Parallel Decreases in the Expression of Receptors for Insulin and Insulin-like Growth Factor I in a Mutant Human Fibroblast Line

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SUMMARY

The receptors for insulin and the insulin-like growth factor (IGF) I are two structurally homologous disulfide-linked multisubunit complexes of apparent Mr = 350,000. The similar subunit structures of these two types of receptors suggested that their genetic expression might be affected by common genetic defects. We have examined this possibility in an insulin-resistant, diabetic patient who exhibits decreased insulin binding activity. The receptors for IGF-I and insulin in skin fibroblasts from this patient were affinity labeled with ^{125}I-IGF-I and ^{125}I-insulin, respectively, and visualized by electrophoresis and autoradiography in polyacrylamide gels. Control fibroblasts exhibited the usual affinity labeling of the disulfide-linked Mr = 350,000 insulin and IGF-I receptor structures. The intensity of labeling of both receptor types in the patient's fibroblasts was less than in control fibroblasts. Binding data indicated that this decrease is due to a decreased receptor number with little or no decrease in affinity for the respective ligands. The high-affinity IGF-II receptor in fibroblasts affinity labeled with ^{125}I-IGF-II or ^{125}I-IGF-I consists of a single polypeptide not disulfide linked to any other membrane component. The molecular size and intensity of labeling of the IGF-II receptor in the patient's fibroblasts were unaltered when compared with those of controls. These observations suggest that a common genetic defect alters the expression of the homologous receptor structures for insulin and IGF-I. DIABETES *32*:541–544, June 1983.

The closely related peptides insulin and the insulin-like growth factors (IGF) I and II control cellular proliferation and metabolism apparently by interacting with specific cell surface receptors. The subunit structure of receptors for insulin, IGF-I, and IGF-II has been identified using receptor affinity-labeling methodology. The IGF-II receptor affinity labeled by crosslinking to ^{125}I-IGF-II consists of a single 250 kD polypeptide not covalently linked to any other membrane component.[1–3] This IGF-II receptor exhibits a higher affinity for IGF-II than for IGF-I, and no affinity for insulin.[1,2] The receptors for insulin and IGF-I are multisubunit complexes strikingly similar to each other in molecular size and subunit structure.[2,4,5] Thus, both receptor types consist of two α subunits of 125–140 kD and two β subunits of 90–98 kD, all disulfide linked in a proposed (β-S-S-α)-S-S-(α-S-S-β) subunit structure.[2,4,5] The β subunits in both receptor types have a site approximately in the middle of their amino acid sequence very susceptible to cleavage by a lysosomal protease and by elastase.[2,6] The disulfides linking the two (α-S-S-β) receptor halves can be dissociated by reduction with dithiothreitol under non-denaturing conditions, whereas the disulfides linking one α and one β subunit can be split by reductants only after denaturation of the receptor molecule with ionic detergents.[7,8] Naturally occurring antireceptor antibodies inhibit simultaneously the binding of insulin and IGF-I to IM-9 lymphocytes.[9] While showing an extensive structural homology, the receptors for insulin and IGF-I can be clearly distinguished from each other by their respective affinity for various ligands. Thus, the insulin receptor exhibits high affinity for insulin and low affinity for IGF-I and IGF-II,[2,3,10] whereas the IGF-I receptor has a higher affinity for IGF-I than for IGF-II, and low affinity for insulin.[2]

The intriguing homology between the structures of receptors for insulin and IGF-I suggests that these two receptor types may be the products of homologous genetic sequences, and their expression may be controlled by similar mechanisms and affected by similar defects. This hypothesis is supported by results in this report which show that a ge-

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netic disorder in a diabetic patient[10] results in an altered expression of receptors for insulin and IGF-I.

METHODS AND MATERIALS

Forearm skin fibroblasts from the patient (D.H.) and control subjects[11] were cultured in minimum essential medium, supplemented with 10% heat-inactivated fetal calf serum and 1 mg/L biotin, at 37°C in a humidified air/CO_2 95:5 atmosphere. Insulin binding studies were performed as previously described.[12] The methods for preparation of fibroblast membranes and affinity-labeling of membrane receptors for insulin, IGF-I, and IGF-II were described before.[2] Electrophoresis of affinity-labeled material was performed by the method of Laemmli.[13] Electrophoretic gels were stained for protein, dried, and subjected to autoradiography for 5 days using the conditions previously described.[6]

Porcine insulin (gift of Dr. R. Chance, Eli Lilly and Company, Indianapolis, Indiana) was labeled with ^{125}I by the immobilized lactoperoxidase method (Enzymobeads, Bio-Rad Labs., Richmond, California) to a specific activity of 80 Ci/g. Human IGF-I and IGF-II (gift of Dr. R. Humbel, Biochemisches Institut der Universitat, Zurich, Switzerland) were labeled with ^{125}I to a specific activity of 210 Ci/g by the chloramine method described previously.[14] Disuccinimidyl suberate was from Pierce Chemical Co. (Rockford, Illinois).

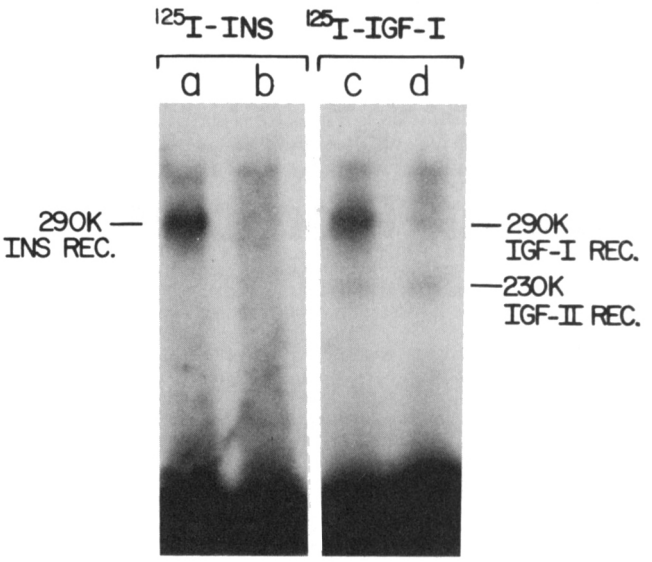

FIGURE 1. SDS-polyacrylamide gels of affinity-labeled receptors for insulin IGF-I and IGF-II. Membranes (150 μg of membrane protein) from control fibroblasts (a, c) and the patient's fibroblasts (b, d) were incubated in the presence of 5 nM ^{125}I-insulin or 5 nM ^{125}I-IGF-I for 90 min at 10°C, and then with 0.20 mM disuccinimidyl suberate for 15 min at 0°C. Affinity-labeled membranes were washed and collected by centrifugation at 30,000 × g for 20 min and electrophoresed on 5% polyacrylamide gels in the presence of 1% sodium dodecyl sulfate. Autoradiograms were obtained from the fixed, dried gels. Autoradiography was for 1 day (c, d) or 3 days (a, b). K is kilodalton.

RESULTS AND DISCUSSION

In a previous study, the binding of insulin to several cell types from an insulin-resistant diabetic patient (D.H.) was found to be deficient when compared with that of control cells.[11] Fibroblasts were established in monolayer cell culture from forearm skin biopsies from the patient.

The insulin receptors in control fibroblasts and in the fibroblasts from the patient were affinity labeled by crosslinking to receptor-bound ^{125}I-insulin with disuccinimidyl suberate[15] and analyzed by dodecyl sulfate-polyacrylamide gel electrophoresis and autoradiography (Figure 1). The unreduced insulin receptor affinity labeled in membranes from control fibroblasts appeared mainly as 290-kD labeled complexes (Figure 1, lane a) that correspond to the proposed $(\alpha\beta_1)_2$ insulin receptor form.[4,6] The insulin receptor affinity labeled in membranes from the patient's fibroblasts had a normal migration pattern in electrophoretic gels (Figure 1, lane b), but the amount of radioactivity associated with the affinity-labeled insulin receptor band corresponding to these cells was less than in controls on a per milligram of membrane protein basis.

Normal human skin fibroblasts contain IGF-I receptors structurally homologous to the insulin receptor, and IGF-II receptors that also bind IGF-I. Both receptor types can be efficiently affinity labeled by crosslinking to ^{125}I-IGF-I.[2] The possibility that the defect in insulin receptors observed in the patient's fibroblasts was associated with a similar defect in IGF-I receptors was examined. Lanes c and d in Figure 1 correspond to equivalent amounts of fibroblast membranes from a control subject and the patient, respectively, crosslinked to membrane-bound ^{125}I-IGF-I. Similar to the insulin receptors in these membrane preparations, the affinity-labeled IGF-I receptors migrate in the electrophoretic gels mostly as 290-kD $(\alpha\beta_1)_2$ labeled complexes. Importantly, the intensity of labeling of the IGF-I receptor bands in the patient's sample was much less than in the control sample (Figure 1). However, the 230-kD IGF-II receptor that also binds to, and can be affinity labeled with, ^{125}I-IGF-I (Figure 1, lanes c and d) or with ^{125}I-IGF-II (not shown) was not defective in the patient's fibroblasts when compared with normal fibroblasts.

The competition curve of insulin binding to fibroblasts (Figure 2) shows a decrease in binding capacity without a decrease in the apparent affinity of the insulin receptor in the patient's fibroblasts when compared with fibroblasts from three normal subjects. This defect observed in fibroblasts after several generations in cell culture is thought to reflect a genetic abnormality rather than the effect of environmental factors.[11] We addressed the question of whether the decrease in IGF-I binding to IGF-I receptors in the patient's fibroblasts was due to a defect in receptor number or in receptor affinity. The ability of unlabeled IGF-I to compete with ^{125}I-IGF-I for binding to, and affinity labeling of, IGF-I receptors was examined in fibroblast membranes from the patient and a normal subject. Membranes were crosslinked to membrane-bound ligands after incubation in the presence of ^{125}I-IGF-I and various concentrations of unlabeled IGF-I. The affinity-labeled membranes were electrophoresed on dodecyl sulfate-polyacrylamide gels in the presence of dithiothreitol, which dissociates the IGF-I receptor complex into its subunits, allowing a better quantitation of the receptor-associated radioactivity.[2] The densitometric analysis of autoradiograms prepared from the resulting gels indicated that IGF-I is equally potent in inhibiting the labeling of the IGF-I receptor in control fibroblasts and in the patient's fi-

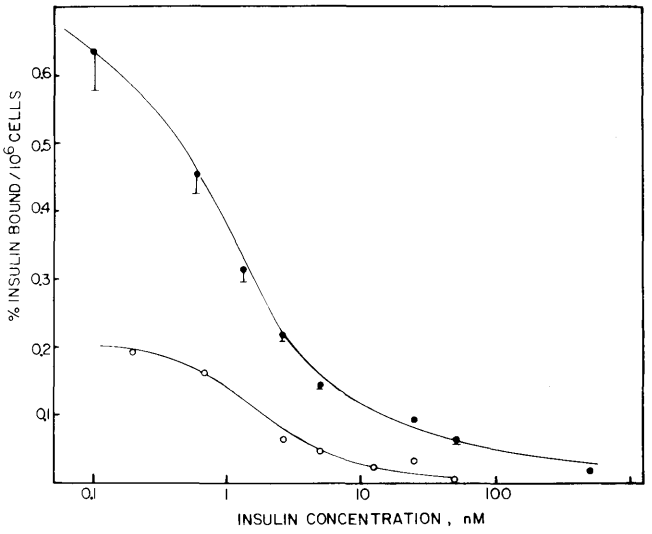

FIGURE 2. ^{125}I-insulin binding to fibroblasts from the patient (○) and three normal subjects (●). Confluent monolayers of cultured fibroblasts, between the sixth and fifteenth doublings, were incubated for 3 h at 16°C in the presence of 70 pM ^{125}I-insulin and in the presence or absence of increasing concentrations of unlabeled insulin. After washing out the unlabeled ligands, the radioactivity associated with the monolayers was determined with a gamma counter.[12] All data were corrected for nonspecific binding defined as the amount of cell-associated radioactivity measured in the presence of 2 μM unlabeled insulin. Data are expressed as percent of the input radioactivity specifically bound to fibroblasts.

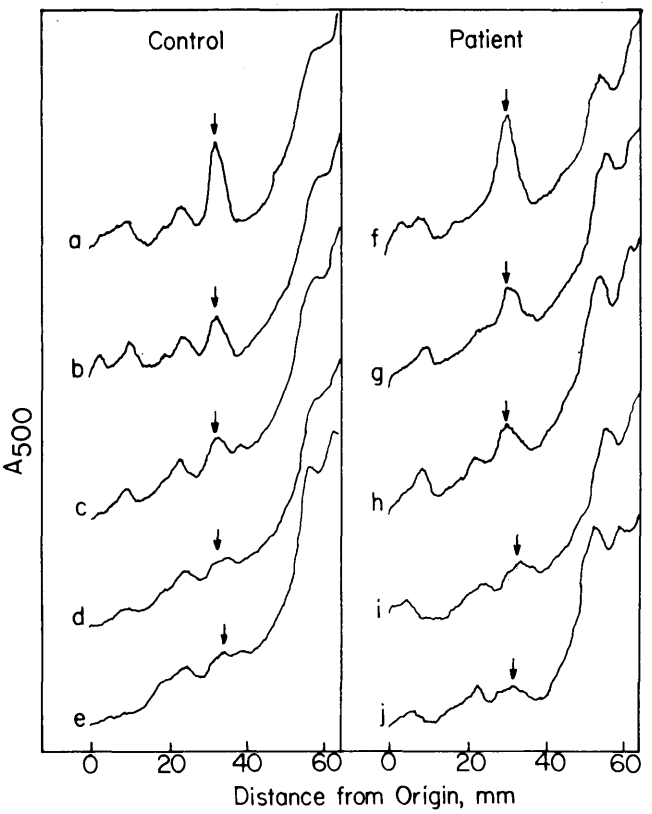

FIGURE 3. Competition of IGF-I with ^{125}I-IGF-I for binding to IGF-I receptors in skin fibroblast membranes. Fibroblast membranes from a control subject (125 μg of membrane protein) and from the patient (250 μg of membrane protein) were incubated for 90 min at 10°C in the presence of 1.0 nM ^{125}I-IGF-I and the following concentrations of unlabeled IGF-I: 0 nM (a, f), 3 nM (b, g), 10 nM (c, h), 30 nM (d, i), and 100 nM (e, j). Membranes were then incubated for 15 min at 0°C in the presence of 0.20 mM disuccinimidyl suberate. Affinity-labeled membranes were solubilized in the presence of 1% sodium dodecyl sulfate and 50 mM dithiothreitol and electrophoresed on 6% polyacrylamide gels. Autoradiograms were prepared from the resulting fixed, dried gels and analyzed by scanning densitometry at 500 nm. Shown are the densitometric profiles corresponding to each individual lane. Arrows point at the affinity-labeled 130-kD α subunit of the IGF-I receptor.

broblasts (Figure 3). Thus, in membranes from both types of fibroblasts the displacement of about 50% of the radioactivity associated with the α IGF-I receptor subunit was effected by 3 nM IGF-I (Figure 3). From these data we conclude that the number of IGF-I receptors rather than their affinity for the ligand is decreased in the fibroblasts of this patient.

These data indicate that the patient described in this report is defective in the level of expression of $(\alpha\beta)_2$ receptor structures for insulin and IGF-I. This situation is in contrast with the decreased affinity of insulin and IGF-I receptors found in fibroblasts of a patient with leprechaunism,[16] which is another example of a parallel defect in these two receptor structures. The two highly homologous insulin and IGF-I receptor structures may be the products of two different genetic sequences generated either by a duplication process or by rearrangement of sequences that code for constant domains and variable regions of these receptors in analogy to the genetic mechanism involved in the expression of immunoglobulin molecules. Alternatively, the receptors for insulin and IGF-I may be products of post-translational modification of the same polypeptide derived from a single genetic sequence. Any of these three alternative processes or their regulatory mechanisms is susceptible to specific pre- or post-translational defects that would affect simultaneously the level of expression of the $(\alpha\beta)_2$ receptor structures for insulin and IGF-I in the patient studied here.

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