antilipolytic effect of concanavalin A. In fact, this insulin mimicker has been shown to prevent internalization of β-adrenergic receptors (25). When all present data with lipolysis are taken together, it seems likely that insulin exerts its antilipolytic effects in human fat cells through several separate molecular events. This hormone effect seems to require a complete insulin physiologic doses rapidly translocates the β-adrenoceptors from the plasma membrane to the interior of human fat cells and that this is accompanied by a decrease in the lipolytic sensitivity of β-agonists without a change in lipolytic responsiveness. This hormone effect seems to require a complete insulin signal from the receptor binding site to the effector and it may constitute one mechanism by which insulin inhibits catecholamine-stimulated lipolysis.

In summary, the present study shows that insulin in physiologic doses rapidly translocates the β-adrenoceptors from the plasma membrane to the interior of human fat cells and that this is accompanied by a decrease in the lipolytic sensitivity of β-agonists without a change in lipolytic responsiveness. This hormone effect seems to require a complete insulin signal from the receptor binding site to the effector and it may constitute one mechanism by which insulin inhibits catecholamine-stimulated lipolysis.

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