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# Acute insulin action requires insulin receptor kinase activity: Introduction of an inhibitory monoclonal antibody into mammalian cells blocks the rapid effects of insulin

**(ribosomal protein S6 phosphorylation/glucose uptake/glycogen synthesis/fat cells)**

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ABSTRACT **The role of the insulin receptor tyrosine kinase (protein-tyrosine kinase, EC 2.7.1.112) in various rapid insulin effects was studied by injecting four different cell types (by osmotic lysis of pinocytotic vesicles) with a monoclonal antibody that specifically inhibits the kinase activity of the insulin receptor and the closely related receptor for insulin-like growth factor (IGF)-I. Injection of this inhibitory antibody resulted in a decreased ability of insulin to stimulate (*i*) the uptake of 2-deoxyglucose in Chinese hamster ovary cells and freshly isolated rat adipocytes, (*ii*) ribosomal protein S6 phosphorylation in CHO cells, and (*iii*) glycogen synthesis in the human hepatoma cell line HepG2. The ability of insulin, IGF-I, and IGF-II to stimulate glucose uptake in TA1 mouse adipocytes was also inhibited. Studies with CHO cells demonstrated that these effects of the inhibitory antibody were specific, since (*i*) there was no change in phorbol ester-stimulated glucose uptake and (*ii*) injection of a noninhibiting antibody to the kinase had no effect on insulin action. These studies indicate that the tyrosine kinase activity of the insulin receptor is important in mediating several rapid insulin effects in a variety of different cell types.**

Insulin elicits a remarkable array of biological effects in a wide range of cell types. Despite intensive effort, however, the molecular mechanism of insulin action remains unclear. Recently, the discovery of an insulin-stimulated tyrosine kinase activity (protein-tyrosine kinase, EC 2.7.1.112) in the insulin receptor $\beta$ subunit led to the proposal that insulin's various biological effects are mediated by the kinase activity of its receptor (for review see ref. 1). However, efforts to establish a role for the kinase in insulin action have not been entirely successful. For example, it has been shown that certain polyclonal antisera are capable of stimulating glucose uptake in rat adipocytes without stimulating receptor phosphorylation or receptor kinase activity (2, 3). Thus, it has been suggested that the receptor kinase may not be important in mediating certain rapid insulin actions.

To directly assess the physiological role of the receptor kinase in the intact cell, we have developed a panel of monoclonal antibodies to the insulin receptor kinase domain (4, 5). Several of these antibodies were found to be extremely potent inhibitors of insulin receptor kinase activity (4, 5), and more recent studies have shown that they also inhibit the kinase activity of the closely related receptor for insulin-like growth factor (IGF)-I (6). These inhibitory antibodies, known as the $\beta_2$ antibodies, were found to bind to an antigenic region containing an important autophosphorylation site of the insulin receptor (tyrosines 1162 and 1163) (7). Thus, it was possible to clarify the role of the receptor kinase by introducing these antibodies into the cytoplasm of insulin target cells. In previous studies, microinjection of one of these inhibitory antibodies into *Xenopus* oocytes was found to inhibit insulin's ability to stimulate oocyte maturation, supporting a role for the kinase in this long-term effect of insulin (4). In the present work, we have used these antibodies to probe the function of the kinase in several rapid effects of insulin. To efficiently introduce the inhibitory antibody into large numbers of various mammalian cell types, we used the method of Okada and Rechsteiner (8), which takes advantage of the process of pinocytosis, which normally occurs in most cells. In this procedure, cells are first exposed to a hypertonic solution containing the desired antibody. Pinocytosis of this solution results in the formation of hypertonic vesicles, which can be selectively lysed by brief exposure of the cells to mildly hypotonic medium. The antibody is thus released into the cytosol, while cell viability is largely unaffected.

In the present studies this technique has been used to introduce the inhibitory antibody into the cytoplasm of several cultured cell lines, as well as primary rat adipocytes. Several rapid and intermediate actions of insulin and IGF-I were found to be inhibited by the antibody, providing direct evidence that the receptor kinase mediates the acute actions of insulin in various cell types.

## MATERIALS AND METHODS

**Materials.** Purified pork insulin was purchased from Elanco (Indianapolis, IN); human IGF-I (a product of recombinant DNA technology) was a generous gift of J. Merryweather (Chiron, Emeryville, CA); purified rat IGF-II (MSA III-2) was a generous gift of S. Peter Nissley (National Institutes of Health, Bethesda, MD); $H_3[^{32}P]PO_4$ (carrier-free), 2-deoxy-D-[1,2-$^3$H]glucose (53 Ci/mmol; 1 Ci = 37 GBq), and D-[6-$^3$H]glucose (25 Ci/mmol) were from ICN; phloretin, glycogen, human $\gamma$-globulins (Cohn fraction II), phorbol 12-myristate-13-acetate (PMA), and silicone oil (dimethylpolysiloxane, 100 centistokes) were from Sigma; poly(ethylene glycol) (PEG) 1000 was from Baker; and Matrigel basement membrane was from Collaborative Research (Waltham, MA). Staphylococcal protein A-purified monoclonal antibodies 17A3 and 1G2 have been described previously (4, 5).

**Antibody Loading of Cultured Cell Lines.** Normal IgG (human or mouse) or purified monoclonal anti-receptor antibody (17A3 or 1G2) was dialyzed against 50 mM Hepes, pH 6.9/150 mM NaCl and then concentrated in a Centricon 30 concentrator (Amicon) to a final concentration of ≈50 mg/ml in 280 $\mu$l. The addition of sucrose (68 mg) and 50% (wt/vol) PEG 1000 (80 $\mu$l) to this antibody concentrate resulted in a final volume of ≈0.4 ml of hypertonic antibody solution with approximate final concentrations of 0.5 M

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Abbreviations: IGF, insulin-like growth factor; PMA, phorbol 12-myristate 13-acetate.

sucrose, 10% PEG 1000, 35 mM Hepes at pH 6.9, 105 mM NaCl, and antibody at 30 mg/ml.

To minimize the amount of hypertonic antibody solutions required, the method of Okada and Rechsteiner (8) was modified for use with cells in suspension. In most experiments, as recommended by Wright (9), a minimum of 0.4 ml of hypertonic solution was used for the treatment of $10^7$ cells. Trypsinized cells ($10^7$ per tube) were washed once with phosphate-buffered saline (PBS; 20 mM sodium phosphate, pH 7.5/150 mM NaCl) and centrifuged 7 min at 700 × *g*. After all traces of the supernatant had been carefully aspirated, cell pellets were resuspended vigorously in 0.4 ml of warm hypertonic antibody solution. After 10 min at 37°C with occasional shaking, the hypertonic cell suspension was diluted with 10 ml of 37°C hypotonic medium (60% serum-free Ham's F-12/40% $H_2O$). After 2 min at 37°C, the cells were centrifuged, the supernatant was removed (for later repurification of unused antibody), and the cells were then treated as required for each bioassay (below).

**Glucose Uptake Assays.** CHO (Chinese hamster ovary) cells were loaded as described above with the desired antibody and resuspended in normal culture medium (Ham's F-12/10% fetal calf serum). Cells were then plated in 24-well tissue culture plates ($4 \times 10^5$ cells per 16-mm well) and incubated 12 hr in 5% $CO_2$ at 37°C. Medium was then changed to fresh medium containing 10 mM 2-deoxyglucose (10). After 4 hr at 37°C, wells were washed twice with warm DB/BSA (140 mM NaCl/2.7 mM KCl/1 mM $CaCl_2$/1.5 mM $KH_2PO_4$/8 mM $Na_2HPO_4$/0.5 mM $MgCl_2$/0.1% bovine serum albumin, pH 7.4) and incubated 30 min at 37°C with 0.5 ml of the same buffer. The desired concentration of insulin or PMA was added for 15 min at 37°C; 0.1 mM 2-deoxy-D-[1,2-$^3$H]glucose (0.4 μCi/ml) was added for the last 10 min of this incubation. Cells were washed twice with ice-cold DB containing 100 μM phloretin and solubilized with 0.03% sodium dodecyl sulfate, and their radioactivities were measured by liquid scintillation counting. Protein assays of cell lysates confirmed that there were no differences in cell number between groups loaded with different antibodies. In some experiments, extra wells of injected cells were plated and tested 12 hr later for their ability to bind $^{125}$I-labeled insulin, as described (7).

Trypsinized TA1 adipocytes (11) were loaded with antibody as above and incubated for 1 hr at 37°C in serum-free medium containing 10 mM 2-deoxyglucose. Insulin- or IGF-stimulated glucose uptake was then measured by the above method, modified for cells in suspension.

Isolated adipocytes were obtained from epididymal fat pads of male rats (300 g) by standard techniques (12, 13) and washed twice in FC/BSA buffer (131 mM NaCl/4.7 mM KCl/2.5 mM $CaCl_2$/1.2 mM $MgSO_4$/2.5 mM $NaH_2PO_4$/10 mM Hepes, pH 7.4/3% bovine serum albumin) containing 10 mM glucose. Tubes containing 0.3 ml of packed cells were loaded with 0.4 ml of antibody solution, and then incubated 1 hr at 37°C in FC/BSA buffer containing 10 mM 2-deoxyglucose. Insulin-stimulated glucose uptake in FC/BSA buffer was then measured by a modification of the method described above, except that reactions were terminated by centrifuging the cells through 100-μl silicone oil layers in 400-μl microcentrifuge tubes.

**Phosphorylation of Ribosomal Protein S6 in CHO Cells.** CHO cells ($2 \times 10^7$ cells per antibody) were loaded with 2 ml of hypertonic solution, resuspended in normal culture medium, and distributed into 100-mm tissue culture plates at high density ($4 \times 10^6$ cells per dish). After 12 hr at 37°C, dishes were washed twice with KRBB/BSA (107 mM NaCl/5 mM KCl/3 mM $CaCl_2$/1 mM $MgSO_4$/7 mM $NaHCO_3$/10 mM glucose/0.1% bovine serum albumin, pH 7.4) and then incubated 3 hr at 37°C with 4 ml of KRBB/BSA containing 200 μCi of $H_3[^{32}P]PO_4$. Cells were then treated with indicated concentrations of insulin for 15 min at 37°C, washed twice with ice-cold PBS, and solubilized with 3 ml of ice-cold 50 mM Hepes/5 mM KCl/5 mM $MgCl_2$/50 mM NaF/40 μM EDTA/1% Triton X-100/1% sodium deoxycholate, pH 7.5. This lysate was centrifuged at 10,000 × *g* for 10 min and the supernatant was layered over 3 ml of 1.6 M sucrose/50 mM Hepes/5 mM $MgCl_2$/0.5 M KCl/40 μM EDTA, pH 7.5 and centrifuged at 140,000 × *g* for 2 hr at 4°C. Ribosomal pellets were washed and analyzed by electrophoresis on 12.5% polyacrylamide gels.

**Glycogen Synthesis by HepG2 Cells.** Human hepatoma cells (HepG2), loaded with the desired antibody, were resuspended in culture medium (Eagle's minimal essential medium/Earle's salts/10% fetal calf serum) and plated onto 24-well culture plates ($4 \times 10^5$ cells per well) coated with 0.1 ml per well of Matrigel basement membrane. After 14 hr at 37°C, wells were washed twice with warm DB containing 1% bovine serum albumin and 1 mM glucose, and then incubated 2 hr at 37°C with 0.5 ml of the same buffer containing insulin and 3 μCi of D-[6-$^3$H]glucose. Cells were washed twice with ice-cold PBS and solubilized with 0.5 ml of 30% KOH containing glycogen at 3 mg/ml. After vigorous shaking for 30 min at 24°C, contents of the wells were transferred to glass tubes, wells were rinsed with another 0.25 ml of KOH/glycogen, and the solutions were boiled 30 min. Tubes were cooled to 4°C, and 1.7 ml of ice-cold ethanol was added. After 16 hr of incubation at 4°C, tubes were centrifuged (2000 × *g*, 30 min, 4°C) and the supernatants were discarded. Pellets were dissolved in 0.75 ml of $H_2O$, and 1.5 ml of ice-cold ethanol was added. After 2 hr at 4°C, precipitates were once again pelleted by centrifugation and supernatants were discarded, and the pellets were dissolved in 0.2 ml of $H_2O$ and their radioactivities were measured by liquid scintillation counting.

## RESULTS

**Glucose Uptake by CHO Cells.** Our initial studies involved the effect of kinase inhibition on one of the most well-characterized and rapid responses to insulin: the stimulation of membrane glucose transport (14, 15). To determine if the inhibition of insulin receptor kinase activity *in vivo* affects insulin responsiveness, CHO cells were loaded with either normal immunoglobulin (IgG) or the inhibitory monoclonal antibody 17A3. In normal CHO cells and in cells injected with control IgG (Fig. 1*A*), 15-min exposure to insulin resulted in a dose-dependent enhancement of the uptake of labeled 2-deoxyglucose: a 2-fold stimulation of uptake occurred at 3 nM insulin. In cells loaded with the inhibitory antibody 17A3, both the sensitivity to insulin and the maximal insulin response were reduced. At low insulin concentrations (3 nM), the response was inhibited 80%. In addition, basal glucose uptake (in the absence of added insulin) was 20% lower in cells loaded with antibody 17A3, possibly due to the inhibition by the antibody of basal receptor kinase activity (4, 5). To determine whether the inhibitory effect of antibody 17A3 was specific, CHO cells were osmotically injected with monoclonal antibody 1G2, which also recognizes the cytoplasmic domain of the insulin receptor but has little effect on receptor autophosphorylation or receptor kinase activity (4, 5). Insulin-stimulated glucose uptake was only slightly inhibited (10% at 3 nM insulin) in cells loaded with this antibody (Fig. 1*A*).

The injection of antibodies that bind to an internal receptor domain could induce receptor clustering and internalization, resulting in decreased receptor number and loss of insulin sensitivity. To test for this possibility, the binding of $^{125}$I-labeled insulin ($^{125}$I-insulin) was studied in additional wells of the same cells injected with antibodies in the above experiments. Cells were incubated with $^{125}$I-insulin (50,000 cpm) for

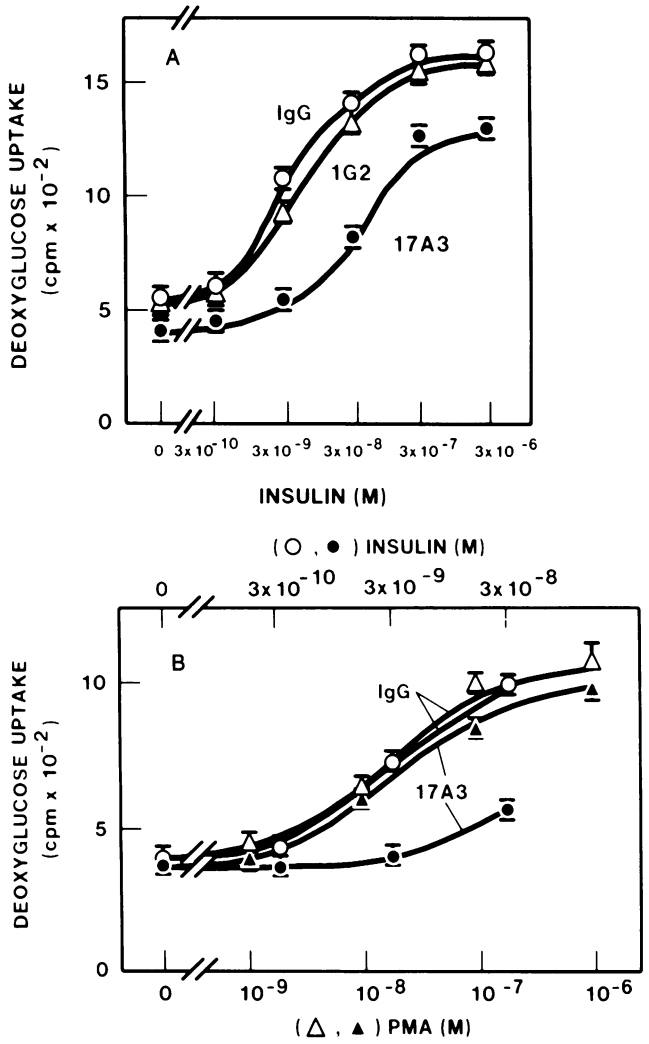


FIG. 1. Effect of inhibitory antibody on glucose uptake by CHO cells. (*A*) Uptake of 2-deoxyglucose was measured in CHO cells injected with normal IgG (○), monoclonal antibody 1G2 (△), or monoclonal antibody 17A3 (●), after 15-min incubation with insulin at the indicated concentrations. (*B*) Uptake of 2-deoxyglucose was measured in CHO cells injected with normal IgG (open symbols) or antibody 17A3 (closed symbols) after 15-min treatment with either insulin (circles) or PMA (triangles) at the indicated concentrations. Values are means ± SD of triplicates.

16 hr at 4°C, in the presence or absence of excess unlabeled insulin (see *Materials and Methods*). Cells loaded with control IgG, antibody 1G2, and antibody 17A3 were found to specifically bind 141 ± 27, 161 ± 7, and 136 ± 12 cpm of $^{125}$I-insulin, respectively, indicating that no significant changes in receptor number had occurred.

The specificity of the inhibitory effects of antibody 17A3 was further tested in studies in which glucose transport was stimulated by an agent that would be expected to act by a mechanism not involving the insulin receptor. Phorbol esters, such as PMA, are known to stimulate glucose uptake in a variety of cell types (16, 17), possibly via the direct phosphorylation of the glucose transporter by protein kinase C (18). Thus, antibody 17A3 was tested for its ability to inhibit the response of CHO cells to phorbol esters. CHO cells, loaded as before with either control IgG or antibody 17A3, were treated for 15 min at 37°C with various doses of insulin or PMA (Fig. 1*B*). As before, the insulin response was greatly reduced in cells loaded with antibody 17A3. In contrast, both the amount of stimulation by PMA and the sensitivity to PMA were not affected by antibody 17A3. A slight downward shift of the dose–response curve for PMA was observed in cells loaded with antibody 17A3; this is presumably a consequence of the ability of 17A3 to decrease the basal glucose uptake in these cells, as mentioned above.

**Phosphorylation of Ribosomal Protein S6 in CHO Cells.** To determine if another acute effect of insulin also involves the receptor kinase, we studied insulin-stimulated phosphorylation of ribosomal protein S6 (19, 20). As before, CHO cells were osmotically injected with either control IgG or antibody 17A3. Twelve hours later, semiconfluent monolayers of injected cells were labeled for 3 hr at 37°C with [$^{32}$P]orthophosphate and then treated with or without insulin for 15 min at 37°C. Polyacrylamide gel analyses of ribosomal preparations from these cells revealed that insulin greatly stimulated the phosphorylation of a protein of molecular weight 34,000. Previous studies with a variety of cell types indicate that this protein is ribosomal protein S6 (19–21). In cells injected with control IgG, S6 phosphorylation was stimulated over 6-fold by insulin, and half-maximal stimulation occurred at 0.5 nM insulin (Fig. 2). The insulin response was dramatically reduced in cells loaded with the inhibitory antibody 17A3: more than 100 nM insulin was required to give a 3-fold stimulation of S6 phosphorylation in these cells (Fig. 2).

**Glycogen Synthesis by HepG2 Cells.** In addition to studying rapid insulin effects, we wished to analyze the effect of kinase inhibition on the intermediate actions of insulin. The human hepatoma cell line HepG2 (22) was chosen for this purpose, since this cell line exhibits insulin-stimulated glycogen synthesis, a major intermediate insulin effect (23, 24).

HepG2 monolayers, loaded as above with either control IgG or the inhibitory antibody 17A3, were tested for their ability to incorporate labeled glucose into glycogen in response to insulin (Fig. 3). Glycogen synthesis was stimulated about 2-fold in control cells, with half-maximal stimulation occurring at about 0.5 nM insulin. Cells injected with the inhibitory antibody 17A3 exhibited an 80% reduction in the insulin response: even at maximal insulin concentrations (300 nM), glycogen synthesis was stimulated only 20%.

**Glucose Uptake by Isolated Rat Adipocytes.** A classic target cell in studies of insulin action is the rat adipocyte, which exhibits a major increase in glucose uptake in response to low doses of insulin (14, 15). To test the role of the kinase in insulin's rapid effects on these cells, freshly isolated rat adipocytes were loaded with control IgG or antibody 17A3 by osmotic lysis of pinocytotic vesicles. Despite their fragility, a majority of the adipocytes remained intact after the treat-

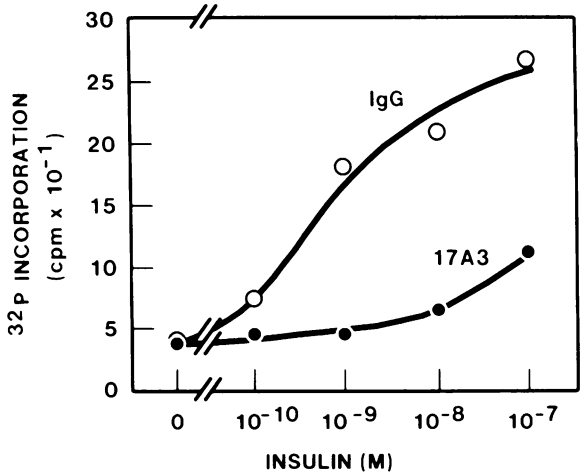


FIG. 2. Effect of inhibitory antibody on insulin-stimulated phosphorylation of ribosomal protein S6 in CHO cells. The labeled ribosomal protein S6 was excised from gels and its radioactivity was measured by liquid scintillation counting. ○ and ● represent CHO cells injected with control IgG and antibody 17A3, respectively. Similar results were obtained in two other experiments.

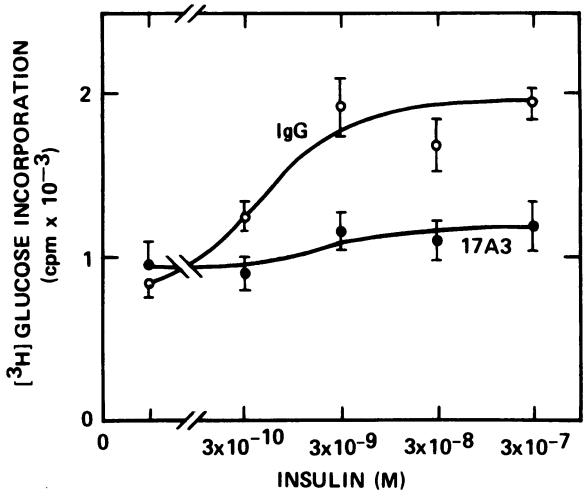


FIG. 3. Effect of inhibitory antibody on insulin-stimulated glycogen synthesis in HepG2 cells. Incorporation of labeled glucose into glycogen was measured in HepG2 cells injected with either control IgG (○) or antibody 17A3 (●) during a 2-hr treatment with insulin at the indicated concentrations. Values are means ± SD of triplicates.

ment and could be analyzed for their ability to transport glucose in response to insulin (Fig. 4). Control cells exhibited a sensitive 3- to 4-fold insulin response. The injection of antibody 17A3 caused an even greater inhibition of insulin responsiveness in these cells than was observed in CHO cells: the response was 90–95% inhibited at moderate insulin concentrations (0.3–3 nM).

**Insulin and IGF Actions on TA1 Adipocytes.** Since antibody 17A3 also inhibits IGF-I receptor kinase activity (6), it was possible to use this antibody to test the role of this kinase in the actions of IGF-I. Similarly, our studies could be used to clarify the actions of a related polypeptide, IGF-II, which binds mainly to its own receptor but also binds with moderate affinity to the IGF-I receptor (25). Antibody 17A3 does not recognize the IGF-II receptor, which is structurally quite distinct from the insulin and IGF-I receptors and does not appear to possess an intrinsic tyrosine kinase activity (26).

These experiments were performed with the TA1 cell line, a mouse cell line that, like 3T3-L1 cells, undergoes a differentiation process from a fibroblast to an adipocyte

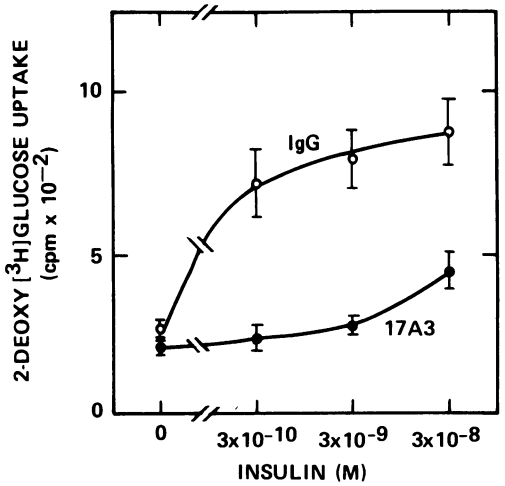


FIG. 4. Effect of inhibitory antibody on insulin-stimulated glucose uptake by isolated rat adipocytes. 2-Deoxyglucose uptake was measured in isolated rat adipocytes injected with either control IgG (○) or antibody 17A3 (●) after treatment with insulin at the indicated concentrations. Values are means ± SD of triplicates.

phenotype (11). For the present studies we used TA1 adipocytes, which exhibit rapid glucose uptake responses to insulin, IGF-I, and IGF-II (27). Over 2-fold increases in glucose uptake were seen in control IgG-injected cells treated with each of the three polypeptides (Fig. 5); half-maximal stimulation occurred at approximately 3 nM insulin, 0.3 nM IGF-I, and 1 nM IGF-II. The introduction of antibody 17A3 into these cells resulted in an inhibition of the response to all three factors: the response was inhibited 78%, 58%, and 69% in cells treated with 3 nM insulin, IGF-I, and IGF-II, respectively.

## DISCUSSION

Insulin's broad range of biological effects, including those studied in the present work, are mediated by a variety of postreceptor mechanisms and involve a broad range of time courses. For example, the stimulation of glucose transport, which occurs within minutes of insulin binding, appears to involve a translocation of glucose transporter proteins from an intracellular site to the plasma membrane (14, 15). In addition, many of insulin's rapid and intermediate effects, which occur minutes to hours after insulin treatment, involve changes in protein phosphorylation. These effects may involve increased protein phosphorylation (as in the case of

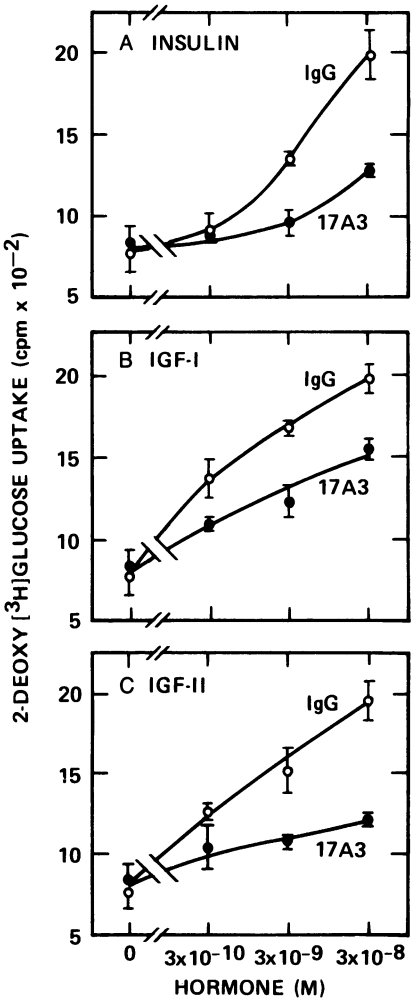


FIG. 5. Effect of inhibitory antibody on stimulation of glucose uptake in TA1 cells by insulin and IGFs. 2-Deoxyglucose uptake was measured in TA1 adipocytes injected with either control IgG (○) or antibody 17A3 (●) after treatment with insulin at the indicated concentrations (*A*), IGF-I (*B*), or IGF-II (*C*). Values are means ± SD of triplicates.

protein S6) (19, 20) or may involve the dephosphorylation of specific proteins (as in the case of glycogen synthase) (20, 28). Finally, long-term insulin effects, such as the stimulation of oocyte maturation, involve changes in RNA and DNA synthesis that require hours to days to reach maximal effect (4). Clearly, the mechanism underlying signal transmission by the insulin receptor must be capable of initiating a broad range of molecular pathways. Our findings suggest that the receptor kinase activity may be the initial step in these pathways. In previous studies, an inhibitory antibody to the kinase was found to block the long-term effect of insulin on oocyte maturation (4); in the present work this antibody blocked the rapid and intermediate effects of insulin on glucose uptake, S6 phosphorylation, and glycogen synthesis. In addition, the present results are supported by independent evidence from site-directed mutagenesis studies in CHO cells, where specific inhibition of insulin receptor kinase activity (by replacement of tyrosines 1162 and 1163 with phenylalanines) decreased insulin's ability to stimulate glucose uptake (7). These various results clearly suggest that receptor kinase activity is a crucial signal that initiates a variety of postreceptor mechanisms responsible for diverse responses to the insulin.

Several lines of evidence indicate that the inhibitory effects of the antibody are specific. First, this antibody does not recognize several other tyrosine kinases, including the epidermal growth factor receptor, the platelet-derived growth factor receptor, and the protein products of the viral oncogenes v-*fms* and v-*ros* (4). Second, a noninhibiting antibody to the receptor kinase domain (1G2) did not affect insulin's ability to stimulate glucose uptake in CHO cells. Third, the inhibitory antibody did not affect the ability of phorbol esters to stimulate glucose uptake by CHO cells.

The present work also demonstrates the utility of this injection approach in clarifying the role of the receptor kinase in insulin action. This approach, in contrast to methods involving expression of mutant receptors, can be readily applied to a broad range of cell types. Thus, in the present work, insulin responses were analyzed in four different cell types, including primary rat adipocytes. The extensive inhibition of the insulin response in rat adipocytes is particularly noteworthy, since this cell type is the same as that previously used in studies questioning the importance of the kinase (2, 3) (see introduction). The finding that the inhibitory effects of the antibody could be demonstrated even in this cell type, as well as in three other cultured cell lines, strongly suggests that the receptor kinase mediates the biological effects of insulin in a wide variety of cell types.

The importance of receptor tyrosine kinase activity in the actions of IGFs was also examined in the present studies. Since the inhibitory antibody 17A3 also recognizes the IGF-I receptor (6), we could demonstrate that the effect of IGF-I on glucose uptake involves IGF-I receptor kinase activity. The actions of IGF-II were also clarified in these experiments. IGF-II can bind with moderate affinity to the IGF-I receptor as well as its own receptor, making it difficult to determine which of the two IGF receptors mediates IGF-II action (25). The antibody injection technique provides a useful method for determining the receptor type through which IGF-II is acting. Since the inhibitory antibody does not recognize the IGF-II receptor, then only IGF-II effects that are mediated by the IGF-I receptor should be inhibited by antibody 17A3. This was indeed the case in the present work, indicating that the effect of IGF-II on glucose uptake in TA1 cells is largely mediated by the IGF-I receptor kinase. Additional studies of the effect of the inhibitory antibody on other biological actions of IGF-II should be useful in answering the continuing question of which IGF-II effects are mediated by its own receptor.

The injection of specific antibodies may also be useful in future studies of postreceptor steps in insulin action. For example, several proteins have been identified in which tyrosines are phosphorylated in response to insulin (29, 30). The injection of antibodies to these proteins could be used to determine their importance in the actions of insulin.

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