Interactions between the Receptors for Insulin and the Insulin-like Growth Factors on Adipocytes*

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Insulin increased the binding of 125I-multiplication stimulatory activity (MSA) by 50% of control within a few minutes at 24 °C. Under steady state conditions, MSA binding was increased in dose-dependent fashion by insulin at concentrations between 0.1 and 0.2 ng/ml, concentrations nearly identical with those observed for the stimulation of glucose oxidation by insulin in these cells. Scatchard analysis of 125I-MSA binding showed a single class of noninteracting sites with an association constant of about 1.2 × 10^6 M^-1. Insulin increased MSA receptor affinity without altering the number of MSA receptors. Kinetic experiments revealed that this increase in affinity was due to a decrease in the apparent dissociation rate constant k_d from 9.5 ± 0.7 × 10^-2 to 5.7 ± 0.5 × 10^-2 min^-1. No significant change was seen with the association rate constant k_a. This effect of insulin on MSA receptors appeared to be specific and involved the insulin receptor. Neither spermine nor vitamin K_3, which stimulate glucose oxidation nor chemically unrelated peptide hormones, such as epidermal growth factor and glucagon, could mimic this effect. Proinsulin, a weak insulin analogue, enhanced 125I-MSA binding but at 50-fold higher concentrations. Antibodies (IgG) to the insulin receptor which exhibit insulin-like activity also produced an increase in MSA binding, where the monovalent Fab fragments of the IgG, which bind to the insulin receptor but do not stimulate glucose oxidation, did not. Treatment of adipocytes with antireceptor Fab did not inhibit the ability of insulin to stimulate glucose oxidation and to enhance MSA binding. Antimicrotubular or antimicrofilament agents also inhibited the insulin-stimulated enhancement of MSA binding. Insulin was ineffective in enhancing 125I-MSA binding at 15 °C or in a crude fat cell membrane preparation.

We and others have shown that many types of mammalian cells have separate receptors for insulin and the IGFs (3, 7-10). By direct binding studies of radioactive-labeled insulin and IGFs, these receptors have been shown to differ in their temperature and pH optima (10-12), in their specificity for insulin and IGF analogues (9-14), and in their reactivity toward antibodies directed against the insulin receptor (15, 16).

Recently, we have shown that the ability of MSA, an IGF isolated from conditioned media of Buffalo rat liver cells, to mimic insulin’s metabolic effects in rat adipocytes, is due to MSA’s binding to the insulin receptor whereas the growth-promoting activity of MSA and insulin were mediated via another receptor, presumably through one of the growth factor receptors (16). During the study of the MSA receptor on rat adipocytes, we noticed that unlabeled insulin does not displace labeled MSA from the adipocytes; rather, it enhances the binding of labeled MSA (16). A similar observation was made by Schoenle et al. (17, 18), Zapf et al. (19, 20), and Meuli and Froesch (21), in earlier studies of IGF-I and IGF-II binding to rat adipocytes. In the present report, we have characterized this effect of insulin on the MSA receptor and found that this effect is mediated via the insulin receptor, suggesting an interaction between the receptors of insulin and MSA at the cellular level.

EXPERIMENTAL PROCEDURES

Materials—Crude collagenase (CL545KB7) and papain (37.4 mg/ml; 19.7 units/mg) was purchased from Worthington; bovine serum albumin (fraction V, lot NS-3309) from Armour; protein-A-Sepharose from Pharmacia; dimethyl phthalate, and glycine from Eastman; soybean trypsin inhibitor, L-cysteine, and iodoacetamide from Sigma; epidermal growth factor from Collaborative Research, Inc.; glucagon and pork proinsulin from Novo Pharmaceutical Co.; vinblastine and porcine insulin (27.5 units/mg) from Lilly; and colchicine and cytochalasin D from Aldrich.

Polypeptides—MSA was purified from serum-free media conditionned by the BRL-3A cell line as previously described (2). The conditioned medium was absorbed onto Dowex at neutral pH and eluted at pH 11. The Dowex eluate was then chromatographed on Sephadex G-75 in 1.0 M acetic acid. MSA, assayed by the stimulation of [3H]thymidine incorporation into DNA in chick embryo fibroblasts, eluted from Sephadex G-75 in 3 broad regions, designated I, II, and III (21).

On gel electrophoresis, there are four species of MSA in the peak II region. Each of these MSAs has equal specific activity in chick embryo fibroblast bioassay, radioreceptor, and radioimmunoassay, and therefore, fractions from G-75 Sephadex containing these 4 species were pooled and used as standard in the radioreceptor assays. For iodination, peak II MSA was subjected to further purification by preparative polyacrylamide gel electrophoresis (pH 2.3, 9 M urea) and a homogenous polypeptide, MSA II-1, was isolated. 125I-MSA was prepared by a previously published modification of the chloramphen-T
procedure at specific activities of 42-75 mCi/g (11).

**Isolation of Antireceptor IgG and Fab Fragments**—The source of antireceptor IgG was serum from a patient (B-2) with the Type B syndrome of extreme insulin resistance and acanthosis nigricans (23, 24). This patient's serum is known to contain a high titer of antibody to the insulin receptor. To purify antireceptor IgG, 3 ml of serum was dialyzed against 24 h at 4 °C into a column containing 5 ml of sheep IgG-Sepharose (25), gel volume of 6 ml, equilibrated with 0.1 M sodium phosphate buffer, pH 7.0. The column then was washed three times with 6 ml/wash of 0.1 M sodium phosphate buffer, pH 7.0. The IgG was eluted with 9 ml of 0.1 M glycine/HCl buffer, pH 2.8. The eluate was concentrated to 2 ml and then dialyzed against 0.1 M sodium phosphate buffer, pH 7.4, overnight. This IgG preparation had a protein concentration of 10 mg/ml determined by the method of Lowry et al. (26) using bovine serum albumin as standard.

Fab fragments of antireceptor IgG were prepared as follows: 2 ml of IgG preparation in 0.1 M sodium phosphate buffer, pH 7.0, were incubated with 4 mg/ml cytochrome, 3 pg/ml of papain, and 1 mg EDTA at 37 °C for 16 h. Iodoacetamide then was added to a final concentration of 5 mM. The reaction mixture was applied to a protein-A-Sepharose column equilibrated in 0.1 M sodium phosphate buffer, pH 7.0, and dialyzed overnight. Any remaining intact IgG or Fc fragments bind to the protein A column. Fab fragments do not bind to the protein A 24) and were eluted with 10 ml of 0.1 M sodium phosphate buffer, pH 7.0. The eluate was then concentrated to 2 ml by ultrafiltration. Fab fragments were purified by filtration on a Sephadex G-200 column (1.5 x 70 cm, bed volume 145 ml) in 0.1 M sodium phosphate buffer, pH 7.0. The major protein peak had an elution volume consistent with the molecular weight of Fab fragments and blocks insulin tracer binding in a variety of cells at concentrations of 1-10 μg/ml.

**Preparation of Isolated Adipocytes and Crude Adipocyte Membrane**—Male Sprague-Dawley rats of 100-160 g were used for all experiments. All studies were performed in the morning on animals that had free access to Purina rat chow. Animals were decapitated and the epididymal fat pads removed. The fat pads were cut into several pieces and digested at 37 °C in Krebs-Ringer bicarbonate buffer containing 2 mg/ml collagenase as described by Rodbell (27, 28). The Krebs-Ringer bicarbonate buffer used contained 118 mM NaCl, 5 mM CaCl₂, 1.3 mM MgSO₄, 1.2 mM KH₂PO₄, and 2.5 mM NaHCO₃. The pH was adjusted to 7.4 with NaOH after equilibrating with 95% O₂, 5% CO₂. After a 60-min incubation at 37 °C, the isolated adipocytes were separated from undigested tissue by filtration through a silk screen. The cells were washed three times in a Krebs-Ringer bicarbonate buffer, pH 7.4, and resuspended in the same buffer containing 2% bovine serum albumin.

Dispersed epididymal adipocytes from 20 Sprague-Dawley rats weighing between 140 and 160 g were collected as described above in a conical tube. After vigorous shaking, the suspension of fat cells was sonicated with a Branson Soniprep 90 at high setting. Then the suspension of ruptured cells was centrifuged at 24,000 × g in a Sorvall superspeed centrifuge. The crude adipocyte membranes were prepared by the method described by Cuatrecasas (29).

**Hormone Binding with Adipocytes**—Unless otherwise noted, all studies of hormone binding were performed in the Krebs-Ringer buffer with 20 mg/ml of albumin, pH 7.4. The incubation volume was 0.5 ml. The final concentration of cells and 125I-MSA were 2-4 x 10⁶ cells and 250-300 pg/ml, respectively. Unlabeled peptides were added as indicated in the text or figures. Incubation time and temperature required for steady-state binding were 40 min and 24 °C for 125I-MSA binding studies. The incubation was terminated by removing 200-μl aliquots from the cell suspension and rapidly centrifuging the cells in 500-μl microfuge tubes to which 200 μl of dimethyl phthalate had been added as described by Gammeltoft and Gliemann (30). All binding studies were performed in duplicate or triplicate.

**Analysis of the Kinetic Studies**—The binding equilibrium constant (K) and the receptor concentration (K₀) were determined by the method of Scatchard analysis (31). The association and dissociation rate constants, kₐ and kₑ, were determined using the following equation (32, 33):

\[
\ln \frac{[HR]_t}{[HR]_0} = (k_a[H]_o + k_e)t
\]

where \([HR]_0\) is the concentration of hormone-receptor complexes at equilibrium, \([HR]_t\) equals the concentration of hormone-receptor complexes at time t, and \([H]_o\) indicates hormone concentration at time 0. Thus, when \(\ln([HR]_t)/([HR]_0 - [HR]_t))\) is plotted as a function of time, the slope of the line in \(k_a[H]_o + k_e\). By plotting the slopes determined by Equation 1 as a function of \([H]_o\) or in this case, \([125I-MSA]\), another straight line is generated. The slope of the line is the \(k_a\) and the y intercept is the \(k_e\).

**RESULTS**

**Effect of Insulin on 125I-MSA Binding to Its Receptor**—125I-MSA binding to isolated rat epididymal adipocytes (Fig. 1) was inhibited by unlabeled MSA concentrations as low as 10 ng/ml. The binding was inhibited by 50% at a MSA concentration of 60 ng/ml. MSA or IGF receptors on other tissues such as fibroblasts or liver (13, 21) can be divided into two types based on the ability of insulin to compete with MSA for binding. In fibroblasts, insulin is equipotent with MSA in inhibiting 125I-MSA binding, whereas in liver, insulin has almost no affinity for MSA receptors. By contrast, in the intact isolated adipocytes, another pattern of MSA binding was observed. Unlabeled insulin did not compete for MSA binding at any of the concentrations tested; rather, the addition of porcine insulin produced a dose-dependent increase in binding of 125I-MSA (Fig. 1). This insulin-induced increase of 125I-MSA binding was due to an increase in specific binding with no significant effect on the nonspecific binding (Fig. 2A).

The insulin effect on MSA binding was dose-dependent and occurred at physiological concentrations of insulin. The half-maximum concentration of insulin needed for the potentiation effect was 0.7 ng/ml (1.2 x 10⁻⁶ M) and the maximum effect was observed at insulin 2-5 ng/ml (5 x 10⁻⁶ M). The mean increase in MSA binding associated with the addition of insulin was 46% and varied in different experiments between 25% and 75%.

**Scatchard Analysis of MSA Binding**—In an attempt to better understand the nature of the increase in binding, com-

![Fig. 1. Effect of various peptide hormones on the binding of 125I-MSA to rat adipocytes. 125I-MSA binding to isolated rat adipocytes was performed under conditions described in "Experimental Procedures." Unlabeled insulin (○-○), glucagon and EGF (□-□), and unlabeled MSA (■-■) were added at the indicated concentrations. The data are from a representative experiment. R₀ is equal to the total binding of tracer MSA in the absence of any added hormones. Glucagon and EGF (epidermal growth factor) were added separately at each of the indicated concentrations.](image-url)
petition experiments were performed with unlabeled MSA in the presence and absence of a fixed concentration of insulin (10 ng/ml) known to maximally increase MSA binding. The increase of $^{125}$I-MSA binding produced by insulin was greatest at low concentrations of MSA: as the concentration of unlabeled MSA was increased, the curves approached one another (Fig. 2A).

Scatchard analysis of MSA binding both in the presence and the absence of insulin yielded straight lines indicating that MSA binds to a single class of receptors (Fig. 2B). In the control cells, the affinity of these receptors was $1.2 \times 10^6$ M$^{-1}$ and the binding capacity was $5 \times 10^5$ molecules of MSA/cell. In the cells treated with 10 ng/ml of insulin, the apparent affinity of MSA for binding to its receptor was increased to $2.5 \times 10^6$ M$^{-1}$. The receptor concentration (x intercept) was unchanged.

Evaluation of $^{125}$I-MSA Degradation—To investigate whether the observed increase in the affinity of MSA for its receptor was due to a decrease in $^{125}$I-MSA degradation in the presence of insulin, we measured the integrity of the $^{125}$I-MSA in the medium by gel filtration. $^{125}$I-MSA was exposed to rat adipocytes in the presence and the absence of 10 ng/ml of insulin for 40 min at 24°C. After removing the adipocytes by centrifugation in dimonyl phthalate, the infranatants were chromatographed on a PD-10 (G-25) gel filtration column. The elution profile of the $^{125}$I-MSA was the same for tracers incubated with cells in the presence and absence of insulin (Fig. 3). In addition, when the $^{125}$I-MSA in the infranatants was used for rebinding to fresh adipocytes, no significant difference was found between tracers exposed to cells or buffer only, indicating that very little degradation had taken place during the incubation (data not shown).

Association and Dissociation Rate Constants—Thus far, the data have indicated that insulin increases the apparent affinity ($K$) of adipocytes for $^{125}$I-MSA. To test whether the increase was due to a change in the association or dissociation rate, kinetic experiments were performed. Association experiments were performed using several concentrations of $^{125}$I-MSA in the presence and absence of 10 ng/ml of insulin in order to see whether the change is due to association or dissociation rates (Fig. 4). Using Equation 1 as stated under “Experimental Procedures,” the data from Fig. 4 were replotted to give Fig. 5. Since the slopes of the lines in Fig. 5 (A + B) are equal to $(k_a[H]_0 + k_d)$, we can calculate the association rate constant ($k_a$) and the dissociation rate constant ($k_d$) by plotting the slopes of lines as a function of $[H]_0$ (Fig. 5C).

In Table I the calculated apparent values of $k_a$ and $k_d$ in the presence and the absence of insulin are shown. A statistically significant change was observed in the $k_a$ but not for $k_d$, suggesting that insulin’s effect on $^{125}$I-MSA binding was due to a change in the apparent dissociation rate constant. Although the affinity constant for binding derived from the
The symbols used for the $^{125}$I-MSA concentrations were as described in Fig. 4. A and B. Lines in the figures were drawn using the method of linear regression. The symbols used are as described in Fig. 4. Lines in the figures were drawn using the method of linear regression.

Table 1

Equilibrium and kinetic parameters of MSA binding to rat adipocytes

The experimental conditions for the kinetic experiments are described in Figs. 4 and 5, and the equilibrium experiments in Fig. 2. Significant values and the $p$ values were determined by the paired-$t$ test.

<table>
<thead>
<tr>
<th>Kinetic experiments (n = 4)</th>
<th>Control</th>
<th>With insulin (10 ng/ml)</th>
<th>Change</th>
</tr>
</thead>
<tbody>
<tr>
<td>Association rate constant, $k_a$</td>
<td>5.9 ± 0.7 $\times$ $10^8$ M$^{-1}$ min$^{-1}$</td>
<td>5.6 ± 0.9 $\times$ $10^8$ M$^{-1}$ min$^{-1}$</td>
<td>N.S.$^*$</td>
</tr>
<tr>
<td>Dissociation rate constant, $k_d$</td>
<td>9.5 ± 0.7 $\times$ $10^2$ min$^{-1}$</td>
<td>5.7 ± 0.5 $\times$ $10^2$ min$^{-1}$</td>
<td>46$^b$</td>
</tr>
<tr>
<td>Equilibrium constant, $K$</td>
<td>6.2 $\times$ $10^8$ M$^{-1}$</td>
<td>9.5 $\times$ $10^8$ M$^{-1}$</td>
<td>40$^b$</td>
</tr>
<tr>
<td>Equilibrium experiments</td>
<td></td>
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</table>
| Receptor concentration, $R_0$ (sites/cell) | 1.2 $\times$ $10^9$ M$^{-1}$ | 2.5 $\times$ $10^9$ M$^{-1}$ | 94 ± 17
| $^a$ N.S., not statistically significant. $^b$ Statistically significant, $p < 0.05$. $^c$ The data expressed as standard error of the mean with an $n$ of 4.

Proinsulin also increased $^{125}$I-MSA binding with 5% of the potency of insulin (Fig. 6). Other peptide hormones, such as glucagon and epidermal growth factor did not enhance $^{125}$I-MSA binding, indicating the specificity of the effect for insulin and the insulin receptor (Fig. 1).

IgG antibodies to the insulin receptor purified from sera of patients with insulin-resistant diabetes have been found to be useful specific probes for the insulin receptor (23, 24). These antibodies inhibit insulin binding and mimic virtually all of insulin’s metabolic effects (23, 24). Furthermore, these antibodies are specific for the insulin receptor and do not inhibit $^{125}$I-MSA binding in rat adipocytes (25). Addition of antinsulin receptor antibody to this assay produced an increase of $^{125}$I-MSA, again mimicking this effect of insulin (Fig. 7). The concentration of the antibody needed to cause the effect was similar to the concentration of the antibody which stimulated glucose oxidation in the adipocyte (16).

We have previously shown that the antireceptor antibody requires bivalence for biological activity; thus monovalent Fab fragments of the antibodies to the insulin receptor are without biological effects, although they retain the ability to inhibit insulin binding (16, 34). Similarly, addition of Fab fragments of antireceptor antibody did not enhance $^{125}$I-MSA binding (Fig. 7). However, when Fab fragment at concentration of 25 $\mu$g/ml, which inhibited $^{125}$I-insulin binding by about 97%, was added with the unlabeled insulin, there was an inhibition of the insulin effect on MSA binding. In order to observe a complete dose-response curve for the increase of $^{125}$I-MSA binding, an 80-fold increase of insulin concentration was needed (Fig. 7).

To further support the specific involvement of the insulin receptor, we tested the effect of spermine and vitamin $K_3$ on $^{125}$I-MSA binding. These agents can stimulate glucose oxidation in adipocytes through a mechanism not involving the insulin receptor (35, 36). Neither spermine nor vitamin $K_3$ at
ments were added simultaneously. '2sII"SA binding are described under "Experimental Procedures." The insulin and Fab fragments were added simultaneously. '2sII"SA binding are described under "Experimental Procedures." Colchicine, vinblastine, and cytochalasin D at the stated concentrations did not have any effect on binding of '2'II"SA alone. Insulin (10 ng/ml) 150 °C  or less (data not shown).

Further evidence that the mechanism of the effect of insulin on MSA binding was performed as described under "Experimental Procedures." Colchicine vinblastine, and cytochalasin D at the stated concentrations did not have any effect on binding of 15 °C  or less (data not shown).

The Influence of Other Factors and Cytoskeletal Agents—Further evidence that the mechanism of the effect of insulin on 15 °C  or less (data not shown).

In addition, we tested agents which affect microtubules, such as colchicine, and agents which affect microfilaments, such as cytochalasin D and vinblastine, to see whether an intact cytoskeleton was required for this insulin effect. Both classes of these drugs were effective in inhibiting this effect of insulin (Table II). In contrast, these agents do not appear to have an effect on insulin binding (37) and do not alter insulin-stimulated glucose oxidation (34).

**DISCUSSION**

Most mammalian cells, including rat adipocytes, have separate receptors for insulin and the insulin-like growth factors (1-3, 13, 38). The insulin receptor binds insulin with a high affinity and a high degree of specificity, but it can bind also with weak affinity to the IGFs (1-3, 10, 11, 13). There appear to be several types of receptors for the insulin-like growth factors. All bind the insulin-like growth factors with high specificity, but they vary slightly in the affinities for individual IGFs (1, 10, 38, 39). The IGF receptors vary considerably in their affinity for insulin. In some cases, the affinity of insulin is nearly identical with that of IGF for the receptors (13, 16) whereas in others it is several orders of magnitude lower (1, 7, 13, 20, 38). At least a part of these overlapping specificities between the insulin and the IGF receptors is due to structural homologies between these peptides (4-6). The role of these two types of related receptors in biological responses, as well as any possible relationship to one another within the cell, is still unclear.

MSA is an IGF isolated from conditioned media of Buffalo rat liver cells and, like other IGFs, has been shown to stimulate cells in culture to grow (11, 22). Also, like other IGFs, MSA can mimic insulin's metabolic effects. Furthermore, Rechner et al. (40) have identified MSA in rat embryonic liver cells suggesting that this might be the rat equivalent of some human IGF. In receptor binding, MSA, like the other IGFs, binds to a MSA/IGF receptor with high affinity. In addition, MSA binds with weak affinity to receptors for insulin (10, 13, 40). We have previously shown that the weak affinity of MSA for the insulin receptor is important in the production of its insulin-like metabolic effect and that blockade of the insulin receptor with a specific antireceptor antibody inhibits both insulin and MSA effect on glucose oxidation to similar extent (16). Conversely, insulin and IGFs effect on growth (thyminide incorporation into DNA) is not affected by the blockade of insulin receptor indicating that this effect of these peptides is not mediated by insulin receptor but probably through one of the growth receptors (16).

While studying the binding characteristic of insulin and MSA, we have observed that in rat adipocytes insulin did not compete for the binding of the MSA. Rather, insulin enhanced the binding of this polypeptide to its receptors (16). Schoenle et al. (17, 18), Zapf et al. (19, 20), and Meuli and Froesch (21) have reported a similar finding while studying IGF-I and IGF-II binding to adipocytes. In the present paper we have investigated this phenomenon in some detail, in hope to better understand the relationship between these receptors.

The mechanism of this insulin effect was analyzed in two ways. First, Scatchard curves showed clearly that $R_0$ (receptor number) for MSA was not changed but $K_D$ (affinity constant) was increased. Secondly, using association data, the analysis suggested that the insulin is altering the affinity of MSA binding. Further, the change appears to be due to a decrease in the apparent dissociation constant. The absolute values derived by the association experiments are not precise due to inaccuracies of timing and assumptions as reflected by the large differences between the $K_D$ calculated by the two methods. Nevertheless, both equilibrium and kinetic analysis suggested a change in affinity and not in receptor numbers. Since the change in the $k_d$ by kinetic analysis is similar to the change in the binding affinity, it seems reasonable to suggest that $k_d$ rather than $k_o$ is altered. Further studies are necessary to clearly establish whether the $K_D$ change is due to changes in $k_d$ or $k_o$. We cannot explain the large discrepancy between the two values of $K_D$ except that the observation of insulin's effect on MSA binding is much more complicated than a pure membrane phenomenon, and may involve other mechanisms related to hormone processing, internalization, etc. Regardless of the exact values or methods used to determine them, we regard estimates of $K_D$ and $R_0$ as only "apparent" constants, since there is much to be learned about these various hormone-receptor interactions. In addition, measurements of deg-
radiation by rebinding experiments and gel filtration did not reveal any significant difference of degradation of 125I-MSA in the presence of insulin which could account for the observation.

The enhancement of MSA binding by insulin is mediated via the insulin receptor. This conclusion is supported by the studies using proinsulin and the antibody to the insulin receptor. With the latter, only the bivalent antireceptor IgG to the insulin receptor was able to mimic insulin in enhancing MSA binding, which indicates that insulin receptor is involved. We have previously shown that both the monovalent and the bivalent antibodies bind to the receptor and inhibit insulin binding, but only the bivalent antibody is biologically active (16, 34). Furthermore, as with other biological effects, treatment of cells with the monovalent Fab fragments of receptor antibody shifted the dose-response curves of insulin to the right, so that higher concentrations of insulin were required to produce the enhancement, i.e. the Fab fragments act as competitive inhibitors at the insulin receptor level. Unrelated hormones, such as glucagon and epidermal growth factor, or agents which stimulate glucose metabolism but do not bind to the insulin receptor were without effect.

Although agents such as vinblastine, colchicine, and cytchalasin D do not affect insulin binding and action in adipocytes (34, 37), they were able to inhibit the effect of insulin on MSA binding. The surprising finding suggests that the enhancement of binding requires the involvement of the cytoskeleton, although it is possible that these agents in some way are altering the properties of the membrane nonspecifically.

The process is also temperature-dependent and requires an intact cell. Any factor that adversely affects the metabolic function of cells with the monovalent Fab fragments of receptor antibody shifted the dose-response curves of insulin to the right so that higher concentrations of insulin were required to produce the enhancement, i.e. the Fab fragments act as competitive inhibitors at the insulin receptor level. Unrelated hormones, such as glucagon and epidermal growth factor, or agents which stimulate glucose metabolism but do not bind to the insulin receptor were without effect.

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