Receptor Aggregation Is Necessary for Activation of the Soluble Insulin Receptor Kinase*

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Purified polyclonal human antibodies (B-8) against the receptor for insulin (anti-R IgG), and their F(ab')2 and Fab' fragments, were used to study a possible role of receptor aggregation in the process that couples insulin binding with the activation of the insulin receptor kinase. Anti-R IgG, F(ab')2, and Fab' fragments were shown to inhibit insulin binding to solubilized partially purified receptor preparations from rat liver. This suggests that the antibodies and fragments bind near or at the insulin-binding site. Only anti-R IgG and its bivalent F(ab')2 fragments were capable of stimulating the receptor kinase activity. Monovalent Fab' fragments were completely devoid of such activity. Cross-linking of anti-R Fab' with goat anti-human Fab' restored the capability of the Fab' fragments to activate the receptor kinase. These data strongly suggest that receptor cross-linking or aggregation constitutes a sufficient trigger to activate the insulin-receptor kinase and could, therefore, be an important step in the transmembrane signaling process. This step presumably precedes the activation of the receptor kinase and the resulting phosphorylation of its protein substrates.

The insulin receptor is a transmembrane glycoprotein composed of two α (135 kDa) and two β (95 kDa) subunits linked through disulfide bridges (1). Insulin action initiates upon binding of the hormone to the outer surface of its membrane receptor (2) and is then transformed into a transmembrane signal which activates a tyrosine kinase associated with the β subunit of the receptor (3-7). To date, most of our knowledge is limited to some understanding of the first and last steps in this process, but almost no clues exist as to the nature of the transmembrane signal itself. The isolation of pure receptors which retain their autophosphorylation activity (5) and the finding that such autophosphorylation is an intramolecular event (8,9) suggest that the information required to transform insulin binding to kinase activation resides within the receptor itself. Recent sequencing of a cDNA probe of the insulin receptor (10) reveals that only a single 23-amino acid stretch of the β subunit transverses the membrane, but this apparent simple communication between the receptor subunits across the membrane gives no clues as to the nature of the coupling process which leads to kinase activation.

Several observations in a variety of systems (11-13) suggest that receptor aggregation or cross-linking play a crucial role in transmembrane signaling. We, therefore, investigated the possible role of receptor aggregation in activating the insulin receptor kinase. For that purpose we made use of polyclonal human anti-receptor antibodies which develop spontaneously in certain patients with extreme insulin resistance (14). These antibodies are appropriate tools for such a study since (a) they bind specifically to the receptor and mimic most biological effects of the hormone (15-17) and (b) they can be presented to the receptor either as bivalent (IgG, F(ab')2) or monovalent (Fab') ligands. In the present work, we show that: (a) purified anti-R IgG and their F(ab')2 fragments act as partial agonists of insulin and partially activate the receptor kinase; (b) monovalent Fab' fragments are devoid of such activity; (c) monovalent Fab' fragments regain capacity to stimulate the receptor kinase when cross-linked with goat anti-human Fab'. These data strongly suggest that receptor cross-linking or aggregation constitutes a sufficient trigger to activate the insulin-receptor kinase and could, therefore, be an important step in the transmembrane signaling process.

**EXPERIMENTAL PROCEDURES**

**Methods**

**Purification of IgG**—A serum containing autoantibodies to the insulin receptor (15) was obtained from a patient (B-8). IgG fraction was obtained from ammonium sulfate precipitate by DEAE-cellulose chromatography (18). An IgG fraction with identical properties was obtained when the patient's serum was acidified to pH 4.0 and treated with Norit A charcoal to delete 'insulin which is possibly present. F(ab')2 fragments were prepared as described (19). Five to 10 mg of IgG (in 0.1 M sodium acetate, pH 4.0) were incubated with solid BSA (1 mg/25 mg of antibodies) at 37 °C for 24 h. The samples were adjusted to pH 8.0 with 2 M Tris and dialyzed against phosphate-buffered saline, pH 7.4, for 48 h.

Fab' fragments were prepared by reduction of F(ab')2 fragments with 10 mM dithiothreitol in 0.2 M Tris, pH 8.6, for 60 min at 22 °C (11). Iodoacetamide (20 mM final concentration) was then added for 30 min. The solution was dialyzed against phosphate-buffered saline, pH 7.4, for 48 h. The purity of IgG, F(ab')2, and Fab' was judged by polyacrylamide gel electrophoresis in sodium dodecyl sulfate under nonreducing conditions. They appeared as 150-, 100-, and 23-30-kDa proteins, respectively.

An IgG fraction was purified from goat anti-human Fab' serum by affinity chromatography over a column of human IgG coupled to Sepharose. After extensive washing with phosphate-buffered saline, elution was carried out with 0.2 M HCl-glycine buffer, pH 2.7. The eluate was neutralized and dialyzed over a 24-h period against 1 liter of phosphate-buffered saline (pH 7.4) which was changed every 8 h.

*The abbreviations used are: IgG, immunoglobulin type G; EGF, (2-hydroxyethyl)-1-piperazineethanesulfonic acid; BSA, bovine serum albumin.

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Insulin Receptor Phosphorylation

**Insulin Receptor Preparation**—Preparation of plasma membranes from rat liver, solubilization of the membranes in Triton X-100, and purification of the insulin receptor on wheat germ agglutinin coupled to agarose was carried out as previously described (20).

Phosphorylation of Tyrosine-containing Polymers—Receptor preparations (15 µl, about 10 µg of protein) were incubated for 16–18 h at 4°C with 15 µl of the appropriate stimulant (e.g., anti-receptor antibody) in Hepes (50 mM), BSA (0.1 mg/ml), pH 7.6.

The samples were placed at 22°C for 30 min in the presence of 10 µl of insulin (3.6 µg/ml in Hepes (50 mM), BSA (0.1 mg/ml), Triton (0.1%), pH 7.6) or buffer only. Phosphorylation was initiated with 20 µl of receptor mix to give the following final concentrations: 50 µM [γ-32P]ATP (15 Ci/mmol), 1.6 mM CTP, 66 mM magnesium acetate, 0.1% Triton X-100, and 3.3 mg/ml (Glu4-Tyr20). Reactions were allowed to proceed for 15–18 min at 22°C and were terminated by applying aliquots onto Whatman No. 3MM filter papers that were extensively washed in 10% trichloroacetic acid, 10 mM sodium pyrophosphate, rinsed in ethanol, dried, and counted by liquid scintillation.

One unit of kinase activity was defined as the amount of enzyme required to incorporate 1 pmol of 32P into Glu4-Tyr20 during 10 min. Experiments were carried out in duplicate. Activities did not vary by more than 10%.

**Autophosphorylation of the Insulin Receptor**—Autophosphorylation was carried out essentially as phosphorylation of exogenous substrates except that (Glu4-Tyr20) was omitted. The reaction was terminated by adding a (× 2.5 concentrated) stopping solution containing 5% (v/v) sodium dodecyl sulfate, 0.06 M Tris, 5 mM ATP, 25% (v/v) glycerol, 1.82 M NaCl, 0.02% (w/v) bromphenol blue, pH 6.8. After heating for 10 min at 95°C, the samples were analyzed by 7.5% sodium dodecyl sulfate-polyacrylamide gel electrophoresis and autoradiography.

**Binding Inhibition of 125I-insulin to Solubilized Receptor Preparation.**—The assay was performed as a modification of the method described in Ref. 21. Briefly, 50-µl aliquots of receptor preparation were incubated with 30 µl of insulin or anti-receptor antibodies for 16–18 h at 4°C. Twenty-µl aliquots of 125I-insulin tracer (180 CI/mmol, 2.5 × 10^−9 M) were added and incubation continued for 30 min at 22°C. Forty µl of reaction mix (identical to that used in the phosphorylation assay) was added and incubation continued for another 30 min at 22°C. Reaction was terminated by adding 280 µl of human IgG (1.5 mg/ml) and 420 µl of polyethylene glycol 6000 (25%), and the samples were placed on ice. Separation of bound from free insulin was immediately carried out as described in Ref. 21. The total time lapse between addition of 125I-insulin and isolation of the 125I-insulin-receptor complexes was kept constant for each tube. Nonspecific binding was determined by adding insulin (10^−6 M) together with the 125I-insulin tracer.

**Materials**

Sera from a patient with autoantibodies to the insulin receptor (B-8) was a generous gift of Dr. P. Gorden, Diabetes Branch, National Institute of Health, Bethesda, MD. Goat anti-human Fab' was kindly provided by Dr. Zelig Eshhar, Department of Chemical Immunology, The Weizmann Institute of Science, Rehovot, Israel. 125I-Monoiodo-labeled (at tyrosine 14 of the A chain) human insulin (2000 CI/mmol) and [γ-32P]ATP (3000 CI/mmol) were from Amersham, Buckinghamshire, England. ATP, CTP, and the synthetic tyrosine-containing polymer (Glu4-Tyr20), were from Sigma. The other materials and reagents used were previously described (20).

**RESULTS**

**Effect of Anti-R IgG and Its Fab' Fragments on Insulin Binding to Solubilized Receptors**—Polyclonal antibodies to the insulin receptor (anti-R) which develop in certain patients with insulin-resistant diabetes mimic most biological effects of the hormone (22). As illustrated in Fig. 1 these antibodies and their monovalent Fab' fragments were capable of inhibiting insulin binding to solubilized lectin-purified receptor preparations from rat liver. Human control IgG was ineffective. Maximal inhibition with IgG or Fab' was about 50% that of insulin. Half-maximal inhibition of insulin binding occurred with 5 × 10^−9 M (30 ng/ml) insulin and with 1 × 10^−8 M (50 µg/ml) Fab' or IgG, respectively. Assuming that approximately 1% of the total IgG are anti-receptor antibodies (11), then the purified anti-R IgG was equipotent with insulin, on a molar basis (~4 × 10^−9 M), in inhibiting insulin binding. The Fab' fragments appeared to be, on a molar basis, about 30% as potent as the IgG.

**Activation of the Receptor Kinase by Anti-R IgG and Its Fragments**—Binding of anti-R (B-8) to solubilized lectin-purified receptor preparations stimulated the insulin receptor tyrosine kinase to phosphorylate exogenously added tyrosine-containing polymers (Glu4-Tyr20), Fig. 2A). Human control IgG was ineffective. Half-maximal activation (at 22°C) occurred after 10-min incubation of receptor preparations with anti-R IgG while 20 min were required to obtain a maximal effect. Maximal activation of the receptor kinase with anti-R IgG occurred at 200 µg/ml (1.3 × 10^−9 M) and was only 50% of that obtained with insulin. Half-maximal activation (at 75 µg/ml, 5 × 10^−7 M) occurred at about the same IgG concentration which produced half-maximal inhibition of insulin binding. To validate such comparison, both binding and kinase studies (Fig. 2B) were carried out under identical conditions (cf. "Methods"). These results suggest that the antibody subpopulation which partially inhibits insulin binding has a similar affinity to the receptor as the subpopulation which partially activates the receptor kinase. The stimulatory effects of anti-R IgG on the receptor kinase activity were further studied. We could demonstrate that the kinase activity assayed in the presence of insulin or anti-R IgG increased linearly with increasing receptor concentrations (Fig. 3), which means that anti-R IgG, like insulin, activated the receptor kinase independently of receptor concentration (Fig. 3A).

We next tested the capability of anti-R fragments F(ab')2 and Fab' to activate the receptor kinase. As seen in Fig. 4A (empty bars), bivalent Fab'2 fragments, like IgG, retained their capacity to act as partial agonists of insulin and stimulated the receptor kinase, suggesting that the Fc fragments of the antibodies are not involved in the process. In contrast, the monovalent Fab' fragments were completely devoid of such stimulatory activity. Although in certain experiments...
concentrations are final in the assay. This is one out of three similar described under “Methods.” Results are mean control IgG described in the legend to Fig. 1. Stimulation of receptor kinase alone. Insulin (10 pL, Receptor preparations (15 ~1) were experiments carried out in duplicate. Maximal activity did not vary by more than 10%. Phosphorylation was then initiated with 20 pL of reaction mix. Maximal (100%;) activity was initiated with 20 pL of reaction mix as described in the legend to Fig. 1. Stimulation of receptor kinase activity was assayed as follows. Receptor preparations (15 ~1) were incubated for 16-18 h at 4 °C with 15 µl of anti-R IgG (A) or human control IgG (D), or with buffer (50 mM Hepes, 1 mg/ml BSA, pH 7.6) alone. Insulin (10 µL, 6 × 10−7 M) was added to samples incubated without antibodies, and buffer was added to the rest. All samples were placed at 22 °C for 30 min, and phosphorylation was then initiated with 20 µl of reaction mix. Maximal (100%) activity was that assayed in the presence of insulin. The indicated antibody concentrations are final in the assay. This is one out of three similar experiments. Maximal activity did not vary by more than 10%.

anti-R Fab’ alone caused a small activation of the receptor kinase (Fig. 5B), these effects were not reproducible and could result due to small contamination with intact IgG.

The loss of stimulatory activity of the Fab’ fragments could not be due to chemical modifications that occur with reduction and alkylation since monovalent Fab’ effectively competed with insulin (Fig. 1) and anti-R IgG on binding to the receptor. The latter was evident as anti-R Fab’ completely abolished the stimulatory effect of anti-R IgG on receptor phosphorylation (not shown). Hence, conversion of bivalent IgG to monovalent Fab’ did not result in any gross alteration in bio-recognition sites of the antibodies.

Inhibition of Insulin-stimulated Kinase Activity by Anti-R IgG and Its Fragments—Since anti-R IgG was only a partial agonist of insulin (Figs. 1 and 2B), the combined effects of insulin and anti-R on the receptor kinase activity were studied. We could demonstrate (Fig. 4A (filled bars), Fig. 4B) that the presence of either anti-R IgG or its F(ab’)2 and Fab’ fragments markedly inhibited the insulin-stimulated portion of the receptor kinase activity (i.e. the activity assayed in the presence of insulin less the activity assayed in its absence) which dropped (Fig. 4A) from 6.6 activity units in the absence of anti-R IgG to 0.54 unit in its presence. This 90% inhibition of the insulin-stimulated portion of the kinase activity (compare open versus filled bars in Fig. 4A) was specific as antibodies derived from human control serum had no such effect. Total kinase activity assayed in the presence of insulin (Fig. 4A, filled bars) was lower in the presence of Fab’ as compared to IgG or F(ab’)2. This is due to the fact that anti-R Fab lacks the capacity to stimulate the receptor kinase activity on its own (in the absence of insulin) as do the intact IgG and F(ab’)2 (Fig. 4A, open bars). Inhibition of kinase activity by anti-R IgG and its fragments was independent of receptor concentration (Fig. 3) and was dose dependent (Fig. 4B) with a half-maximal effect at 50 and 90 µg/ml of anti-R IgG or Fab’, respectively.

Effect of Cross-linked Fab’ Fragments on Receptor Kinase Activity—To explore the role of valency in maintaining the bioactivity of the antibodies, reconstitution of valency was attempted by exposing the receptor to the monovalent antibodies and then cross-linking them by addition of a second antibody. Addition of anti-Fab’ restored the capability of the monovalent antibody fragments to enhance receptor kinase activity. The enhanced activity was detected by increased autophosphorylation of the 95,000 subunit of the receptor itself (Fig. 5A) and by increased phosphorylation of exogenous substrates, respectively. This could be explained by the fact that goat anti-Fab’ antibodies cross-linked at least part of anti-R Fab’ fragments to other nonrelevant fragments present in the patient’s serum. Doing so, they reduced the effective concentration of cross-linked anti-R Fab’ and prevented fruitful activation of the receptor kinase. The capacity of cross-linked Fab’ to inhibit insulin-stimulated kinase activity was similar to that of native Fab’. F(ab’)2 and crosslinked Fab’ fragment from normal serum were both ineffective.

**DISCUSSION**

Antibodies against the receptor for insulin (B-8) were used to study a possible role of receptor aggregation in the process that couples insulin binding with the activation of the insulin

**FIG. 2.** A, stimulation of receptor kinase activity by anti-R IgG. Receptor preparations (15 µl) were incubated for the indicated times at 22 °C with 15 µl of anti-R IgG (■) or human control IgG (□). Phosphorylation was then initiated with 20 µl of reaction mix as described under “Methods.” Results are mean ± S.D. of two experiments carried out in duplicate. B, comparison of inhibition of insulin binding and kinase activity by anti-R IgG. Inhibition of 125I-insulin binding by anti-R IgG (A) or human control IgG (Δ) was carried as described in the legend to Fig. 1. Stimulation of receptor kinase activity was assayed as follows. Receptor preparations (15 µl) were incubated for 16-18 h at 4 °C with 15 µl of anti-R IgG (●), human control IgG (○), or with buffer (50 mM Hepes, 1 mg/ml BSA, pH 7.6) alone. Insulin (10 µL, 6 × 10−7 M) was added to samples incubated without antibodies, and buffer was added to the rest. All samples were placed at 22 °C for 30 min, and phosphorylation was then initiated with 20 µl of reaction mix. Maximal (100%) activity was that assayed in the presence of insulin. The indicated antibody concentrations are final in the assay. This is one out of three similar experiments. Maximal activity did not vary by more than 10%.

**FIG. 3.** Effect of receptor concentration on antibody-stimulated tyrosine kinase activity. Receptor preparations (0.57 mg/ml) were diluted as indicated. Fifteen-µl samples were incubated for 16-18 h at 4 °C with 15 µl of anti-R IgG (1.1 mg/ml) (■, □), or human control IgG (1.1 mg/ml) (●, Δ), or buffer (●, ○). Ten µl of insulin (6 × 10−7 M) (■, ●, □, ○) or buffer (□, O, Δ) were added, and the samples were placed at 22 °C for 30 min. Tyrosine kinase activity was assayed as described under “Methods.” This is representative of two similar experiments, each carried out in duplicate which didn’t vary by more than 6%.
and with insulin M), respectively. This is representative of three experiments assayed in duplicates that varied by less than its fragments. The assay was performed as described under "Methods." Anti-R IgG, F(ab')2, and Fab' were diluted in Hepes (50 mM) and BSA (0.1 mg/ml) to give final concentrations of 0.7, 0.25, and 0.3 mg/ml, respectively. Final concentrations of human control IgG, F(ab')2, and Fab' were 0.65, 0.28, and 0.21 mg/ml, respectively. Control samples were incubated with buffer alone. Empty and filled bars represent tyrosine kinase activity of samples incubated without and with insulin (10^{-7} M), respectively. This is representative of three experiments assayed in duplicates that varied by less than 3%. B, inhibition of insulin-dependent phosphorylation by anti-R IgG and its Fab' fragment. Receptor preparations (15 μl) were incubated (16-18 h at 4 °C) with 15 μl of anti-R IgG (1.4 mg/ml), Fab' (1.4 mg/ml), or human control IgG (2.2 mg/ml). Ten μl of insulin (6 × 10^{-7} M in 50 mM Hepes, 1 mg/ml BSA, Triton 0.25%, pH 7.6) or buffer only were added, and the samples were placed at 22 °C for 30 min. Phosphorylation at 22 °C was initiated with 20 μl of reaction mix and was carried out for 15 min. Maximal (100%) insulin-dependent phosphorylation was determined in samples incubated without antibodies and was defined as the activity assayed in the presence of insulin (5.7-7.3 units) less the activity assayed in its absence (0.4-0.8 unit). Inhibition was defined as per cent reduction in insulin-dependent phosphorylation due to the presence of antibodies. Antibody concentrations are final in assay. The results are the mean of two experiments which did not vary by more than 12%.

Receptor kinase. Activation of the receptor kinase by anti-R IgG results due to direct interaction between the insulin receptor and the antibodies since (i) the receptors are solubilized and partially purified and are presumably free from membrane components which could, in principle, mediate antibody-receptor interaction; and (ii) these antibodies (B-8) were previously shown to immunoprecipitate such soluble receptors (20). Similar antibody concentrations are required to activate the receptor kinase and to inhibit insulin binding, which suggests that activation occurs as a result of antibody binding near or at the insulin-binding site.

A deeper insight into the nature of interaction between insulin, antibodies, and receptors in solution reveals that these interactions are rather complex. First, anti-R IgG acts only as a partial agonist of insulin both in inhibition of insulin binding and stimulation of receptor kinase activity. This could result from binding of certain antibody subpopulations to determinants which are sufficiently distant from the insulin-binding site. These antibodies fail to block insulin binding or stimulate the receptor kinase while preventing the attachment of other antibody species which are capable of doing so.

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\caption{A, stimulation of tyrosine kinase activity by anti-R IgG and its fragments. The assay was performed as described under "Methods." Anti-R IgG, F(ab')2, and Fab' were diluted in Hepes (50 mM) and BSA (0.1 mg/ml) to give final concentrations of 0.7, 0.25, and 0.3 mg/ml, respectively. Final concentrations of human control IgG, F(ab')2, and Fab' were 0.65, 0.28, and 0.21 mg/ml, respectively. Control samples were incubated with buffer alone. Empty and filled bars represent tyrosine kinase activity of samples incubated without and with insulin (10^{-7} M), respectively. This is representative of three experiments assayed in duplicates that varied by less than 3%. B, inhibition of insulin-dependent phosphorylation by anti-R IgG and its Fab' fragment. Receptor preparations (15 μl) were incubated (16-18 h at 4 °C) with 15 μl of anti-R IgG (1.4 mg/ml), Fab' (1.4 mg/ml), or human control IgG (2.2 mg/ml). Ten μl of insulin (6 × 10^{-7} M in 50 mM Hepes, 1 mg/ml BSA, Triton 0.25%, pH 7.6) or buffer only were added, and the samples were placed at 22 °C for 30 min. Phosphorylation at 22 °C was initiated with 20 μl of reaction mix and was carried out for 15 min. Maximal (100%) insulin-dependent phosphorylation was determined in samples incubated without antibodies and was defined as the activity assayed in the presence of insulin (5.7-7.3 units) less the activity assayed in its absence (0.4-0.8 unit). Inhibition was defined as per cent reduction in insulin-dependent phosphorylation due to the presence of antibodies. Antibody concentrations are final in assay. The results are the mean of two experiments which did not vary by more than 12%.

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\end{figure}
Second, the effects of insulin and anti-R on receptor kinase activity are not additive. An antibody-receptor complex shows only ~50% of the maximal insulin-stimulated kinase activity and is still capable of insulin binding, yet it does not resume a full kinase activity even in the presence of saturating concentrations of the hormone. Therefore, an antibody-receptor complex appears to have three unique features: a partial kinase activity, a partial insulin-binding activity, and no capability to couple insulin binding to a full kinase activation. These features are not easily interpreted, and their potential physiological significance is not clear yet. Nevertheless, they seem to indicate that an antibody-receptor complex maintains a conformation which presumably differs from that of a receptor-insulin complex with a full kinase activity. The simultaneous interaction of insulin, antibodies, and receptors and their effects on the receptor kinase are currently under study.

The capability of anti-R antibodies to activate the receptor kinase depends on their valency. This is indicated by the finding that while both intact IgG or its bivalent Fab′2 fragments retain their stimulatory potency, the monovalent Fab′ fragments are devoid of such activity. The inactive Fab′ monomers still retain their capacity to bind to the receptor and to inhibit binding of insulin or anti-R IgG which suggest that "occupancy" of the receptor is necessary but not sufficient for signal transduction. Cross-linking of the Fab′ monomers reconstitutes their bivalency and their capacity to stimulate the receptor kinase. This led us to conclude that microaggregation or microclustering of receptor subunits which share identical antigenic determinants precedes activation of the receptor kinase by anti-receptor antibodies. That this process occurs in solution suggests it is confined to the receptor itself and is not mediated by other cellular components. Microaggregation could, in principle, take place between subunits within the same receptor tetramer (αβ2) or between subunits of two adjacent receptors. That activation of the receptor kinase by anti-R is independent of receptor concentration tends to favor the former mechanism, although more extensive studies are needed to reach a definitive conclusion. It is now well established that antibody-mediated receptor aggregation in intact cells is both necessary and sufficient to induce insulin's bioeffects (11). Our results are, therefore, compatible with a model where antibody binding followed by receptor aggregation leads to kinase activation and induction of insulin's metabolic effects (Fig. 6, route a). There are, however, other types of anti-receptor antibodies that induce receptor aggregation and mimic insulin's bioeffects but fail to stimulate receptor phosphorylation either in intact cells (23) or in cell-free systems (17). These antibodies will presumably act by bypassing the kinase activation step (Fig. 6, route b).

The question remains as to what extent this model applies to the mode of action of insulin itself and whether a hormone such as insulin is able to induce receptor aggregation without external cross-linking. In principle, insulin binding could induce conformational changes similar to those induced upon binding of a stimulatory antibody, leading to subunit microaggregation and kinase activation. Alternatively, insulin could trigger receptor kinase activity by a completely different process since microaggregation is not a unique mechanism even for triggering of receptor kinases by antibodies. For example, Schreiber et al. (24) have recently shown that monovalent Fab′ fragments derived from monoclonal IgM antibodies against the EGF receptor inhibit EGF binding and stimulate the EGF receptor kinase activity but do not induce receptor clustering. These findings point to the possible existence of more than a single mode of transmembrane signaling and suggest that the nature of the coupling process will depend not only on the receptor but on the nature of the stimulating ligand as well.

Finally, are activations of the receptor kinase and receptor phosphorylation necessary steps in insulin action? Evidence has been presented that two polyclonal anti-receptor antisera (vide supra) which mimic insulin's metabolic effects (11, 19) did not stimulate the receptor kinase when assayed in intact cells (23) or a cell-free system (17). On the other hand, it looks as if the receptor kinase may mediate the metabolic effects of insulin as severe defects in receptor phosphorylation are found in certain patients with extreme insulin resistance (25, 26) and in streptozotocin-induced diabetic rats (27). In addition, some agents which mimic insulin effects, such as vanadate (28) and trypsin (29), also stimulate the insulin receptor kinase. Our current results demonstrate a complete correlation between antibody valency, its potency to stimulate receptor kinase activity, and its capacity to mimic insulin's bioeffects (11, 19) and are, therefore, compatible with the idea that receptor phosphorylation could have a physiological role in mediating insulin action.

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REFERENCES

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