The Subunit Structures of Two Distinct Receptors for Insulin-like Growth Factors I and II and Their Relationship to the Insulin Receptor*

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Intact cells and membranes isolated from several human and rodent tissues have been affinity-cross-linked to human 125I-insulin-like growth factor I (IGF-I) and 125I-insulin-like growth factor II (IGF-II). Dodecyl sulfate-polyacrylamide gel electrophoresis of the affinity-labeled material resolves two types (type I and type II) of labeled membrane components that fulfill the properties expected for high affinity growth factor receptors. Type I receptors consist of three disulfide-linked forms (Mr = 350,000, 320,000 and 290,000) structurally similar to the insulin receptor forms in membrane preparations from various tissues (Massague, J., Pilch, P. F., and Czech, M. P. (1980) Proc. Natl. Acad. Sci. U.S.A. 77, 7137-7141). The proposed subunit stoichiometries of these type I IGF receptor forms are (β-S-S-α)-S-S-(α-S-S-β), (β-S-S-α)-S-S-(α-S-S-β), and (β-S-S-α)-S-S-(α-S-S-β), respectively, based on two-dimensional electrophoretic analysis. The α and β subunits migrate with apparent Mr = 130,000 and 98,000, respectively, and the β subunit is a proteolytic fragment of the β subunit. The disulfide-linked Mr = 350,000 receptor species probably represents the native receptor complex. The type I IGF receptors exhibit a higher affinity for IGF-I than for IGF-II, and a low affinity for insulin. The type II receptor consists of one single labeled species (Mr = 258,000-268,000) that is not disulfide-linked to any other membrane component. This type II IGF receptor has a higher affinity for IGF-II than for IGF-I, and has no significant affinity for insulin. This receptor type apparently corresponds to the same structure previously identified as a membrane receptor for multiplication-stimulating activity (Massague, J., Guillette, B. J., and Czech, M. P. (1981) J. Biol. Chem. 256, 2122-2125). The ability of IGF-I and IGF-II to compete with 125I-insulin for affinity labeling the high affinity insulin receptor in all tissues examined is lower than that of insulin. In conclusion, we have identified a specific growth factor receptor with high affinity for IGF-I that consists of a heterotetrameric disulfide-linked subunit composition virtually identical with the insulin receptor structure. A second growth factor receptor with high affinity for IGF-II is structurally distinct from these disulfide-linked receptor types.

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Insulin-like growth factors I and II from human serum (1) are two closely related peptides with amino acid sequences considerably homologous to that of insulin (2, 3). IGF-I and IGF-II stimulate sugar and amino acid transport, sulfation of rat costal cartilage, and macromolecule synthesis in several tissues (4). They are also potent mitogens for certain cell types (4). Many biological properties of IGF-I and IGF-II are also shared by somatomedin C (5, 6), somatomedin A (7, 8), and rat multiplication-stimulating activity (9). Recent amino acid-sequence studies have shown that rat somatomedin C* and multiplication-stimulating activity (10) are the rat counterparts of human IGF-I and IGF-II, respectively.

Like insulin, the primary event in the action of IGF-I and IGF-II on target cells appears to be their binding to specific receptors on the cell surface. A basic question concerning the mechanism of action of these three structurally related polypeptide hormones is whether their respective receptors share also common structural and biological properties. Radioligand binding studies in several laboratories (4, 7, 11) have shown that the binding of IGPs to various cell and membrane systems is rather complex and suggests a considerable heterogeneity in IGF receptor forms and distribution among tissues. Studies of this kind have also indicated that the IGF-binding sites in some tissues exhibit a certain degree of affinity for insulin, and the high affinity insulin receptor can specifically interact with IGF-I and IGF-II (4, 11). Until recently, direct structural information clarifying the nature and distribution of the receptor(s) for IGF-I and IGF-II had been lacking. In a preliminary report, we have described the characteristics of a receptor structure affinity-labeled by cross-linking to 125I-multiplication-stimulating activity (12). We report here the structural characteristics of two types of affinity-labeled membrane components that exhibit high affinity and specificity for IGF-I and IGF-II. The relative affinities of these two putative receptors for IGF-I and IGF-II, their subunit composition, tissue distribution, and relationship to other known receptor structures are also described.

MATERIALS AND METHODS

Preparation of Cells and Membranes—White fat cells were isolated by the collagenase method (13) from epididymal fat pads of 100-200-g rats. Membranes from rat adipocytes and human placenta were prepared as described before (14). Rat and mouse liver membranes were prepared by the method of Neville (15). Fibroblasts from normal human foreskin skin biopsies were grown to confluence at 37 °C in McCoy’s 5A medium containing 25 mM sodium bicarbonate, 10% fetal bovine serum in air/CO2, 19:1 atmosphere. Human skin fibroblast membranes were obtained by homogenization of mechanically detached cultures in 0.25 M sucrose, 10 mM Tris, 1 mM EDTA, pH 7.4.

1 The abbreviations used are: IGF, insulin-like growth factor; MSA, multiplication-stimulating activity.

2 J. Rubin and R. A. Bradshaw, personal communication.
with a tight fitting Dounce homogenizer. The homogenate was centrifuged at 3,000 x g for 10 min and the resulting supernatant was centrifuged at 30,000 x g for 20 min. The material pelleted in the latter centrifugation was resuspended in 0.25 M sucrose, 10 mM Tris, 1 mM EDTA, pH 7.4, layered on top of a 20-50% (w/w) sucrose gradient and centrifuged at 100,000 x g for 60 min. The membranous fraction that equilibrated at 35-37% (w/w) sucrose was pelleted at 30,000 x g for 30 min after dilution with 10 mM Tris, 1 mM EDTA, pH 7.4, and used in the affinity-labeling experiments. Protease inhibitors 1 mM phenethylsulfonyl fluoride and 0.1 mg/ml soybean trypsin inhibitor were occasionally used during preparation of membranes from these tissues with no significant effect on the pattern of specifically affinity-labeled membrane proteins.

Murine 3T3-L1 fibroblasts (American Type Culture Collection) were grown at 37 °C in Dulbecco's modified Eagle's medium supplemented with 10% calf serum. A crude membrane fraction was obtained by centrifugation of the resulting homogenate at 30,000 x g for 60 min. The membranous fraction that equilibrated at 31-32% sucrose was collected after centrifugation at 100,000 x g for 60 min. The fraction equilibrating at 31-32% sucrose was collected and the resulting supernatant was centrifuged at 100,000 x g for 60 min. The fraction equilibrating at 31-32% sucrose was collected and the resulting supernatant was centrifuged at 100,000 x g for 60 min. The fraction equilibrating at 31-32% sucrose was collected and the resulting supernatant was centrifuged at 100,000 x g for 60 min. The resulting pellet was resuspended in homogenization medium, layered on top of a 20-40% (w/w) sucrose gradient and centrifuged at 100,000 x g for 60 min. The fraction equilibrating at 31-32% sucrose was collected and the resulting supernatant was centrifuged at 100,000 x g for 60 min. The membranous fraction that equilibrated at 35-37% (w/w) sucrose was pelleted at 30,000 x g for 30 min after dilution with 10 mM Tris, 1 mM EDTA, pH 7.4, and used in the affinity-labeling experiments. Protease inhibitors 1 mM phenethylsulfonyl fluoride and 0.1 mg/ml soybean trypsin inhibitor were occasionally used during preparation of membranes from these tissues with no significant effect on the pattern of specifically affinity-labeled membrane proteins.

Insulin-like Growth Factor Receptor Structures

Membrane preparations from rat adipocyte, rat liver, human placenta, and human skin fibroblast were incubated with 5 nM [125I]-IGF-I or 5 nM [125I]-IGF-II and then cross-linked to the membrane-bound hormone by incubation with 0.2 mM disuccinimidyl suberate. Two groups of labeled bands appear on autoradiograms of dodecyl sulfate-polyacrylamide gels electrophoresed with the membranes affinity-labeled with [125I]-IGF-I (Fig. 1a). One group (type I affinity-labeled membrane components) consists of three labeled species with apparent molecular masses of 110, 100, and 90 kDa. The other group (type II affinity-labeled membrane components) consists of three labeled species with apparent molecular masses of 90, 80, and 70 kDa.}

RESULTS AND DISCUSSION

Membrane preparations from rat adipocyte, fibroblast, sarcoma, melanoma, and human skin fibroblast were homogenized in the latter buffer and a crude membrane fraction was obtained by centrifugation at 30,000 x g for 20 min, after removing most of the nuclei, mitochondria, and other heavy fractions by centrifugation at 3,000 x g for 20 min. Following affinity-labeling, the lymphocyte-type cells were directly solubilized in the presence of 2% sodium dodecyl sulfate, 50 mM Tris, pH 6.8, and centrifuged at 100,000 x g for 20 min, and the supernatant was kept for gel electrophoresis.

Electrophoresis and Autoradiography—The cross-linked samples were boiled for 5 min in the presence of 1% sodium dodecyl sulfate, 50 mM Tris, pH 6.8, with or without 50 mM dithiothreitol, and subjected to electrophoresis on polyacrylamide gels according to Laemmli (20). Gels were 5% polyacrylamide (100:1 acrylamide/bisacrylamide ratio) unless otherwise indicated. After electrophoresis, gels were stained for protein, dried, and subjected to autoradiography as described before (12). Marker proteins were: myosin (M, = 250,000), &beta;galactosidase (M, = 116,000), phosphorylase b (M, = 94,000), bovine serum albumin (M, = 68,000), ovalbumin (M, = 45,000), and carbonic anhydrase (M, = 31,000). The M, values of affinity-labeled species were obtained after graphic extrapolation of the plot log M, versus R, values corresponding to the protein standards.

Reagents—Human IGF-I and IGF-II were a gift of Prof. R. E. Humbel (Biochemisches Institut der Universitat, Zurich, Switzerland) and were labeled with I21I by the chloramine-T method previously used (4) to a specific activity of 180-270 Ci/g. Porcine insulin (gift of R. Chance, Eli Lilly) was labeled with 125I by the lactoperoxidase method (Enzymobeads, Bio-Rad) to a specific activity of 80-110 Ci/g. Disuccinimidyl suberate was from Pierce Chemical Co.

**Affinity-labeling Protocols—**Membranes (150-200 pg of membrane protein) in 200 μl of Krebs-Ringer phosphate buffer, pH 7.4, were incubated at 10 °C for 90 min with [125I]-labeled hormones in the presence or absence of nonradioactive hormones at the indicated final concentrations. At the end of this incubation, the excess unbound hormone was washed out by dilution with ice-cold Krebs-Ringer phosphate buffer, pH 7.4, and centrifugation at 12,000 g for 3 min. The membrane samples were resuspended in the latter medium, and disuccinimidyl suberate was freshly dissolved in dimethyl sulfoxide and added at final 0.20 mM concentration under the conditions previously described (19). The cross-linking reaction was allowed to proceed for 15 min at 0 °C before it was terminated by quenching the unreacted disuccinimidyl suberate with an excess Tris buffer, pH 7.4. The incubation mixture was centrifuged at 12,000 x g for 5 min and the pellet was dissolved in electrophoresis buffer.

Intact cells resuspended in Krebs-Ringer phosphate buffer, pH 7.4, with 4% bovine serum albumin were affinity-labeled using a protocol similar to that used for affinity-labeling isolated membranes. Incubation with [125I]-labeled and unlabeled hormones was conducted at 23 °C for 60 min, and the cross-linking reaction was stopped by addition of excess Tris buffer, pH 7.4. Samples (100 μg of membrane protein) of the cross-linked membranes were electrophoresed on 5% polyacrylamide slab gels according to Laemmli (20), in the absence (lanes 1 to 4) or presence (lanes 5 to 8) of 50 mM dithiothreitol (DTT). Autoradiograms from the fixed, dried gels are shown. Two distinct types (types I and II) of affinity-labeled bands were revealed on the autoradiograms as described in the text.
plexes dissociate into free polyacrylamide gel electrophoresis in the presence of dithiothreitol (21). These structural features are apparently shared by receptor subunits, probably by reduction of intrachain disulfide bonds (21). Concomitant with an increase of the apparent affinity-labeled by IZ5I-IGF-II. The conditions used for this experiment were the same as for the experiment in Fig. 1a. However, the relative amount of radioactivity associated with the IGF-I labeled species was higher when membranes were affinity-labeled with IZ5I-IGF-II than with IZ5I-IGF-I. Conversely, the labeling of type II species was more intense when membranes were cross-linked with IZ5I-IGF-II than with IZ5I-IGF-I.

The pattern of labeled bands shown in Fig. 1 remained the same when different membrane preparations from a given tissue or different preparations of IZ5I-labeled peptides were used during the course of this study. Preliminary experiments indicated that the concentration of disuccinimidyl suberate used in the affinity-labeling protocol could be varied from 0.05 to 0.5 M without any apparent change in the molecular weight of the affinity-labeled species or in the protein staining pattern of the gels. Therefore, it seems unlikely that any of the type I or type II affinity-labeled species results from the artificial cross-linking of two or more adjacent membrane components. Electrophoresis of affinity-labeled membranes from various tissues before conditions resolving peptides in the M, = 40,000–400,000 range did not show any specifically labeled peptide other than those described above or their respective reduction counterparts (not illustrated).

The electrophoretic characteristics of the type II IZ5I-IGF-I labeled species are similar to those of the IGF-I receptor for multiplication-stimulating activity affinity-labeled in membranes from several rat and human tissues (12). Furthermore, both unlabeled IGF-I and MSA compete with IZ5I-IGF-I, IZ5I-IGF-II, or IZ5I-MSA for the affinity labeling of these species on rat liver and adipocyte membranes, suggesting that both kinds of ligand share this putative growth factor receptor. In insulin present at 10–4 M during incubation of membranes with IZ5I-IGF-I or IZ5I-MSA does not compete for the labeling of the type II labeled species (12). Human placenta membranes affinity-labeled with IZ5I-IGF-I were subjected to a bidimensional electrophoresis analysis to further document the subunit composition of the disulfide-linked type I labeled species, as well as to establish more rigorously their structural relationship with the known insulin receptor complex. The affinity-labeled placenta membranes were first electrophoresed on a dodecyl sulfate-polyacrylamide gel in the absence of dithiothreitol. This first dimension electrophoresis resolved the type I and type II labeled bands depicted in Fig. 1a, lane 3, as well as two very minor labeled species with M, = 205,000 and 155,000, respectively (Fig. 2). This first gel was subjected to a second dimension electrophoresis in the presence of dithiothreitol. The characteristics of the labeled species separated after this treatment support the hypothesis that the M, = 350,000, 320,000, and 290,000 membrane components labeled by IZ5I-IGF-I have a subunit stoichiometry equivalent to their respective (αβ)2, (αβ)β1, and (αβ)3 insulin receptor counterparts. Thus, the main radioactive species released by dithiothreitol from all three labeled species with Mr = 350,000, 320,000, and 290,000 putative IGF receptors was a region of the labeled species varied upon addition of dithiothreitol 5–8).

The tissue distribution, apparent M, and sensitivity to dithiothreitol of the membrane components labeled by IZ5I-IGF-II were indistinguishable from those labeled by IZ5I-IGF-I (Fig. 1). However, the relative amount of radioactivity associated with the IGF-I labeled species was higher when membranes were affinity-labeled with IZ5I-IGF-II than with IZ5I-IGF-I (Fig. 1a, lanes 3 and 4 versus Fig. 1b, lanes 3 and 4). Conversely, the labeling of type II species was more intense when membranes were cross-linked with IZ5I-IGF-II than with IZ5I-IGF-I.

1 J. Massague and M. P. Czech, unpublished results.
Small amounts of \((\alpha\beta)\) and \((\alpha\beta_1)\) insulin receptor half-fragments that are not disulfide-linked as complete receptor complexes are present in native membranes from rat liver (25) and human placenta. The apparent \(M_r\) of these native receptor fragments is increased by low concentrations of dithiothreitol, probably due to reduction of intrapeptide disulfide bonds (20). At high concentrations, dithiothreitol promotes the complete dissociation of these \((\alpha\beta)\) and \((\alpha\beta_1)\) insulin receptor fragments into their respective \(\alpha\) and \(\beta\) receptor subunits. Native membranes also appear to contain unassembled \((\alpha\beta)\) and \((\alpha\beta_1)\) IGF receptor fragments. Supportive evidence for this postulate is provided by the detection of reduced \(\alpha\) IGF receptor subunit in the second electrophoresis dimension (Fig. 2, species 13 and 14) at positions expected for the precursor unassembled \((\alpha\beta)\) and \((\alpha\beta_1)\) IGF receptor fragments (positions \(M_r = 205,000\) and 155,000, respectively, on the first dimension gel).

The affinity-labeling of the \(\beta\) subunit from type I IGF receptors was more clearly observed when individual receptor complexes were isolated from a first gel and re-electrophoresed in the presence of dithiothreitol (Fig. 3, inset). Under these conditions, the proposed \((\alpha\beta)\), \((M_r = 350,000)\) receptor form, but not the \((\alpha\beta_1)\); \((M_r = 290,000)\) receptor form yielded labeled receptor subunit. Compared to the labeling of the \(\alpha\) subunit, the affinity-labeling of the \(\beta\) subunit of the type I IGF receptor using the present methodology is apparently rather low (Fig. 2). A similar result is obtained when the insulin receptor is cross-linked to \(^{125}\text{I}\)-insulin by disuccinimidyl suberate (14, 19, 21, 24). In the case of the insulin receptor, the \(\alpha\) and \(\beta\) receptor subunits present in the native \((\alpha\beta_1)\) form of this receptor can be purified using insulin-agarose affinity-chromatography (26, 27). The results obtained using this methodology directly indicate the presence of equimolar amounts of both subunits in the receptor complex (27). A similar conclusion can be drawn from the immunoprecipitation of biosynthetically or chemically labeled insulin receptors using anti-insulin receptor autoantibodies from patients with severe insulin resistance and acanthosis nigricans (28, 29).

Whereas the structural characteristics of the insulin receptor have been documented by these several independent methodologies, the subunit stoichiometry of the \((\alpha\beta_1)\) IGF receptor complex proposed here is based only in the strict analogy between the electrophoretic properties of this affinity-labeled IGF receptor species and the insulin receptor. Confirmation of this proposal must await direct chemical characterization of the type I IGF receptor.

The affinity-labeling of all type I and type II IGF receptor forms was abolished by the presence of 1 \(\mu\)M IGF-I during incubation of human skin fibroblast membranes with \(^{125}\text{I}\)-IGF-I (Fig. 3, lanes 2 and 6). The labeling of type I IGF receptors was only slightly decreased by the presence of 1 \(\mu\)M insulin during incubation with \(^{125}\text{I}\)-IGF-I (Fig. 3, lanes 3 and 7). An insulin concentration of 10 \(\mu\)M displaced most of the \(^{125}\text{I}\)-IGF-I bound to this receptor type (Fig. 3, lanes 4 and 8). However, 1 or 10 \(\mu\)M insulin did not substantially affect the labeling of type II IGF receptors by \(^{125}\text{I}\)-IGF-I (Fig. 3, lanes 3, 4, 7, and 8). A similar pattern of \(^{125}\text{I}\)-IGF-I displacement by IGF-I and insulin was observed with IGF receptors in human placenta membranes (IGF receptor type I and type II) and on rat liver and rat adipocyte membranes (IGF receptor type II) (data not shown).

The high affinity insulin receptor present on human skin fibroblasts can be affinity-labeled by cross-linking to \(^{125}\text{I}\)-insulin with disuccinimidyl suberate (Fig. 4, lanes 1 and 5). Like the insulin receptor on membranes from other tissues, the affinity-labeled fibroblast insulin receptor appears as \((\alpha\beta_1)\), \((\alpha\beta_1)\), and \((\alpha\beta_1)\), disulfide-linked complexes that can be
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Human skin fibroblasts membranes were incubated with 5 nM 125I-IGF-I in the absence of unlabeled hormones or in the presence of IGF-I or insulin (INS) at the concentration indicated on top of each lane. After cross-linking to membrane-bound hormones by disuccinimidyl suberate as specified under “Materials and Methods,” the membranes were boiled in the presence of 1% sodium dodecyl sulfate and electrophoresed (100 μg of membrane protein/lane) on 5% polyacrylamide gels (lanes 1 to 4). Samples on lanes 5 to 8 are the respective counterparts of samples on lanes 1 to 4 added with 50 mM dithiothreitol (DTT) during solubilization in 1% dodecyl sulfate. The proposed subunit stoichiometries (see text) of the disulfide-linked labeled species that appear on the autoradiogram from a fixed dried gel are indicated.

FIG. 3. Displacement of 125I-IGF-I from membrane components of human skin fibroblasts by IGF-I and insulin. Human skin fibroblasts membranes were incubated with 5 nM 125I-IGF-I in the absence of unlabeled hormones or in the presence of IGF-I or insulin (INS) at the concentration indicated on top of each lane. After cross-linking to membrane-bound hormones by disuccinimidyl suberate as specified under “Materials and Methods,” the membranes were boiled in the presence of 1% sodium dodecyl sulfate and electrophoresed (100 μg of membrane protein/lane) on 5% polyacrylamide gels (lanes 1 to 4). Samples on lanes 5 to 8 are the respective counterparts of samples on lanes 1 to 4 added with 50 mM dithiothreitol (DTT) during solubilization in 1% dodecyl sulfate. The proposed subunit stoichiometries (see text) of the disulfide-linked labeled species that appear on the autoradiogram from a fixed dried gel are indicated.

FIG. 4. Displacement of 125I-insulin from the insulin receptor in human skin fibroblast membranes by insulin and IGF-I. Human skin fibroblast membranes were incubated with 10 nM 125I-insulin (INS) in the absence of unlabeled hormones, or in the presence of insulin or IGF-I at the concentrations indicated on top of each lane. After cross-linking of membranes to membrane-bound hormone by 0.2 mM disuccinimidyl suberate, portions (100 μg of membrane protein) of the affinity-labeled membrane samples were solubilized in the presence of 1% sodium dodecyl sulfate and electrophoresed on 5% polyacrylamide gels (lanes 1 to 4). Membrane samples on lanes 5 to 8 received identical treatment as samples on lanes 1 to 4, respectively, except that they were added with 50 mM dithiothreitol (DTT) during solubilization in sodium dodecyl sulfate. An autoradiogram from a fixed, dried gel is shown. The subunit stoichiometry of the insulin receptor complexes is indicated.

FIG. 5. Graded competition for the labeling of the adipocyte a insulin receptor subunit by unlabeled insulin and IGF-I. Rat adipocyte membranes were incubated for 60 min at 10°C with 5 nM 125I-insulin (INS) in the presence of the indicated molar concentrations of nonradioactive insulin or IGF-I. At the end of the incubation, the unbound hormones were washed out at 4°C and the membrane-associated radioactivity was determined with a gamma counter (a). Membranes were immediately treated with 0.2 mM disuccinimidyl suberate, and the cross-linked material was electrophoresed in 7% polyacrylamide gels in the presence of sodium dodecyl sulfate and 50 mM dithiothreitol. After electrophoresis, gels were fixed, dried, and subjected to autoradiography. Shown is the portion of the resulting autoradiogram that corresponds to the M, = 100,000-150,000 region of the gels (b).

Dissociated into free receptor subunits by incubation with dithiothreitol in the presence of sodium dodecyl sulfate. The presence of 1 μM unlabeled insulin during incubation of skin fibroblast membranes with 125I-insulin prevents the radioactive labeling of the insulin receptor species (Fig. 4, lanes 2 and 6). The affinity-labeling of the human skin fibroblast insulin receptor is also prevented by IGF-I added in excess during incubation of membranes with 125I-insulin (Fig. 4, lanes 3, 4, 7, and 8). Under similar conditions, IGF-I also prevents the affinity-labeling of insulin receptor on rat adipocyte (Fig. 5) and human placenta membranes but not on rat liver membranes (not illustrated). The relative potency of IGF-I to prevent the labeling by 125I-insulin of the insulin receptor in rat adipocyte membranes is about 100-500 times lower than that of unlabeled insulin (Fig. 5). These data correlate well with the relative affinity of IGF-I for the insulin receptor in adipocytes as determined by binding studies (4).

These competition studies described above indicate that (a) the type I IGF receptor and the insulin receptor show structural similarities but are two distinct species, (b) the type I insulin receptor exhibits a high affinity for IGF-I and a low affinity for insulin, (c) the type II IGF receptor exhibits a high affinity for IGF-II and no significant affinity for insulin, and (d) human skin fibroblasts, human placenta, and rat adipocyte insulin receptors exhibit a low affinity for IGF-I while the rat liver insulin receptor does not have any apparent affinity for IGF-I. Interestingly, this apparent heterogeneity among insulin receptors from different tissues is not detected when their affinity for insulin itself is examined (30).

The relative intensity of labeling of type I IGF receptor and type II IGF receptor by 125I-IGF-I was different from their relative intensity of labeling by 125I-IGF-II (compare Fig. 1, a and b). This observation suggested a different affinity of each IGF receptor type for IGF-I and IGF-II. A more complete documentation of the affinity of both IGF receptor types for these two ligands is provided in Figs. 6 and 7. In these experiments, membranes from human skin fibroblasts and rat liver were incubated with 125I-IGF-I or 125I-IGF-II in the
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Fig. 6. Relative potencies of IGF-I and IGF-II to displace $^{125}$I-IGF-I from type I IGF receptors in human skin fibroblast membranes. Membranes were incubated with 0.5 nM $^{125}$I-IGF-I in the presence of various concentrations of unlabeled IGF-I (lanes a to f) or unlabeled IGF-II (lanes g to l). After cross-linking to membrane-bound hormone, membranes were electrophoresed in the absence of dithiothreitol on dodecyl sulfate-polyacrylamide gels (5% polyacrylamide). Autoradiograms from the fixed, dried gels were obtained. A portion of autoradiogram corresponding to the type of this receptor is shown.

Fig. 7. Relative potencies of IGF-I and IGF-II to displace $^{125}$I-IGF-I and $^{125}$I-IGF-II from type II IGF receptors in human skin fibroblast and rat liver membranes. Membranes were incubated with 0.5 nM $^{125}$I-IGF-I (series 1, 2, 5, and 6) or 0.5 nM $^{125}$I-IGF-II (series 3 and 4) in the presence of various concentrations of unlabeled IGF-I (series 1, 3, and 5) or unlabeled IGF-II (series 2, 4, 5, and 6). After cross-linking to membrane-bound hormone, membranes were electrophoresed in the absence of dithiothreitol on dodecyl sulfate-polyacrylamide gels (5% polyacrylamide). After electrophoresis, the gels were fixed, dried, and subjected to autoradiography. The portions of the resulting autoradiograms that contain the type II $^{125}$I-IGF labeled band are shown.

The type I and type II IGF receptor structures affinity-labeled in membranes from rat liver and adipocytes, and from human placenta and skin fibroblasts, have also been observed in other tissues and cultured cell lines of human and rodent origin screened for the presence of these receptor structures. Table I summarizes the relative amount of insulin receptor and of the two types of IGF receptors present in some of these systems as estimated by affinity-labeling of the corresponding whole cell or isolated membrane preparations. The human RPMI 6666 and RPMI 7666 lymphoblasts exhibit insulin receptors, but not IGF receptors, on their surface. Mouse 3T3-L1 fibroblasts exhibit a low number of affinity-labeled insulin and type I IGF receptors, but the intensity of labeling of the type II IGF receptor in these cells is similar to that in rat adipocytes. 3T3-L1 fibroblasts are characterized by their ability to differentiate to an adipocyte phenotype (16) sensitive to short term metabolic effects of insulin (17). It has been shown (17, 32) that the number of high affinity insulin-binding sites, but not that of epidermal growth factor-binding sites, increases upon differentiation of 3T3-L1 fibroblasts into adipocytes. Consistent with these observations, we have observed a more intense labeling of the insulin receptor in 3T3-L1 adipocytes than in 3T3-L1 fibroblasts (Table I). This apparent increase in insulin-binding capacity was accompanied by an equivalent increase in the amount of labeling associated with the type I IGF receptor (Table I). These observations suggest that differentiation of 3T3-L1 cells includes the induction of these receptors for IGF-I and IGF-II.

Tissue distribution and relative amounts of types I and II IGF receptors and insulin receptor

| Tissue Type | IGF-I | IGF-II | Insulin |
|-------------|-------|--------|---------|
| Human Placenta | High Low High |
| Skin fibroblasts | Low High Low |
| IM-9 (lymphocytes) | High High Low |
| RPMI 6666 (lymphoblasts) | Low Low Low |
| RPMI 7666 (lymphoblasts) | Low Low Low |
| TE67 (osteogenic sarcoma) | Low Low Low |
| A9 (melanoma) | Low Low Low |
| Nonhuman Rat adipocytes | High High High |
| Rat liver | High High High |
| Rat lymphocytes | Low Low Low |
| Rat H-35 hepatoma | High Low Low |
| Mouse liver | High High High |
| Mouse 3T3-L1 fibroblasts | High High Low |
| Mouse 3T3-L1 adipocytes | High High High |
| Rabbit superior cervical ganglia | Low Low Low |

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(αβ)2 insulin and IGF receptor structures possibly through a mechanism common for both receptor types. Little or no increase of the amount of labeling associated with the type II IGF receptor was observed upon fractionation of 3T3-L1 fibroblasts to adipocytes. Like rat and mouse liver membranes, H-35 rat hepatoma cells do not exhibit labeling of type I IGF receptors. The limited number of insulin receptors in H-35 hepatoma cells as determined by radioiodide binding studies (31) results in a low intensity of insulin receptor labeling in these cells (Table I). Interestingly, H-35 hepatoma cells present a large number of type II IGF receptors both on the cell surface and in isolated membrane preparations. Types I and II of IGF receptors and insulin receptor are found in variable proportions in A875 melanoma cells, TE85 sarcoma cells, IM-9 lymphocytes, and rat lymphocytes, as well as in membrane preparations from rabbit superior cervical ganglia (Table I).

The two types of membrane components affinity-labeled by 125I-IGF-I and 125I-IGF-II have the properties expected for physiologically relevant IGF receptors. They exhibit high affinity and specificity for IGF-I and IGF-II. They are present on the surface of intact target cells (Fig. 8). Yet, these two receptor types differ from each other in subunit composition, highest affinity ligand, and tissue distribution. The IGF binding kinetics observed in other laboratories for tissues containing different relative amounts of type I and type II IGF receptors is readily predicted from the results presented here. For example, the finding that IGF-II is more potent than IGF-I in displacing 125I-IGFs bound to rat liver membranes (11) is consistent with our demonstration that this tissue is devoid of the type I receptor. Also, IGF-I is more potent than IGF-II in displacing 125I-IGF-I bound to human skin fibroblast membranes, consistent with the presence of some type I receptor in those cells (11). High concentrations of insulin compete for the binding of 125I-IGFs to membranes from human skin fibroblasts (33) but not from rat liver (11, 34). This point agrees with our results showing that the type I growth factor receptor has some affinity for insulin but the type II receptor does not have any significant affinity for his hormone.

The use of affinity-labeling methodology to link 125I-IGF-I to components of BRL 3A2 rat liver cells and human placenta membranes has been recently reported (35, 36). These data are consistent with the results presented in this study on the type I growth factor receptor structure. The structural characteristics of the receptors for IGF-I, IGF-II, and insulin revealed by the present affinity-labeling methodology raise several basic questions concerning the evolution, genetic expression of the type I IGF receptor and the high affinity insulin receptor, as well as their apparent ability to undergo a similar unique proteolytic transformation, suggest that these two kinds of receptors may have similar amino acid sequences and may have evolved from a common ancestor molecule. This hypothesis would also imply that the genetic expression of the type I IGF receptor and the insulin receptor on a given cell type may involve specific rearrangements of common DNA sequences. The direct assessment of the presence of type I and type II IGF receptors on different cellular systems by the affinity-labeling methodology used in the present study may prove useful for the clarification of these issues.

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Fig. 8. Affinity labeling of insulin and IGF receptors on the cell surface of intact rat adipocytes and human skin fibroblasts. Rat adipocytes were isolated from epididymal fat pads by the collagenase method (13). Cultures of human skin fibroblasts were detached from the plates by incubation at 37 °C for 20 min with 1 mM EDTA, 138 mM NaCl, 1.2 mM KCl, 10 mM sodium phosphate, pH 7.4, followed by gentle scraping and homogenization to disperse cell aggregates. The isolated rat adipocytes and human skin fibroblasts were incubated with 2 nM 125I-insulin (INS), 2 nM 125I-IGF-I, or 2 nM 125I-IGF-II at 23 °C for 30 min in 1.0 ml of Krebs-Ringer phosphate buffer, pH 7.4, containing 4% bovine serum albumin. Cells were then incubated at 15 °C for 15 min in the presence of 0.5 mM disuccinimidyl suberate. The cross-linking reaction was stopped by addition of 4.0 ml of 0.25 M sucrose, 10 mM Tris/HCl, 1 mM ethylene glycol bis(β-aminoethoxy)ether)-N,N,N',N'-tetraacetic acid, pH 7.2, and Polytron homogenization of the cells. A crude membrane fraction was obtained from the homogenate by centrifugation at 30,000 × g for 20 min after separating most of the nuclei, mitochondria, and unbroken cells by centrifugation at 5,000 × g for 10 min. This membrane fraction was solubilized in the presence of 1% sodium dodecyl sulfate and subjected to electrophoresis on 5% polyacrylamide gels. The autoradiogram from a fixed, dried gel is shown.
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