Purification and Characterization of the Receptor for Insulin-like Growth Factor I†

David O. Morgan,‡,§ Kurt Jarnagin,‖ and Richard A. Roth*,‡

Department of Pharmacology, Stanford University School of Medicine, Stanford, California 94305, and Department of Physiology and Hormone Research Laboratory, University of California, San Francisco, California 94143

Received March 25, 1986; Revised Manuscript Received May 23, 1986

ABSTRACT: The receptor for insulin-like growth factor I (IGF-I) was purified from the rat liver cell line BRL-3A by a combination monoclonal anti-receptor antibody column and a wheat germ agglutinin column. Analyses of these receptor preparations on reduced sodium dodecyl sulfate–polyacrylamide gels yielded protein bands of M_r 136K (α subunit) and M_r 85K and 94K (β subunit). These receptor preparations bound 5 times more IGF-I than insulin, and the binding of both labeled ligands was more potently inhibited by unlabeled IGF-I than by insulin. These results indicate that these receptor preparations contained predominantly the IGF-I receptor. This highly purified receptor preparation was found to possess an intrinsic kinase activity; autophosphorylation of the receptor β subunit was stimulated by low concentrations of IGF-I (half-maximal stimulation at 0.4 nM IGF-I). Twentyfold higher concentrations of insulin were required to give comparable levels of stimulation. A monoclonal antibody that inhibits the insulin receptor kinase was found to inhibit the IGF-I receptor kinase with the same potency with which it inhibits the insulin receptor. In contrast, monoclonal antibodies to other parts of the insulin receptor only poorly recognized the IGF-I receptor. A comparison of V8 protease digests of the insulin and IGF-I receptors again revealed some similarities and also some differences in the structures of these two receptors. Thus, the IGF-I receptor is structurally, antigenically, and functionally similar to but not identical with the insulin receptor.

Insulin-like growth factor I (IGF-I) is a polypeptide hormone whose amino acid sequence is about 50% homologous to that of proinsulin (Rinderknecht & Humbel, 1978). IGF-I, at high concentrations, can also bind to the insulin receptor and elicit biological responses through this receptor with about 1% of the potency of insulin (Froesch et al., 1985). In addition, various cells have a distinct receptor for IGF-I which binds IGF-I with high affinity and insulin with a weaker affinity (Rechler & Nissley, 1985). In vivo, IGF-I appears to be a primary regulator of growth, whereas insulin primarily functions as a regulator of more acute metabolic responses. However, with cells in culture, examples have been found of insulin regulating cellular growth through its own receptor and IGF-I regulating acute metabolic responses through its distinct receptor (Froesch et al., 1985; Rechler & Nissley, 1985). The different effects of these hormones on various cell types may in part be determined by the levels of the two receptor types on a particular cell.

In addition to sharing a similar spectrum of biological responses and a similar amino acid sequence, IGF-I and insulin have receptors with a similar general structure [for a review, see Rechler & Nissley (1985)]. Both receptors have α subunits (M_r ~130K) which are readily labeled by cross-linking to their respective radiolabeled ligands. By immunoprecipitation of either biosynthetically labeled or surface-labeled cells, both receptors have also been shown to contain a β subunit of M_r ~95K. In both receptor types, these two subunits are linked via disulfide bonds to form tetrameric ($\alpha_2\beta_2$) complexes of M_r ~350000. Both receptors are synthesized as a single precursor polypeptide of M_r 190000 which is processed to yield the α and β subunits. By analogy to the insulin receptor, it has also been argued that the IGF-I receptor has an intrinsic tyrosine-specific kinase activity because of the following: (1) membrane preparations of a rat liver cell line which are high in IGF-I receptors but low in insulin receptors exhibit an IGF-I stimulatable phosphorylation of a M_r 98000 protein (presumably the β subunit of the IGF-I receptor) (Sasaki et al., 1985); (2) these same preparations exhibit an IGF-I-stimulated phosphorylation of a tyrosine-containing synthetic substrate (Zick et al., 1984); (3) in placenta extracts, IGF-I stimulates phosphorylation of the β subunit of the IGF-I receptor with half-maximal stimulation occurring at concentrations of IGF-I which half-maximally inhibit binding of ^{125}I-IGF-I to its receptor (Rubin et al., 1983); and (4) immunoprecipitates of IGF-I receptor retain kinase activity (Jacobs et al., 1983).

These results support but do not prove the hypothesis that the IGF-I receptor has an intrinsic kinase activity. First, these studies utilized receptor preparations that were fairly impure (less than 1% of the protein present was the receptor) (Rubin et al., 1983; Sasaki et al., 1985; Zick et al., 1984). Second, in these various experiments, insulin was observed to be more potent at stimulating phosphorylation of both the IGF-I receptor and exogenous substrates than would be expected on the basis of its ability to bind to the IGF-I receptor (Rubin et al., 1983; Sasaki et al., 1985; Zick et al., 1984). In addition, there was no detectable stimulation of kinase activity by IGF-I in the experiments with isolated IGF-I receptor in immunoprecipitates (Jacobs et al., 1983). Finally, recent studies of the characteristics of the IGF-I receptor kinase in these impure preparations indicated that it was very similar to the insulin receptor kinase in terms of substrate specificity, activation by autophosphorylation, and nucleotide and cation preference (Sasaki et al., 1985). These studies raised the possibility that the presence of insulin receptor in these partially purified preparations was responsible for the observed kinase activity.

Additional evidence to support the hypothesis that the IGF-I receptor is a kinase required its purification to homogeneity. Recently, we developed a panel of monoclonal antibodies to

† This work was supported by NIH Research Grants AM 34926 and 21344, Research Career Development Award (to R.A.R.) AM 01393, an Alberta Heritage Foundation Award (to D.O.M.), and a Juvenile Diabetes Foundation Fellowship (to K.J.).

* Correspondence should be addressed to this author.
‡ Stanford University School of Medicine.
§ Department of Physiology, University of California.
‖ Hormone Research Laboratory, University of California.

0006-2960/86/0425-5560$01.50/0

the cytoplasmic domain of the insulin receptor (Morgan et al., 1986; Morgan & Roth, 1986). Immunoaffinity purification with several of these antibodies has provided highly purified insulin receptor preparations from a variety of species and tissue types (Morgan et al., 1986; Roth et al., 1986). Since several of these antibodies were found to have high affinity for the IGF-I receptor, it was possible to utilize these antibodies to purify the IGF-I receptor. Since these same antibodies also bind the insulin receptor, a rat liver cell line (BRL-3A) was used as the source of receptors since it has few insulin receptors but abundant IGF-I receptors (Sasaki et al., 1985). The IGF-I receptor from these cells was purified to homogeneity and found to have an IGF-I-stimulatable kinase activity and a structure which is similar to but distinct from that of the insulin receptor.

Experimental Procedures

Materials

Pork insulin was purchased from Elanco, and IGF-I (prepared by recombinant DNA technology) was a gift of J. Merryweather, Chiron Corp. Insulin and IGF-I were iodinated by the following procedure to specific activities of 50–100 Ci/g. One to five micrograms of hormone in 20 μL of 0.5 M sodium phosphate, pH 7.4, was combined with 10 μL (1 mCi) of $Na^{125}I$ and 20 μL of chloramine-T (0.06 mg/mL) in the same buffer. After 30 min at 24 °C, labeled hormone was separated from free NaI by passage over a 9-mL Sephadex G-25 column. [γ-^{32}P]ATP (3000 Ci/mmol) is from Amersham; reagents for polyacrylamide gel electrophoresis are from Bio-Rad. Monoclonal antibodies to the insulin receptor have been characterized previously (Morgan et al., 1986; Morgan & Roth, 1986). All other materials were obtained as in Morgan and Roth (1985, 1986). Rat liver insulin receptor was purified as described (Morgan et al., 1986; Roth et al., 1986).

Methods

Receptor Purification. Confluent roller bottles of BRL-3A cells were solubilized in a minimal volume of ice-cold 50 mM *N*-(2-hydroxyethyl)piperazine-*N*′-2-ethanesulfonic acid (Hepes), pH 7.6, 1% Triton X-100, 1 mg/mL bacitracin, and 1 mM phenylmethanesulfonyl fluoride (PMSF). Lysates were clarified by centrifugation (30000*g*, 3 h, 4 °C) and loaded onto a 10-mL Affigel column (Bio-Rad) composed of 10 monoclonal anti-receptor antibodies as described (Morgan et al., 1986). The column was washed with buffer A (50 mM Hepes, pH 7.6, 150 mM NaCl, and 0.1% Triton X-100) containing 1 mg/mL bacitracin and 1 mM PMSF and then with buffer A containing 1 M NaCl. Receptor was eluted with 1.5 M $MgCl_2$, 120 mM sodium tetraborate, and 0.1% Triton X-100, pH 6.5, diluted 10-fold in buffer A, and loaded onto a 2-mL wheat germ agglutinin (WGA) Affigel column. After the column was washed with buffer A, the receptor was eluted with 0.3 M *N*-acetyl-D-glucosamine in buffer A. Preliminary studies of these preparations indicated the presence of a small amount of insulin receptor, which was removed by passing the preparation over a 2-mL Affigel column composed of monoclonal anti-receptor antibody 15B5, which has a high affinity for the insulin receptor and a low affinity for the IGF-I receptor. Purified IGF-I receptor and rat liver insulin receptor were iodinated with Bolton–Hunter reagent (NEN) to a specific activity of approximately 10 Ci/g.

Ligand Binding Studies. IGF-I binding and insulin binding were measured on purified receptor immobilized on microtiter wells coated with anti-receptor antibodies. Wells of a 96-well poly(vinyl chloride) plate were coated with 50 μL of rabbit anti-mouse IgG (10 μg/mL in 20 mM $NaHCO_3$, pH 9.6) for 4 h at 24 °C. Wells were washed twice with wash buffer [20 mM sodium phosphate, pH 7.4, 150 mM NaCl, 0.05% Tween-20, and 0.1% bovine serum albumin (BSA)], and incubated 4 h at 24 °C with 50 μL of monoclonal antibody 17A3 (3×10^{-8} M) in buffer A containing 1% BSA. Wells were washed twice and incubated 16 h at 4 °C with 50 μL of purified receptor (of which about 1 ng binds to the well) in buffer A containing 1% BSA. Wells were washed twice and incubated 60 min at 24 °C with various concentrations of unlabeled ligand, followed by the addition of ^{125}I-IGF-I or ^{125}I-insulin (50000 cpm) for 90 min at 24 °C. Wells were washed twice, cut off, and counted.

Receptor Autophosphorylation. Reaction mixtures of 20 μL of buffer A containing 0.1 mg/mL BSA and 5–10 ng of purified receptor were incubated 60 min at 24 °C with 2 mM $MnCl_2$ and desired concentrations of IGF-I or insulin, followed by a 60-min incubation at 24 °C with 10 μM [γ-^{32}P]ATP (20–40 Ci/mmol). Reactions were then analyzed by polyacrylamide gel electrophoresis and autoradiography. The 85–94-kilodalton (kDa) β-subunit bands were excised and counted. The effect of antibody 17A3 on kinase activity was measured by including the desired concentration of protein A purified 17A3 in the initial 60-min incubation.

Protease Mapping. ^{125}I-Labeled receptor preparations (600000 cpm) were subjected to polyacrylamide gel electrophoresis after denaturation and reduction by boiling in 1% sodium dodecyl sulfate (SDS) and 5% 2-mercaptoethanol. The separated α and β subunits were excised from the unfixed gel, by using prestained molecular weight markers (BRL) as a guide. Proteins were eluted from crushed gel slices by soaking 16 h at 4 °C in 200 μL of buffer A and then centrifuging to pellet the gel. Aliquots (20 μL) of these eluted subunits were then combined with 10 μL of buffer A containing the desired concentration of Staphylococcus *aureus* V8 protease (Sigma). After 30 min at 37 °C, reactions were analyzed by electrophoresis on 15% polyacrylamide gels.

Results

IGF-I Receptor Structure. Soluble lysates of the rat liver cell line BRL-3A (which are high in IGF-I receptor) were purified by sequential affinity chromatography on a column composed of monoclonal anti-receptor antibodies and a wheat germ agglutinin column. Small amounts of insulin receptor in these IGF-I receptor preparations were removed by passage of the material over an affinity column containing an antibody which preferentially binds the insulin receptor. The purified IGF-I receptor was compared to the rat liver insulin receptor by ^{125}I labeling and analysis by SDS gel electrophoresis and autoradiography (Figure 1). The two rat receptor types had a similar pattern of two major subunits: one at M_r 135K and a doublet at roughly M_r 85K and 94K. These molecular weights correspond to previously known molecular weights of the receptor α and β subunits, respectively. Slight differences in molecular weights were observed between the insulin and IGF-I receptor β subunits. Since insulin receptors from different tissues also exhibit slightly different molecular weights on SDS gels, it is not possible to conclude solely from the molecular weight which receptor is the IGF-I receptor and which is the insulin receptor.

IGF-I Binding Activity. To distinguish between the receptors for insulin and IGF-I, binding studies were performed with ^{125}I-labeled IGF-I and insulin. The BRL-3A receptor preparation was found to bind 5 times more labeled IGF-I than insulin (Figure 2A). Since IGF-I receptors also have a weak affinity for insulin, competition studies were performed to determine whether the observed binding of ^{125}I-insulin was due

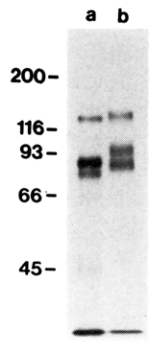

FIGURE 1: Subunit composition of insulin and IGF-I receptors. Purified receptor preparations from rat liver (lane a) and purified IGF-I receptor from BRL-3A cells (lane b) were labeled with ^{125}I with Bolton–Hunter reagent, reduced, and electrophoresed on a 7.5% polyacrylamide gel. An autoradiograph of the dried gel is shown. Molecular weights of protein standards ($\times 10^{-3}$) are shown.

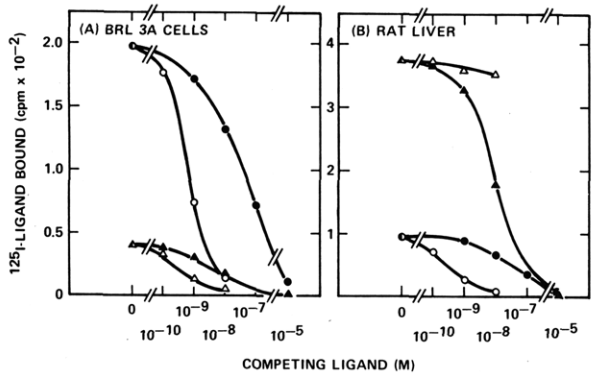

FIGURE 2: Binding of IGF-I and insulin to purified receptor preparations. Purified IGF-I receptor from BRL-3A cells (panel A) or insulin receptor from rat liver (panel B) was incubated with 0.8 nM ^{125}I-insulin (triangles) or 0.4 nM ^{125}I-IGF-I (circles) in the presence of the indicated concentrations of unlabeled insulin (closed symbols) or IGF-I (open symbols). Bound radioactivity was measured as described under Methods.

to the presence of a small amount of insulin receptor or ^{125}I-insulin binding to the IGF-I receptor. The binding of ^{125}I-IGF-I to the BRL-3A preparation was displaced with high affinity by IGF-I (half-maximal inhibition at 0.6 nM IGF-I) and displaced poorly by insulin (I_{50} = 30 nM insulin); thus, a high-affinity IGF-I receptor binding site is present in these preparations. Scatchard analysis of IGF-I binding data (not shown) indicates that the receptor preparations bound approximately 0.5–1.5 mol of IGF-I/mol of receptor. The small amount of ^{125}I-insulin binding to this receptor preparation was displaced by low concentrations of IGF-I (I_{50} = 0.5 nM) and high concentrations of insulin (I_{50} = 7 nM), indicating that the observed binding of labeled insulin was also to the IGF-I receptor.

In contrast to the results with the receptor preparations from BRL-3A cells, the rat liver receptor preparations contained mainly the insulin receptor. The rat liver preparations bound 5 times more insulin than IGF-I, and the ^{125}I-insulin binding was displaced by low concentrations of insulin (I_{50} = 9 nM insulin) and not displaced by IGF-I at concentrations of 10 nM (Figure 2B). Rat liver insulin receptor preparations also bound a small amount of ^{125}I-IGF-I. This binding appeared to be due to the presence of a small amount of IGF-I receptor in these preparations, since IGF-I was more potent than insulin at inhibiting its binding (Figure 2B).

Receptor Autophosphorylation. Studies of receptor autophosphorylation were then performed to determine if this highly purified IGF-I receptor possessed intrinsic kinase activity. Incubation of this preparation with Mn^{2+}, [γ-^{32}P]ATP, and increasing concentrations of IGF-I resulted in a dose-dependent increase in the labeling of the receptor β subunit (Figure 3). The two bands of the β-subunit doublet were stimulated equally. The response to IGF-I was quite sensitive: half-maximal stimulation occurred at 0.4 nM IGF-I, a value close to the potency of IGF-I in the displacement of ^{125}I-IGF-I binding to this receptor. Insulin was over 17-fold less potent in stimulating autophosphorylation; half-maximal stimulation occurred at about 7 nM, a value in close agreement with the ability of insulin to inhibit ^{125}I-insulin binding to this receptor. The maximum incorporation of phosphate into the receptor was approximately 2–4 mol of phosphate/mol of receptor.

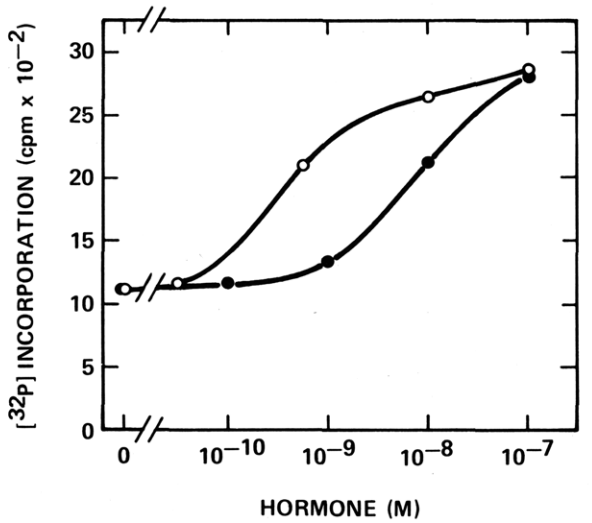

FIGURE 3: Stimulation of BRL-3A IGF-I receptor autophosphorylation by IGF-I and insulin. Purified IGF-I receptor was incubated with Mn^{2+}, [γ-^{32}P]ATP, and the indicated concentrations of IGF-I (○) or insulin (●). Reaction products were analyzed by electrophoresis, and the 85–94-kDa β-subunit bands were excised and counted by liquid scintillation. Values are means of duplicates, and similar results were obtained in four separate experiments.

Receptor Antigenic Structure. Previous studies with a panel of monoclonal antibodies to the insulin receptor kinase domain have shown that only one of the four major antigenic regions in this domain is conserved in the IGF-I receptor (Morgan & Roth, 1986). Antibodies to this region, the β_2 antibodies, were found to bind the IGF-I receptor to the same extent as they bound the insulin receptor (Morgan & Roth, 1986). Since the antibodies to the β_2 region are potent inhibitors of receptor autophosphorylation, it was possible in the present work to more carefully compare their affinity for the insulin and IGF-I receptors by analyzing their dose-dependent inhibition of receptor autophosphorylation. The β_2 antibody 17A3 inhibited the phosphorylation of the purified IGF-I receptor with the same affinity as it inhibited the rat liver insulin receptor: both receptor types were inhibited half-maximally at an antibody concentration of 4 nM (Figure 4).

Protease Mapping. Structural relationships between the receptors for IGF-I and insulin were further studied by analyzing the pattern of fragments generated by limited proteolysis of the two receptor types. The ^{125}I-labeled α and β subunits of each receptor type were separated on SDS–polyacrylamide gels, eluted from the gel, treated with various concentrations of *Staphylococcus aureus* V8 protease, and electrophoresed on gels. The resulting patterns of proteolytic fragments obtained from the two receptor types were remarkably similar, although some small differences in fragment size and number were observed (Figure 5). For example, the 26-kDa α-subunit fragment was more heterogeneous in the IGF-I receptor

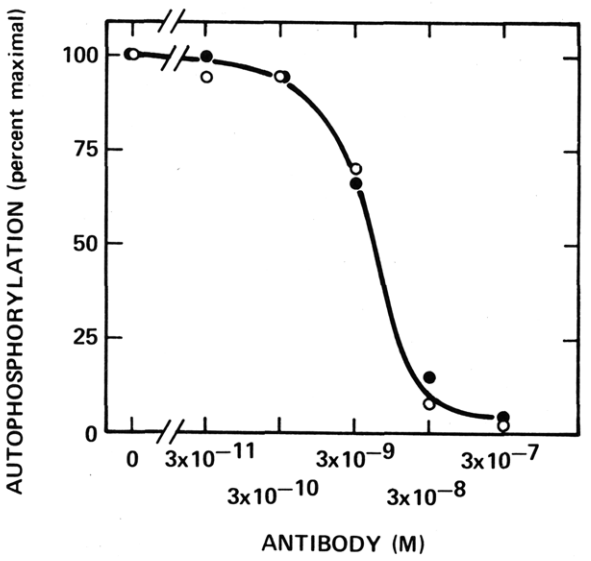

FIGURE 4: Inhibition of receptor phosphorylation by monoclonal antibody 17A3. Either purified IGF-I receptor (5–10 ng) from BRL-3A cells (○) or rat liver insulin receptor (●) was incubated 60 min with Mn^{2+}, the indicated concentration of antibody 17A3, and either IGF-I (30 nM, ○) or insulin (30 nM, ●). [γ-^{32}P]ATP was then added for an additional 60 min, and reaction products were analyzed by electrophoresis. The β-subunit bands were excised from the dried gel and counted by liquid scintillation; 100% values were 1888 (○) and 527 cpm (●). Values are means of duplicates.

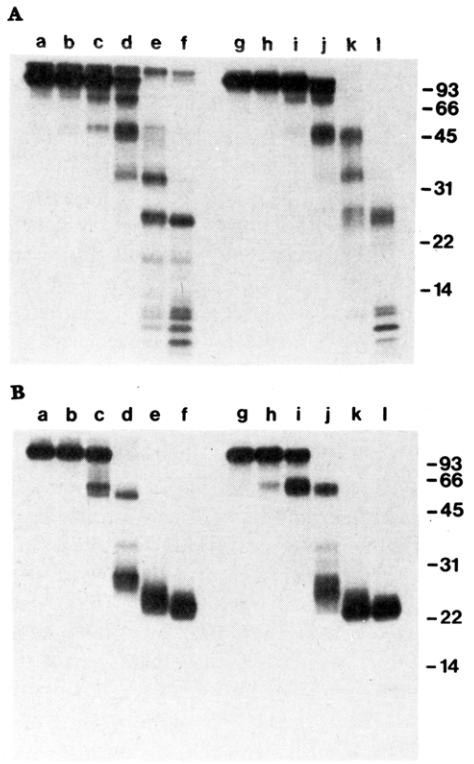

FIGURE 5: Protease mapping of receptor subunits. Purified and ^{125}I-labeled insulin receptor from rat liver (lanes a–f) or IGF-I receptor from BRL-3A cells (lanes g–l) was denatured, reduced, and subjected to polyacrylamide gel electrophoresis. Separated α (panel A) and β (panel B) subunits were then eluted from gel slices and treated with the following concentrations of *Staphylococcus aureus* V8 protease: 0 (lanes a and g), 0.03 (lanes b and h), 0.3 (lanes c and i), 3 (lanes d and j), 30 (lanes e and k), and 300 μg/mL (lanes f and l). Proteolyzed subunits were then electrophoresed on 15% polyacrylamide gels; autoradiographs of dried gels are shown. Molecular weights ($\times 10^{-3}$) of protein standards are shown.

(Figure 5A, lanes f and l), and the ~60-kDa β-subunit fragment of the IGF-I receptor had a slightly lower mobility than the corresponding fragment of the insulin receptor (Figure 5B, lanes d and j).

Discussion

In the present work, we have purified the IGF-I receptor from the BRL-3A cell line by utilizing a monoclonal antibody affinity column. The purified receptor preparation bound approximately 5 times more labeled IGF-I than insulin. Moreover, the binding of both of these ligands was 20–50 times more potently inhibited by IGF-I than insulin. These results indicate that there was no detectable insulin receptor in these preparations. These purified preparations of IGF-I receptor were found to have an IGF-I-stimulatable kinase activity. The concentration of IGF-I which half-maximally stimulated the autophosphorylation reaction (0.4 nM) was close to the concentration of IGF-I which half-maximally inhibited labeled IGF-I binding (0.6 nM). Although insulin also stimulated the autophosphorylation reaction, the concentration of insulin required for half-maximal stimulation was about 20 times greater than the required concentration of IGF-I. Moreover, the concentration of insulin required to half-maximally stimulate autophosphorylation (7 nM) was the same as the concentration of insulin required to half-maximally inhibit the binding of labeled insulin to the IGF-I receptor (7 nM). These results indicate that insulin is acting through the IGF-I receptor. These results are in contrast to prior results using less purified receptor preparations where it was found that insulin was only 2 or 3 times less potent than IGF-I at stimulating phosphorylation (Zick et al., 1984; Sasaki et al., 1985). It may be that these earlier studies are complicated by the presence of contaminating insulin receptor.

Polyacrylamide gel analyses of the highly purified IGF-I receptor from the rat hepatoma cells indicate that the IGF-I receptor is composed of two major subunits: α (M_r 136K) and a doublet of β (M_r 85K and 94K). This structure agrees with the previously derived structure of the IGF-I receptor from human IM-9 lymphocytes, human placenta, and various cell lines (Rechler & Nissley, 1985). The β subunit of the IGF-I receptor in IM-9 lymphocytes was also found to be a doublet (Jacobs et al., 1983). One of these two bands may have represented a small amount of contaminating insulin receptor. However, this appears unlikely in the present studies since the two bands of the β-subunit doublet are present in equal amounts and since their autophosphorylation is equally stimulated by IGF-I. Instead, these two bands could result from a partial proteolysis of the β subunit or differential glycosylation. It is even possible that these two bands may represent different IGF-I receptors since evidence has been presented which suggests that several forms of this receptor exist (Jonas & Harrison, 1985).

In addition to sharing a structural and functional similarity with the insulin receptor, the IGF-I receptor also shares antigenic cross-reactivity with antibodies to the insulin receptor. Prior studies with polyclonal antibodies from various patients had indicated that some of these antisera reacted with both the IGF-I and insulin receptors, whereas others reacted only with the insulin receptor (Kasuga et al., 1983; Jonas et al., 1982). In these studies, it was not possible to determine whether the same antibody molecules were reacting with both receptors. More recently, several monoclonal antibodies to extracellular determinants of the insulin receptor were also shown to react with the IGF-I receptors (Roth et al., 1983; Kull et al., 1983). However, these antibodies had a 100–200-fold higher affinity for the insulin receptor than the IGF-I receptor. In prior studies, we examined the binding of the

IGF-I receptor by monoclonal antibodies to four distinct antigenic regions of the cytoplasmic domain of the β subunit of the insulin receptor (Morgan & Roth, 1986). Antibodies to one particular antigenic region, the β_2 region, were found to precipitate the two receptors equally. In the present studies, antibodies to this region were also found to inhibit with identical potencies the kinase activities of the two receptors. Since the β_2 region is intimately involved in the kinase activity of the receptor, these studies suggest that this region of the two receptors is most highly related. Recently, it has become possible to identify the residues involved in the β_2 region. Site-directed mutagenesis was used to construct mutant insulin receptor cDNAs for expression in Chinese hamster ovary cells (Ellis et al., 1986). Insulin receptors in which tyrosines-1162 and -1163 were replaced with phenylalanines were recognized very poorly by antibodies to the β_2 region, although antibodies to other antigenic regions recognized these mutated receptors normally. The kinase activity of this mutant receptor, both in vivo and in vitro, was also severely reduced. Clearly, the β_2 region is an important region in insulin receptor kinase function; the present results demonstrate that the antigenic region recognized by the β_2 antibodies is highly conserved in the IGF-I receptor. Similarly, anti-peptide antibodies directed against another potentially important region of the insulin receptor kinase (residues 952–967) have recently been shown to cross-react with the IGF-I receptor (Herrera et al., 1986). That the kinase activities of the two receptors are highly related is also suggested by studies indicating that the two receptor kinases share substrate specificities, as well as nucleotide and cation preference (Sasaki et al., 1985). Interestingly, the same β_2-antigenic region which is involved in the kinase activity also appears highly conserved in insulin receptors from other species, since antibodies to this site recognize insulin receptors from frogs, chickens, and mice with high affinity (Morgan et al., 1986). These results suggest that the kinase domain may be important in the function of these receptors.

In summary, the present work indicates that the receptors for IGF-I and insulin are highly homologous. Similarities in subunit structure, kinase activity, proteolytic digestion fragments, and antigenic structure demonstrate that the two receptors are closely related. A more detailed comparison of these two receptors must await the cloning and sequencing of the cDNA that encodes the IGF-I receptor.

ACKNOWLEDGMENTS

We thank J. Merryweather for the generous gift of IGF-I, K. Bird for excellent secretarial assistance, J. Beaudoin for assistance in receptor purification, and W. J. Rutter for advice and support during the course of these studies.

Registry No. IGF-I, 67763-96-6; IGF-I receptor kinase, 103843-29-4; insulin, 9004-10-8; insulin receptor kinase, 88201-45-0.

REFERENCES

Ellis, L., Clauser, E., Morgan, D. O., Edery, M., Roth, R. A., & Rutter, W. J. (1986) *Cell (Cambridge, Mass.) 45*, 721–732.

Froesch, E. R., Schmid, C., Schwander, J., & Zapf, J. (1985) *Annu. Rev. Physiol. 47*, 443–467.

Herrera, R., Petruzzelli, L. M., & Rosen, O. M. (1986) *J. Biol. Chem. 261*, 2489–2491.

Jacobs, S., Kull, F. C., Jr., Earp, H. S., Svoboda, M. E., Van Wyk, J. J., & Cuatrecasas, P. (1983) *J. Biol. Chem. 258*, 9581–9584.

Jonas, H. A., & Harrison, L. C. (1985) *J. Biol. Chem. 260*, 2288–2294.

Jonas, H. A., Baxter, R. C., & Harrison, L. C. (1982) *Biochem. Biophys. Res. Commun. 109*, 463–470.

Kasuga, M., Sasaki, N., Kahn, C. R., Nissley, S. P., & Rechler, M. M. (1983) *J. Clin. Invest. 72*, 1459–1469.

Kull, F. C., Jacobs, S., Su, Y.-F., Svoboda, M. E., Van Wyk, J. J., & Cuatrecasas, P. (1983) *J. Biol. Chem. 258*, 6561–6566.

Morgan, D. O., & Roth, R. A. (1985) *Endocrinology (Baltimore) 116*, 1224–1226.

Morgan, D. O., & Roth, R. A. (1986) *Biochemistry 25*, 1364–1371.

Morgan, D. O., Ho, L., Korn, L. J., & Roth, R. A. (1986) *Proc. Natl. Acad. Sci. U.S.A. 83*, 328–332.

Rechler, M. M., & Nissley, S. P. (1985) *Annu. Rev. Physiol. 47*, 425–442.

Rinderknecht, E., & Humbel, R. E. (1978) *J. Biol. Chem. 253*, 2769–2776.

Roth, R. A., Maddux, B., Wong, K. Y., Styne, D. M., Van Vliet, G., Humbel, R. E., & Goldfine, I. D. (1983) *Endocrinology (Baltimore) 260*, 1865–1867.

Roth, R. A., Morgan, D. O., Beaudoin, J., & Sara, V. (1986) *J. Biol. Chem. 261*, 3753–3757.

Rubin, J. B., Shia, M. A., & Pilch, P. F. (1983) *Nature (London) 305*, 438–440.

Sasaki, N., Rees-Jones, R. W., Zick, Y., Nissley, S. P., & Rechler, M. M. (1985) *J. Biol. Chem. 260*, 9793–9804.

Zick, Y., Sasaki, N., Rees-Jones, R. W., Grunberger, G., Nissley, S. P., & Rechler, M. M. (1984) *Biochem. Biophys. Res. Commun. 119*, 6–13.