Defective Processing of Insulin-Receptor Precursor in Cultured Lymphocytes from a Patient with Extreme Insulin Resistance

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Abstract

We have studied a patient with extreme insulin resistance, acanthosis nigricans, and decreased erythrocyte insulin binding. EBV-transformed lymphocytes from this patient exhibited markedly reduced binding of ^{125}I-insulin. Radioiodination of cell surface receptors followed by immunoprecipitation with anti-receptor antibodies revealed the presence of increased amounts of a 210-kD protein but no detectable α or β subunits. Continuous labeling with 2-[^{3}H]mannose revealed the synthesis of a 190-kD precursor and a 210-kD protein. The 210-kD protein was phosphorylated in an insulin-dependent manner at high insulin concentrations. These results suggest that in this patient the biosynthesis of 190-kD receptor precursor, its terminal glycosylation, and intracellular transport to the cell surface proceed normally, while proteolytic maturation to α and β subunits does not occur. We postulate that this defect either results from mutation(s) within the insulin-receptor gene, which render the precursor resistant to cleavage, or from a defect in the receptor processing enzyme.

Introduction

Severe insulin-resistant diabetes is a characteristic feature of some genetic syndromes that include type A extreme insulin resistance, leprechaunism, Rabson-Mendenhall syndrome, and some forms of lipoatrophic diabetes (1–7). The insulin resistance is thought to be caused by primary defects in the insulin receptor as has been shown by studies dealing with cultured fibroblasts and/or EBV-transformed lymphocytes established from the patient's peripheral blood cells. However, there seems to be considerable heterogeneity with respect to the nature of the underlying abnormalities that occur in these syndromes (8–10).

From pulse-chase studies of the biosynthesis of the insulin receptor, Hedo et al. have provided evidence for at least two different types of defect (8). One form is associated with the absence of the receptor and its precursor, which indicates possible defects at the transcriptional or translational level. The other form appears to result from altered posttranslational processing of receptor protein that interferes with its targeting or function. These suppositions have been supported by recent measurements by Northern blot analysis of the amounts of receptor mRNA that appears in transformed lymphocytes, taken from a series of such patients (11).

In this report we describe a woman from a consanguineous marriage whose clinical manifestations were compatible with the diagnosis of type A extreme insulin resistance (12). We have demonstrated that the insulin-receptor precursor is synthesized normally in this patient but has not undergone proteolytic maturation to mature α or β subunits on the cell surface.

Methods

Patient. The patient was a 23-yr-old Japanese woman who was the product of a marriage between first cousins. She was born after a full-term, uncomplicated delivery; birth weight was 1,750 g. At birth she was noted to have increased body hair. At the age of six, diabetes mellitus was diagnosed, and the patient was noted to have acanthosis nigricans, a disturbance of growth, and impaired intelligence. At the age of 23, the patient was referred to the Kyushu University Hospital. On physical examination she was only 133 cm tall and weighed 36 kg and had acanthosis nigricans on the neck and both axillae, hirsutism, and megadontia accompanied the short stature. The external genitalia were normal. An ultrasonogram revealed bilateral polycystic ovaries. Menses had not yet begun. Brain computed tomography scan showed no abnormality. A glucose tolerance test revealed that plasma glucose levels were 130 mg/dl at fasting and 329 mg/dl at 120 min. The serum insulin concentration was increased from 242 μU/ml at fasting to 1,421 μU/ml at 120 min. An intravenous insulin injection (0.1 U/kg) by bolus showed no significant decrease in plasma glucose. Serum testosterone was 2.9 ng/ml (normal, 0.66–1.95 ng/ml). Neither anti-insulin nor anti-insulin-receptor antibodies were detectable in serum. Insulin binding to erythrocytes was markedly decreased due to the reduction in receptor number. She was the second child and her elder sister had similar clinical manifestations.

Cells. Lymphoblastoid cell lines were established by infecting cells with EBV (4). Cells were grown in continuous culture in RPMI 1640 medium containing 10% fetal bovine serum. ^{125}I-insulin binding to intact cells was measured as described elsewhere (3).

Partial purification of insulin receptors. Cultured lymphocytes were solubilized in 50 mM Tris-HCl buffer, pH 7.6, containing 1% Triton X-100 and 1 mM PMSF at 4°C for 60 min. After removing insoluble material by centrifugation, the supernatant was partially purified with wheat germ agglutinin (WGA)[1]-agarose (13). ^{125}I-insulin binding to

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1. *Abbreviations used in this paper:* WGA, wheat germ agglutinin.

WGA-purified receptors was carried out according to the method of Hedo et al. (14). Autophosphorylation of WGA-purified receptors was performed by a modification of Grunberger's method (15). After termination of phosphorylation receptors were immunoprecipitated with an anti-insulin-receptor antibody B (16) obtained from a patient with type B extreme insulin resistance and analyzed by SDS-PAGE in 7.5% resolving gel.

Cell surface iodination. Cell surface proteins were iodinated using $Na^{125}I$, glucose oxidase, and lactoperoxidase (17). The labeled receptors were solubilized in Triton X-100, immunoprecipitated, and analyzed by SDS-PAGE.

Biosynthetic labeling. Receptor biosynthesis was studied with the continuous labeling technique of Hedo et al. (18), using 2-[^{3}H]-mannose to label the carbohydrate moiety of the receptor.

Results and Discussion

^{125}I-insulin binding to intact cultured lymphocytes from the patient was remarkably decreased, indicating a primary receptor disorder, presumably of genetic origin (Fig. 1 *A*). When the insulin receptor was partially purified by WGA-agarose chromatography after solubilizing the cells, insulin binding was also markedly reduced (Fig. 1 *B*). To examine whether this markedly lowered insulin binding might be due to a decrease in the number of insulin receptors on the cell surface, we performed surface labeling using $Na^{125}I$ and lactoperoxidase, and then immunoprecipitated the receptor with an insulin-receptor autoantibody. As shown in Fig. 2 *A*, α or β subunits of the insulin receptors from this patient were not labeled as were those of a normal subject. Furthermore, a significant increase in the amount of receptor-related protein with an M_r of 210 kD was found. The radioactivity incorporated into this band in the patient was about twice that seen in the normal subject. However, the total amount of radioactivity immunoprecipitated by an anti-insulin-receptor antibody appeared to be decreased in the patient. According to the studies of Hedo et al. concerning insulin receptor biosynthesis, a single polypeptide chain proreceptor with an M_r of 190 kD appears first and then undergoes terminal glycosylation and proteolytic cleavage as it is transported to the surface, which yields the mature α and β subunits on the plasma membrane (18). They also described an additional protein band with an M_r of 210 kD, which was suggested to be a fully glycosylated but uncleaved form of the precursor on the cell surface because this component was labeled by surface iodination and the tryptic fragments of this protein contained peptides from both subunits. The antibody that we used cross-reacted with the 210-kD molecule from the patient, so it is unlikely that the antibody would not see the 135- and 95-kD subunits from the patient. In addition, we obtained the same results of surface labeling using another anti-insulin-receptor antibody B-9, which was kindly provided by Dr. C. R. Kahn (data not shown).

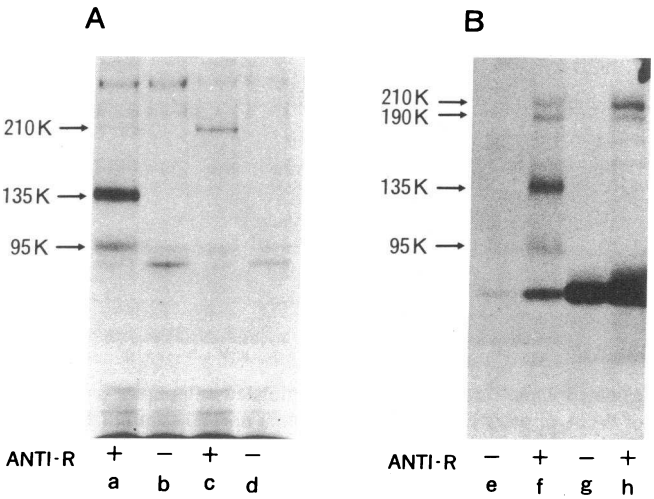

Figure 2. Cell surface labeling (*A*) and biosynthetic labeling (*B*) of insulin receptors in cultured lymphocytes. (*A*) Cultured cells from a normal subject (lanes *a* and *b*) or the patient (lanes *c* and *d*) were iodinated using $Na^{125}I$, glucose oxidase, and lactoperoxidase. Labeled insulin receptors were solubilized, immunoprecipitated with anti-receptor antiserum (lanes *a* and *c*) or normal serum (lanes *b* and *d*), and analyzed by SDS-PAGE followed by autoradiography. (*B*) Cultured cells from a normal subject (lanes *e* and *f*) or from the patient (lanes *g* and *h*) were labeled with 1 mCi of 2-[^{3}H]mannose (1 μCi/10^5 cells), and were incubated at 37°C for 12 h. Thereafter, labeled insulin receptors were solubilized, immunoprecipitated with normal serum (lanes *e* and *g*) or anti-receptor antiserum (lanes *f* and *h*), and analyzed by SDS-PAGE in 7.5% gel followed by autoradiography.

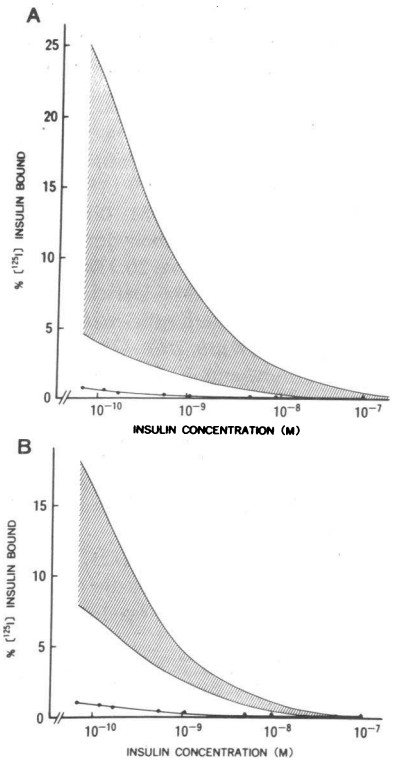

Figure 1. Binding competition curves of intact cultured lymphocytes (*A*) and WGA-purified insulin-receptor preparations (*B*) from the patient. Cultured lymphocytes (10^7 cells/ml) or WGA-purified insulin receptors (5 μg/tube) were incubated with ^{125}I-insulin (6.7×10^{-11} M) and unlabeled insulin (0–1.7×10^{-6} M). The shaded area represents the range of insulin binding in normal subjects. ●, the patient.

To examine the processing of insulin receptors in the patient's cells, biosynthetic labeling by 2-[^{3}H]mannose was carried out (Fig. 2 *B*). In normal cells two protein bands with M_r values of 190 and 210 kD, in addition to the α and β subunits, were detected. On the other hand, α and β subunits were not detected in the patient's cells, while the amount of the 190-kD precursor was comparable to that of normal cells. Furthermore, the band at M_r = 210 kD was also increased, in agreement with the results of the surface iodination studies. These findings suggested that the precursor protein was not undergoing proteolytic cleavage after its synthesis and that, consequently, the uncleaved form of the receptor was increased. As demonstrated in surface labeling and biosynthetic labeling, the

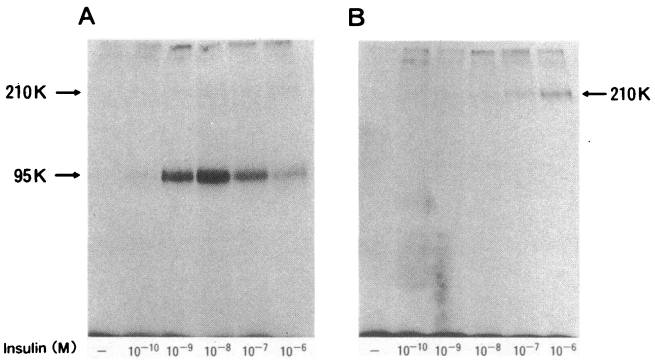

Figure 3. Dose-response of insulin-stimulated autophosphorylation of WGA-purified insulin receptors from a normal control (*A*) or from the patient (*B*). The samples were preincubated with various concentrations of insulin at 4°C overnight. Phosphorylation reactions were studied at 22°C by the addition of γ-[^{32}P]ATP and 3 mM $MnCl_2$ for 15 min. Thereafter, the samples were immunoprecipitated with anti-insulin-receptor antibody and analyzed by SDS-PAGE in 7.5% gel followed by autoradiography.

total amount of radioactivity of receptors (in all forms) appeared to be decreased in this patient. One possibility that would explain this finding is a difference in the degradation rate between the 210-kD protein and mature receptors.

Fig. 3 demonstrates the pattern of autophosphorylation of insulin receptors solubilized from either normal or patient's cells. In the receptor preparation from normal cells, the autophosphorylated bands corresponding to β subunits were easily demonstrated while in the patient's cells the protein of M_r = 210 kD was clearly phosphorylated in an insulin-dependent manner. A higher concentration of insulin was required to give a maximal response. These findings suggest that the uncleaved form of the receptor still retains some enzymatic activity, but has decreased affinity for insulin. However, because the insulin binding was too low, it is difficult to calculate the *Kd* of insulin binding by Scatchard analysis.

On the basis of these results we can propose three possible causes of the receptor defect in this patient: (*a*) a mutation within the cleavage site of the insulin receptor, (*b*) a mutation at a different site within the precursor that causes conformational changes making the receptor precursor resistant to cleavage, as was recently reported for the proinsulin molecule in a family with hyperproinsulinemia (19), and (*c*) a defect in the cellular converting enzyme that catalyzes this proteolytic cleavage event.

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