In the present studies, nine different monoclonal antibodies to the extracellular domain of the insulin receptor were tested in three different cell types for their ability to stimulate the intrinsic tyrosine kinase activity of the receptor. Previous studies had suggested that several of these monoclonal antibodies stimulate biological responses without stimulating the intrinsic tyrosine kinase activity of the receptor (Hawley, D. M., Maddux, B. A., Patel, R. G., Wong, K. Y., Manula, P. W., Firestone, G. L., Brunetti, A., Verspohl, E., and Goldfine, I. D. (1989) J. Biol. Chem. 264, 2438-2444 and Soos, M. A., O'Brien, R. M., Brindle, N. P. J., Stigter, J. M., Okamoto, A. K., Whitaker, J., and Siddle, K. (1989) Proc. Natl. Acad. Sci. U. S. A. 86, 5217-5221). In the present study, a more sensitive assay was utilized, and these same monoclonal antibodies, when added to intact cells, were found to stimulate the phosphotransferase activity of the receptor. This increase in activity was reversed by phosphatase treatment of the receptor. In contrast, monoclonal antibodies which had no insulin-mimetic activities did not stimulate the receptor's kinase activity. In addition, Western blot analyses of lysates with anti-phosphotyrosine antibodies showed that insulin-mimetic, but not non-insulin-mimetic antibodies, stimulated tyrosine phosphorylation of the receptor as well as an endogenous substrate (phosphoprotein M, ≈ 160,000). Finally, these antibodies were found to stimulate the tyrosine phosphorylation of another endogenous substrate of the insulin receptor kinase, the type I phosphatidylinositol kinase. These studies support the hypothesis that monoclonal antibodies, like insulin, stimulate biological responses via their ability to stimulate the tyrosine kinase activity of the receptor.

The binding of insulin to its receptor initiates the diverse biological actions of insulin. The exact mechanism whereby the receptor transduces this signal has not been elucidated. However, significant insights have been gained (for reviews, see Refs. 1, 2). The receptor is a disulfide-linked heterotetrameric membrane glycoprotein consisting of two extracellular α (Mr = 135,000) and two transmembrane β (Mr = 95,000) subunits. Binding of insulin to the α subunit activates a tyrosine-specific phosphotransferase activity in the cytoplasmic domain of the β subunit. The receptor then autophosphorylates and this autophosphorylation increases the receptor's ability to phosphorylate various exogenous substrates. Although the exact mechanism whereby the kinase mediates the generation of a signal is not known, a great deal of evidence supports the hypothesis that kinase activation is an essential early event in the transduction of the insulin signal (1, 2).

Anti-insulin receptor antibodies have been extensively used to probe the structure and function of the insulin receptor (3-15). Several monoclonal and polyclonal antibodies to the extracellular domain of the receptor have been found to stimulate various biological responses. Although initial studies suggested that some of these polyclonal antibodies stimulate an insulin-like response without activating the receptor kinase (3, 4), subsequent studies have challenged this (5, 16). More recently, several monoclonal antibodies have also been reported to stimulate various biological responses without activating the receptor kinase (13-15). Paradoxically, some of these monoclonal antibodies were incapable of stimulating biological responses in cells expressing mutant receptors lacking kinase activity (16). We have, therefore, performed the present experiments in order to further elucidate the mechanism whereby these antibodies stimulate a biological response. By employing a more sensitive assay for receptor kinase activation, we have found that these insulin-mimetic antibodies can stimulate the kinase activity of the receptor in intact cells as well as the tyrosine phosphorylation of the receptor and endogenous substrates. The ability of the various monoclonal antibodies to stimulate the receptor's kinase activity correlates with their ability to stimulate a biological response. These studies support the hypothesis that insulin-mimetic antibodies stimulate biological responses via their ability to stimulate the tyrosine kinase activity of the receptor.

EXPERIMENTAL PROCEDURES

Materials—CHO-T and HIR cells and monoclonal antibodies 5D9, MC51, 2G7, and 1G2 were as described (7, 16, 17). Antibodies 25-49, 18-44, 83-14, 47-9, and 83-7 were provided by Dr. K. Siddle, University of Cambridge (6). Antibodies MA-5, MA-10, and MA-20 were provided by Dr. I. D. Goldfine, Mount Zion Hospital, San Francisco (10, 13). NIH 3T3 HIR cells were provided by Dr. J. Whittaker, University of Chicago (18). Porcine insulin was purchased from Flanco, gel reagents were from Bio-Rad. Affinity purified anti-mouse Ig and mouse IgG were from Pel Freez (Rogers, AR). All other reagents were from Sigma or from sources stated below. 

Kinase Assays—The cells in the indicated size plates were grown to confluence in Ham's F-12 (CHO-T and HIR cells) or Dulbecco's modified Eagle's modified Eagle's medium supplemented with 10% fetal bovine serum (GIBCO) and 100 units/ml penicillin and 100 μg/ml streptomycin (UCSF Tissue Culture Facility, SF, CA) at 37 °C in an atmosphere of 95% air and 5% CO2. Cells were washed once with phosphate-buffered saline (37 °C) and then placed in serum-free medium containing 20 mM Hepes, pH 7.3. After 10 min at 37 °C, the

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indicated concentration of experimental additions were added. After incubation for the indicated time at 37 °C, cells were washed twice with Hepes-buffered saline (ice cold) and lysed with the indicated volume of lysis buffer (50 mM Hepes, pH 7.6, 150 mM NaCl, 1% Triton X-100, 5 mM EDTA, 5 mM EGTA, 20 mM sodium pyrophosphate, 50 mM sodium fluoride, 0.1 mM orthovanadate, 20 mM sodium fluoride, and 1 mg/ml bacitracin).

Assays of lysates for kinase activity were performed using a modification of a plate immunoprecipitation assay described previously (19). Microtiter (96-well) polyvinylidene fluoride plates (Falcon) were coated with 50 μg of the indicated anti-insulin receptor antibody, mouse monoclonal antibody or normal mouse IgG (both 10 μg/ml) in 20 mM NaHCO3, pH 9.6 and incubated overnight at 4 °C. In experiments employing IG2 antibodies, the wells were first precoated with affinity purified rabbit anti-mouse IgG to increase the signal. These wells were then treated with 1 μl normal mouse IgG to saturate the anti-mouse IgG. The wells were then washed three times with wash buffer (50 mM Hepes, pH 7.6, 150 mM NaCl, 0.1% Triton X-100, 0.1% Tween-20, and 0.1% bovine serum albumin), coated with 40 μl of cell lysate, and incubated overnight at 4 °C. The wells were washed three times with wash buffer and then incubated with 20 μl of kinase reaction mix (50 mM Hepes, pH 7.6, 5 mM MgCl2, 5 mM MnCl2, 1 mg/ml poly(Glu,Tyr) (4:1), 2 μCi [γ-32P]ATP (3000 Ci/mmol)). For the phosphatase experiments, the wells were incubated with either 40 μl of 0.1 μM Tris, pH 8.0 or alkaline phosphatase (11.1 units/well) (EC 3.1.3.1, Type VII from bovine intestinal mucosa) (Sigma) in 0.1 M Tris, pH 8.0, for 10 min at 25 °C. The wells were washed three times with wash buffer, and then incubated with kinase reaction mix. After incubation for the indicated time at 25 °C, 10 μl of the reaction mix was spotted on Whatman 3MM paper strips. After air drying, the strips were soaked in 10% trichloroacetic acid at 0 °C for 30 min, boiled in 5% trichloroacetic acid for 5 min, then dried with two washes of 36% ethanol and then one wash with acetone. Incorporated 32P was determined by Cerenkov counting. To determine the relative amounts of receptor precipitated in the kinase assay, duplicate wells were incubated with 40,000 cpm 125I anti-insulin receptor monoclonal antibody (either 83-7, 2G7, or 29B4) in 40 μl of wash buffer at 4 °C and washed and counted in a gamma counter. Similarly, for the phosphatase experiments, phosphatase and control buffer treated wells were incubated with 40,000 cpm anti-insulin receptor monoclonal antibody overnight at 4 °C, washed, cut out, and counted.

Thymidine Incorporation—Measurements of thymidine incorporation were as described (20). Cultured monolayers of cells in 24-well plates were incubated for 36 h at 37 °C in serum-free medium containing 20 mM Hepes, pH 7.3. Fresh serum-free medium and experimental additions were added and the cells incubated for an additional 8 h. Cells were then pulsed with 0.75 μCi of [methyl3H]thymidine (ICN) (20 Ci/mmol) for 45 min, washed once with Hepes-buffered saline (ice cold) and then lysed with 0.025% SDS: Trichloroacetic acid (final concentration, 10%) was added to the lysate, and the resulting precipitate was collected by filtration on Whatman glass fiber filters, washed with 5% trichloroacetic acid (0%, and counted.

Western Blot—Stimulation of receptor and endogenous protein tyrosine phosphorylation in CHO was assessed by immunoblotting with antiphosphotyrosine antibodies. Confluent monolayers in 24-well plates were incubated as described above for the kinase assay and lysed. After reduction and denaturation by heating 2 min at 100 °C in 0.5% SDS and 2.5% β-mercaptoethanol (final concentrations), samples were mixed with 5×-loading buffer, boiled, transferred to nitrocellulose, and probed with polyclonal (21) or monoclonal (22) antiphosphotyrosine antibodies. The bound rabbit or mouse immunoglobulin was detected using alkaline phosphatase conjugated anti-rabbit or anti-mouse immunoglobulin, respectively (Promega Biotech, Madison, WI). Antibody Labeling—Monoclonal antibodies were labeled with 125I by incubating 70 μg of purified antibody in 70 μl of 0.5 M Na2HPO4, pH 7.4, with 0.5 mCi Na125I (carrier free, Amersham Corp.) for 15 min at 25 °C in a 12×75-mm glass test tube precoated with 10 μg of Immuno Gel (Diagnostics Co.). The reaction was then terminated by transferring the reaction mix to a tube containing 220 μl of 0.02 M Na2HPO4, pH 7.4, 2.5 mg/ml bovine serum albumin, 10 mg/ml cytochrome c, and 0.1% Ki. 125I Antibody (10-30 Ci/g) was then separated from unincorporated 125I by chromatography on a Sephadex G-25 column (1.5×5-cm, Pharmacia LKB Biotechnology Inc.).

Results

Kinase Assays—Intact CHO cells overexpressing the human insulin receptor (CHO-T) (17) were incubated for 30 min at 37 °C with either insulin or the insulin-mimetic monoclonal antibody 25-49 (14). After washing and lysing the cells, the receptor in the lysate was isolated by adsorption to microtiter wells previously coated with a monoclonal antibody to the cytoplasmic domain of the receptor (2G7) which does not inhibit the kinase activity of the receptor (7). The adsorbed receptor was then tested for its kinase activity with the exogenous substrate, poly(Glu,Tyr) 4:1. The amount of receptor adsorbed to the wells was also determined by measuring the binding of another monoclonal antibody to the receptor, and the kinase activity measured was normalized to the amount of receptor present. This was found to be necessary since the amount of receptor adsorbed to the wells was increased from 30 to 75% by various monoclonal antibodies. The kinase activities of adsorbed receptor from insulin and antibody-treated cells were both increased in comparison to nontreated cells and the amount of 3P incorporated into substrate increased linearly for at least 80 min under the conditions of this assay (Fig. 1). In subsequent assays the reactions were terminated after 40 min.

A time course of the activation of the receptor kinase in CHO-T cells by 10 nM insulin indicated that insulin maximally stimulated the activity of the receptor approximately 10-fold within 2 min (Fig. 2). The activity of the receptor remained elevated for at least 60 min. Ten nM of the insulin-mimetic monoclonal antibody 25-49 maximally stimulated the receptor kinase activity 3-fold at 20 min. The activity of the antibody-stimulated receptor remained elevated for at least 60 min. For subsequent experiments cells were therefore treated with insulin or antibody for 30 min prior to lysis.

The dose dependence of the activation of the receptor kinase was then studied. One-hundred nM insulin maximally stimulated the receptor kinase 13-fold,

The maximal stimulation in four experiments varied from 10- to 25-fold. This variation was primarily due to the different levels of basal kinase activity of the receptor.
receptor kinase at any of the concentrations tested (Fig. 3). Eight different monoclonal antibodies to the extracellular portion of the insulin receptor were then screened for their ability to stimulate the receptor kinase in CHO-T cells at a concentration of 10 nM (Table I). Two antibodies, 47-9 and MA-10, did not significantly stimulate the kinase activity of the receptor. The other antibodies stimulated the kinase activity of the receptor to variable degrees: monoclonal antibodies 25-49 and 83-14 were most potent, MA-5 was slightly less potent, and monoclonal antibodies 5D9, MC51, and 18-44 were considerably less potent. To verify these results, these same monoclonal antibodies were tested for their ability to stimulate the receptor kinase in an assay in which the receptor was adsorbed to microtiter wells by a different monoclonal antibody (1G2) which binds to a distinct antigenic region of the receptor and also does not inhibit the kinase activity of the receptor (7). Again, monoclonal antibodies 47-9 and MA-10 did not stimulate the receptor kinase whereas the other antibodies did in the order 83-14, 25-49, MA-20, 18-44, 5D9, MC51 (Table I).

These same monoclonal antibodies were then tested for their ability to stimulate the kinase activity of the insulin receptor in another cell line, NIH 3T3 HIR, which also expresses the human insulin receptor cDNA (18). The antibodies which stimulated the receptor kinase in the CHO-T cells again stimulated the receptor kinase in the NIH 3T3 HIR cells in the same order of potency as before and the antibodies which did not stimulate the receptor kinase in CHO-T cells were negative in the 3T3 HIR cells (Table I).

Since both the CHO-T and 3T3 HIR cells express a large number of insulin receptors (~10^6 receptors/cell) (17,18), it was of interest to see whether these antibodies could also stimulate the receptor kinase in cells expressing fewer receptors. We therefore tested the antibodies on a cell line (CHO-HIR) which expresses ~50,000 receptors/cell (16). In these cells, 10 nM insulin activated the kinase activity of the receptor ~80-fold. The higher activation observed in these cells in comparison to CHO-T and 3T3 HIR was due to a lower basal kinase activity of the CHO-HIR receptors. However, the ability of the different monoclonal antibodies to stimulate the receptor kinase in these cells was the same as observed with the other cell types (Table I).

Phosphatase Treatment of Activated Receptor Kinase—We next tested whether the increased kinase activity observed with the insulin-mimetic antibodies was caused by a phosphorylation of the receptor. After treating the adsorbed activated receptor with a phosphatase, the phosphatase was washed out and the receptor tested for kinase activity. Phosphatase treatment completely reversed the increased activity of insulin and antibody-stimulated receptor (Fig. 4). These decreases in receptor kinase activity were not a result of the receptor dissociating from the wells during the phosphatase treatment since phosphatase treatment caused no decrease in the binding of an [3H]-labeled monoclonal antibody to the receptor (Fig. 4). In addition, phosphatase treatment did not damage the receptor since phosphatase-treated receptor could be activated on the well with insulin and ATP to the same
Insulin-mimetic Receptor Antibodies Stimulate Receptor Kinase Activity

**Table 1**

Activation of the insulin receptor kinase by various antibodies in CHO-T, NIH 3T3 HIR, and CHO-HIR cells

Cells were incubated with the indicated additions, lysed, and the insulin receptor adsorbed to microtiter wells coated with either antibody 2G7 or 1G2 as indicated. The specific phosphotransferase activity of the adsorbed insulin receptor (determined by subtraction of the kinase activity measured in wells coated with normal mouse IgG) was measured and normalized for the amount of receptor present. Results shown are means ± S.E. (n = 4 for all the studies except the CHO-T adsorbed with 1G2 where n ranged from 2 to 5) and are the fold stimulation over controls without any addition.

<table>
<thead>
<tr>
<th>Addition</th>
<th>CHO-T 2G7</th>
<th>CHO-T 1G2</th>
<th>3T3 HIR 2G7</th>
<th>CHO-HIR 1G2</th>
</tr>
</thead>
<tbody>
<tr>
<td>10^{-11} M insulin</td>
<td>1.2 ± 0.1</td>
<td>1.6 ± 0.1</td>
<td>1.2 ± 0.1</td>
<td>5.8 ± 0.3</td>
</tr>
<tr>
<td>10^{-9} M insulin</td>
<td>3.4 ± 0.6</td>
<td>5.8 ± 0.6</td>
<td>2.8 ± 0.3</td>
<td>40.6 ± 4.2</td>
</tr>
<tr>
<td>10^{-8} M insulin</td>
<td>10.9 ± 2.1</td>
<td>15.2 ± 3.1</td>
<td>12.0 ± 1.7</td>
<td>61.9 ± 10.2</td>
</tr>
<tr>
<td>10^{-7} M NlgG</td>
<td>19.1 ± 3.8</td>
<td>19.3 ± 1.0</td>
<td>24.1 ± 2.3</td>
<td>80.8 ± 4.8</td>
</tr>
<tr>
<td>10^{-6} M MA20</td>
<td>1.3 ± 0.2</td>
<td>0.9 ± 0.2</td>
<td>1.0 ± 0.1</td>
<td>1.3 ± 0.1</td>
</tr>
<tr>
<td>10^{-5} M 25-49</td>
<td>6.0 ± 1.2</td>
<td>8.0 ± 0.9</td>
<td>5.3 ± 0.7</td>
<td>7.3 ± 0.6</td>
</tr>
<tr>
<td>10^{-4} M 18-44</td>
<td>2.2 ± 0.3</td>
<td>3.9 ± 0.5</td>
<td>1.7 ± 0.1</td>
<td>5.0 ± 0.7</td>
</tr>
<tr>
<td>10^{-3} M 83-14</td>
<td>6.1 ± 0.9</td>
<td>11.7 ± 1.0</td>
<td>3.7 ± 0.4</td>
<td>9.6 ± 1.6</td>
</tr>
<tr>
<td>10^{-2} M 5D9</td>
<td>1.3 ± 0.1</td>
<td>1.7 ± 0.1</td>
<td>1.0 ± 0.1</td>
<td>1.2 ± 0.2</td>
</tr>
<tr>
<td>10^{-1} M MC51</td>
<td>1.8 ± 0.3</td>
<td>3.7 ± 0.1</td>
<td>2.2 ± 0.3</td>
<td>2.3 ± 0.4</td>
</tr>
<tr>
<td>10^{-9} M MA5</td>
<td>2.0 ± 0.4</td>
<td>3.4 ± 0.4</td>
<td>2.0 ± 0.2</td>
<td>2.1 ± 0.2</td>
</tr>
<tr>
<td>10^{-8} M MA10</td>
<td>4.9 ± 1.0</td>
<td>ND</td>
<td>3.2 ± 0.2</td>
<td>ND</td>
</tr>
<tr>
<td>10^{-7} M MA20</td>
<td>1.1 ± 0.1</td>
<td>0.9 ± 0.1</td>
<td>0.9 ± 0.1</td>
<td>1.0 ± 0.1</td>
</tr>
</tbody>
</table>

* Cells.
* Adsorbed by.
* ND, not determined.

Detection of Antibody-stimulated Receptor Autophosphorylation and Endogenous Substrate Phosphorylation by Western Blotting—Lysates of insulin and antibody-treated CHO-T cells were also analyzed by Western blotting with anti-phosphotyrosine antibodies. Insulin-stimulated tyrosine phosphorylation of a 95,000 molecular weight band, presumably the β subunit of the receptor, was first detected with 100 μM insulin (lane k) and increased at higher concentrations of insulin (lanes i and j) (Fig. 5A). Tyrosine phosphorylation of several other bands was also stimulated by insulin, including one of M, = 160,000 (lanes i and j) (Fig. 5A). The rank order of potency of the different antibodies in stimulating tyrosine phosphorylation by Western blotting was the same as that observed in the kinase assays. Like insulin, the ability of the agonist monoclonal antibody MA-5 to stimulate the tyrosine phosphorylation of the receptor was potentiated by the inclusion of vanadate, a tyrosine phosphatase inhibitor, in the media of the cells (Fig. 5B).

**Fig. 4.** Effect of phosphatase treatment on the kinase activity of insulin and antibody-treated insulin receptor. CHO-T cells in 60-mm plates were treated for 30 min with either buffer, 10 nM of either insulin, or the indicated monoclonal antibody. The cells were then lysed, the receptors adsorbed on to microtiter wells coated with 2G7 and then the adsorbed receptors were treated with either phosphatase (○) or buffer (□). The adsorbed receptors were then tested for either kinase activity (top), or receptor number (bottom) as described in the legend to Fig. 1. Data shown are means ± S.D. of triplicate determinations.
stimulate a biological response in the CHO-T cells. Prior studies have shown that the expressed human insulin receptors in CHO cells can mediate the stimulation of [methyl-\(^3\)H] thymidine incorporation (20). Monoclonal antibody 25-49, which stimulates biological responses in other cell types (11, 14), stimulated thymidine incorporation in a dose-dependent manner in CHO-T cells (Fig. 7). Monoclonal antibodies MA-10 and 47-9, which do not stimulate responses in other cell types (11-13), did not significantly stimulate thymidine incorporation in the CHO-T cells (Fig. 7). Five other monoclonal antibodies were also tested at 10 nM for stimulation of thymidine incorporation in CHO-T cells. These antibodies stimulated a response to variable degrees; antibody 18-44 was almost as potent as 25-49; 83-14 and MA-20 were next, and 5D9 and MC51 were least potent.

**DISCUSSION**

In the present studies, nine different monoclonal antibodies to the extracellular domain of the insulin receptor were tested in three different cell lines for their ability to stimulate the intrinsic tyrosine kinase activity of the insulin receptor. Seven of these monoclonal antibodies were found to stimulate the tyrosine kinase activity of the receptor to various degrees (Figs. 1-3, Table I). This increase in kinase activity was dependent on the phosphorylation state of the receptor since it could be reversed by phosphatase treatment (Fig. 4). These same antibodies also stimulated an increase in tyrosine phosphorylation of the receptor as detected by Western blot analysis (Fig. 5). Finally, the monoclonal antibodies which stimulated the tyrosine phosphorylation of the receptor also stimulated the tyrosine phosphorylation of a protein of \( M_r = 160,000 \) and the type I phosphatidylinositol kinase (Figs. 5 and 6). Both of these proteins have previously been concluded to be endogenous substrates of the insulin receptor (23-26). The simplest interpretation of these data is that these monoclonal antibodies, like insulin, stimulate the autophosphorylation of particular tyrosine residues in the receptor (27-29).
which in turn increases the kinase activity of the receptor on substrates.

The ability of these different monoclonal antibodies to stimulate receptor kinase activity and receptor tyrosine phosphorylation correlates with their ability to stimulate thymidine incorporation in CHO-T cells (Fig. 7) and their previously reported biological actions on other cells (11, 13–15, 30, 31). That is, two monoclonal antibodies (MA-10 and 47–9) which do not stimulate responses (and in fact are antagonists of insulin) did not stimulate either the kinase activity of the receptor or its phosphorylation (Table I, Figs. 1–5). Of the seven insulin-mimetic monoclonal antibodies, the most potent antibodies at stimulating biological responses (25–49, 83–14, MA-20, 18–44, MA-5) were also most potent at stimulating the receptor kinase (Table I) and receptor tyrosine phosphorylation (Fig. 5). For example, in NIH 3T3 HIR cells, antibody 18–44 was reported to maximally stimulate 2-deoxyglucose uptake to ~35% the maximal stimulation observed with antibodies 25–49 and 83–14 (14). This is in good agreement with the present studies in which 18–44 was found to stimulate the receptor kinase to ~15% the maximal stimulation observed with antibodies 25–49 and 83–14 (Table I). In addition, MA 5 was found to stimulate the activation of S6 kinase in the NIH 3T3 HIR cells with a time course slower than insulin’s (31). This agrees with the present finding that antibody activation of the receptor kinase is slower than insulin’s activation (Fig. 2).

In the present studies, the detection of the stimulation of receptor kinase activity by the same antibodies which were previously found to be negative (13–15) may in part be due to the utilization of an assay which is more sensitive than the one previously used. In this assay, intact cells are first treated with insulin or antibody, the cells are lysed and the receptor is isolated on microtiter wells coated with other monoclonal antibodies to the receptor. The isolated receptor is then tested for phosphotransferase activity on an exogenous substrate, poly(Glu, Tyr). This assay is capable of detecting an increase in receptor kinase activity with as little as 30 pM insulin. Thus, this assay is considerably more sensitive than the previously employed assay of immunoprecipitation of receptor from 32P-labeled cells which requires 1 nM insulin to detect a response. In addition, the assay of in vivo labeled receptor has a high background since much of the 32P incorporated into the receptor is phosphoserine (31). Thus, studies of the effects of the antibodies on the incorporation of 32P into the receptor may be obscured by this high background. In addition, it is possible that the assay used in the present study overcomes additional technical problems which have prevented the detection of the effects of these antibodies previously. However, another recent study which employed Western analyses with antiphosphotyrosine antibodies also succeeded in detecting an increase in tyrosine phosphorylation of the receptor with several of the same monoclonal antibodies utilized in the present studies (32).

The present studies therefore indicate that monoclonal antibodies which stimulate biological responses do so in part by their ability to stimulate the kinase activity of the receptor. The mechanism whereby these antibodies stimulate the receptor kinase may be different from that of insulin. Indeed, the stimulation of the receptor kinase by antibody was slower than by insulin (Fig. 2). Also, even the most biologically potent monoclonal antibody did not stimulate receptor kinase activity to the same extent as insulin even though in some studies these antibodies have been found to be almost as potent as insulin in stimulating certain biological responses (10, 11, 14, 15, 30, 31). Thus, it is possible that the insulin-mimetic monoclonal antibodies have other effects on the receptor which contribute or detract from their ability to stimulate a biological response. For example, several of these antibodies appear to stimulate the aggregation of the receptor on the cells. In addition, some of the antibodies have been shown to be potent inducers of the endocytosis and degradation of the receptor (33, 34). Such processes can effect the magnitude of the biological responses elicited by these various antibodies.

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