Rapid Publications

Insulin Resistance by Uncleaved Insulin Proreceptor

Emergence of Binding Site by Trypsin

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Two sisters presented with severe insulin resistance and markedly decreased insulin binding to erythrocytes, cultured fibroblasts, and transformed lymphocytes. The dose-response curve of insulin-stimulated amino acid uptake in the fibroblasts was shifted to the right. The molecular weight of the insulin receptor on the transformed lymphocytes from the patients was 210,000 and could not be dissociated to α- and β-subunits by dithiothreitol treatment. However, the proreceptor was cleaved by trypsin, and this led to production of a 135,000-*M*, α-subunit. Insulin binding to the trypsin-treated cells increased to the normal level, and insulin action was normalized. These results suggest that the failure of proreceptor cleavage produces hormone-resistant states and that a proreceptor syndrome may be a unique disease entity for hormone resistance. *Diabetes* 37:653–56, 1988

Polypeptide hormones and hormone receptors are generally produced from their precursors by cleavage in their appropriate sites (1–3). Failure to cleave these prohormones leads to secretion of prohormones with much decreased biological activity resulting in the disease states (4,5). A similar phenomenon may occur in hormone receptors, producing hormone-resistant states by the presence of proreceptor in the plasma membranes with decreased affinity to hormones. We describe a family with insulin proreceptors showing severe insulin resistance. Proreceptor cleavage by trypsin led to the emergence of insulin binding sites and normalization of insulin action.

MATERIALS AND METHODS

Patients. Two sisters, 27 (J.T.) and 23 (Y.T.) yr old, the products of a consanguineous marriage, were found to be growth retarded in their infancy and had been diabetic since the ages of 10 and 6 yr, respectively. They also presented with primary amenorrhea, hirsutism, and acanthosis nigricans. Because they had severe hyperinsulinemia (2010–2240 pM at fasting and 6500–8600 pM after oral glucose loading; normal 22–108 and 144–502 pM, respectively), and exogenous insulin (0.1 μU/kg) lowered blood glucose only to 85% of the original level (normal <50%), the patients had severe insulin resistance. Fasting blood glucose was 7.0–12.0 mM for J.T. and 4.6–7.2 mM for Y.T.

Insulin-binding studies. Insulin binding to erythrocytes, transformed lymphocytes, and cultured fibroblasts was previously described (6,7). Binding studies were carried out at 15°C for 3.5 h for erythrocytes and transformed lymphocytes and 4°C for 16 h for cultured fibroblasts. The binding to cultured fibroblasts was performed in monolayers.

α-Aminoisobutyric acid (AIB)-uptake studies. AIB uptake was measured by the method of Knight et al. (8). Fibroblasts were preincubated with insulin at the indicated concentrations for 3 h, and then [^{3}H]AIB (New England Nuclear, Boston, MA) and unlabeled AIB (final concn 8 μM) were added. AIB uptake was terminated by washing rapidly with ice-cold buffer.

Surface labeling. The cells were iodinated with 50–100 μCi/ml Na^{125}I (New England Nuclear) by 25 μg/ml lactoperoxidase (Sigma, St. Louis, MO). After solubilization and immunoprecipitation with anti-insulin-receptor antibody plus pansorbin (Calbiochem-Behring, La Jolla, CA), the sample was treated with Laemmli buffer containing 100 mM dithiothreitol (DTT; Sigma) and was applied to sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) (9). Anti-insulin-receptor antibody was obtained from a patient with type B insulin-resistance syndrome associated with Sjögren syndrome.

Cross-linking studies. The cells were incubated with ^{125}I-labeled insulin for 16 h at 4°C and cross-linked with 1 mM disuccinimidyl suberate (Pierce, Rockford, IL). The cells were then solubilized and precipitated with anti-insulin-receptor antibody, and the steps that followed were similar to those of the surface-labeling study.

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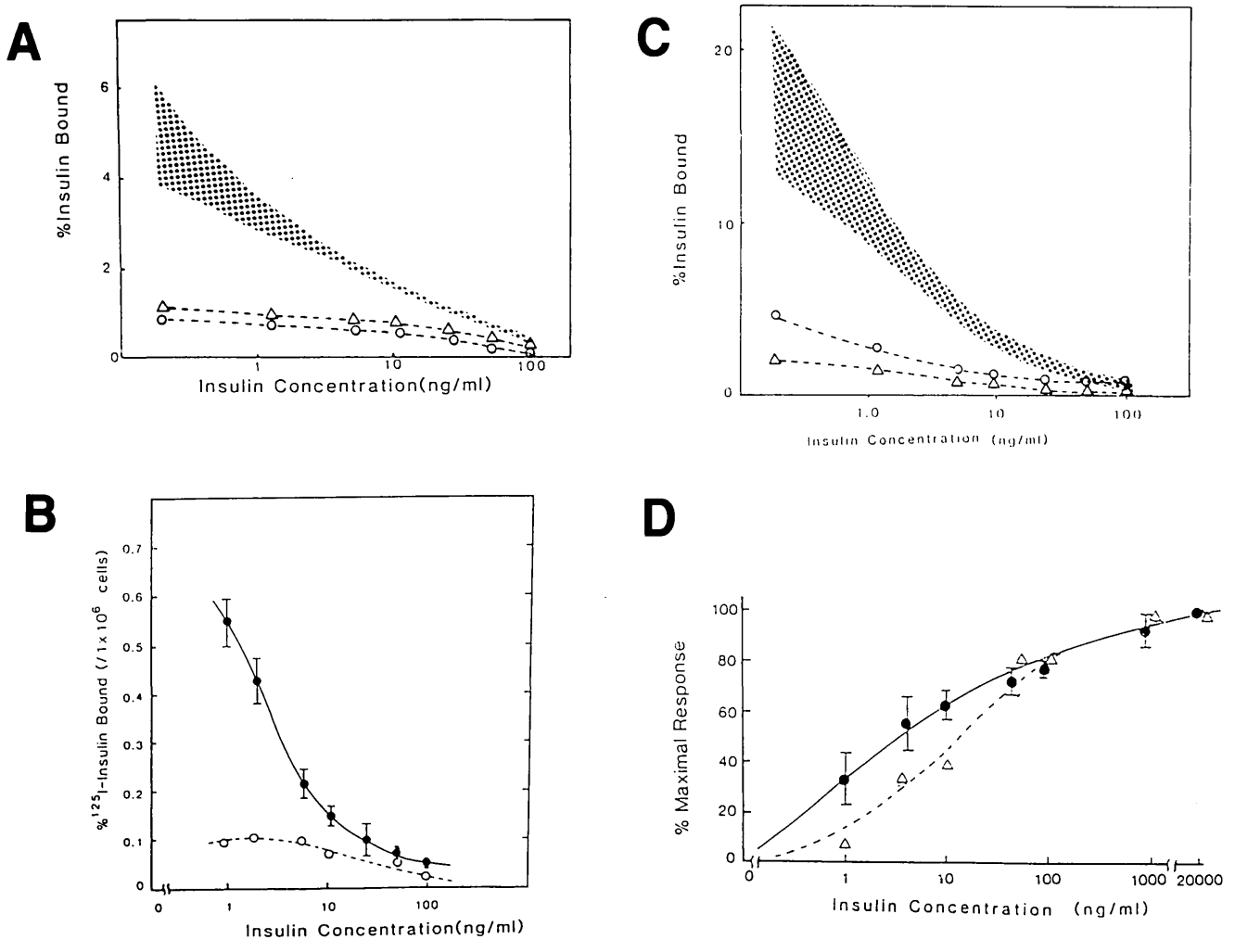

FIG. 1. Insulin binding and insulin action in cells. *A*: insulin binding to erythrocytes from patients Y.T. (△) and J.T. (○) and normal controls (*shaded areas*). Results are expressed in terms of binding of 2.4 × 10^9 cells/ml. *B*: insulin binding to cultured fibroblasts. ^{125}I-insulin binding to cultured fibroblasts from Y.T. (○) and control subjects (●) was carried out in monolayers after 16 h incubation at 4°C. Results are expressed as percentage of ^{125}I-insulin bound per 1.0 × 10^6 cells. *C*: insulin binding to transformed lymphocytes from J.T. (○), Y.T. (△), and controls (*shaded area*). Results are expressed in terms of binding of 5 × 10^6 cells/ml. *D*: α-aminoisobutyric acid–uptake studies in fibroblasts from Y.T. (△) and control subjects (●). Maximal insulin stimulation was comparable in 2 cell lines. Conversion factor for insulin concentration from nanograms per milliliter to SI units is 0.167 nM.

RESULTS

Insulin binding to erythrocytes and cultured fibroblasts was markedly decreased as shown in Fig. 1. Furthermore, the dose-response curve of insulin stimulation in amino acid uptake was shifted to the right because of the decreased insulin binding (Fig. 1). Decreased insulin binding together with acanthosis nigricans led to the diagnosis of a primary insulin-receptor defect, i.e., type A insulin-resistance syndrome (10). To further characterize insulin receptors in these subjects, we transformed lymphocytes with Epstein-Barr virus and labeled surface proteins on the transformed lymphocytes with ^{125}I. The labeled material was immunoprecipitated with anti-insulin-receptor antibody and was applied to SDS-PAGE after treatment with DTT. The result was a large-molecular-weight (210,000-M_r) protein for the patient in contrast to the 135,000-M_r α-subunit for the normal control (Fig. 2). The large-molecular-weight protein recognized by anti-insulin-receptor antibody was assumed to be an insulin proreceptor, and the production of a proreceptor by uncleavage at the Arg-Lys-Arg-Arg region between two subunits was postulated.

Treatment of transformed lymphocytes with trypsin at 0.025% concentration and subsequent cross-linking with ^{125}I-insulin showed conversion of the large-molecular-weight proreceptor to a 135,000-M_r α-subunit (Fig. 2). However trypsin, at >0.025% concentration, further digested the α-subunit in both the control subject and the patient. In accordance with appearance of a normal α-subunit, insulin binding to transformed lymphocytes after treatment with trypsin at 0.025% became normal, i.e., a 7.5-fold increase in binding (Fig. 3); however, in other concentrations of trypsin, either >0.025 or <0.025%, insulin binding was low. Similar results were demonstrated in cultured fibroblasts from these patients. Furthermore, the dose-response curve of insulin-stimulated amino acid uptake in the fibroblasts was normalized from ED_{50} 2.7 to 0.7 nM. Thus, conversion of proreceptor into an α-subunit led to emergence of insulin binding sites and normalization of insulin action. Other enzymes, including chymotrypsin and pepsin, failed to produce an α-subunit or to increase insulin binding. Recently, prohormone-processing enzymes have been characterized and purified (11). The enzymes cleave hormone precursors at pairs of basic res-

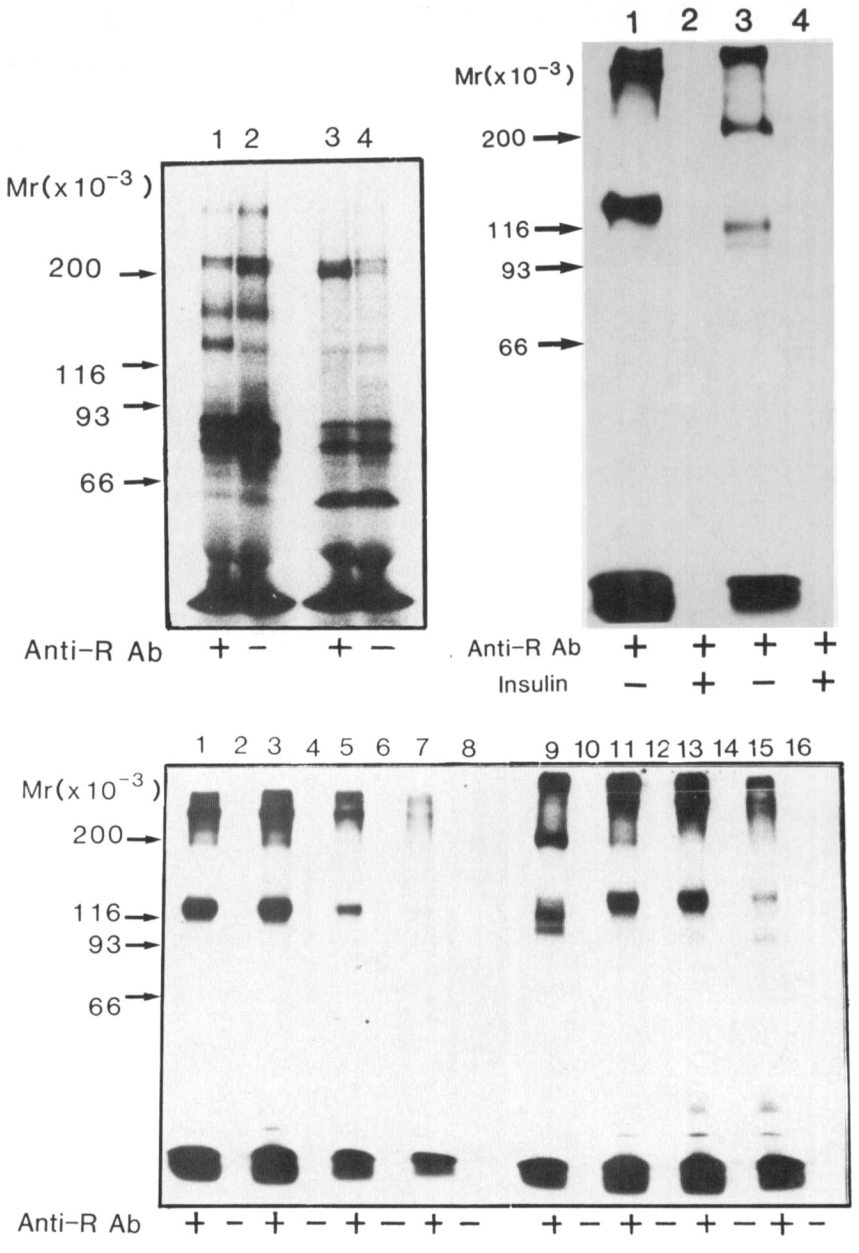

FIG. 2. Pattern of sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE). *Left*: surface labeling of transformed lymphocytes with $Na^{125}I$. After immunoprecipitation with (+) or without (−) anti-insulin–receptor antibody (Anti-R Ab) plus pansorbin, sample was treated with Laemmli buffer containing 100 mM dithiothreitol and was applied to SDS-PAGE (19). *Lanes 1* and *2*, normal controls; *lanes 3* and *4*, patient Y.T. *Right*: insulin-receptor cross-linking study. *Lanes 1* and *2*, normal controls; *lanes 3* and *4*, patient Y.T. In *lanes 2* and *4*, 3 μM insulin was present when cells were incubated with ^{125}I-labeled insulin. *Bottom*: insulin-receptor cross-linking study after trypsin treatment. Transformed lymphocytes from control subject (*lanes 1–8*) and lymphocytes from patient (Y.T.; *lanes 9–16*) were treated with trypsin for 5 min at 25°C at 0 concentration (*lanes 1,2,9,10*), 0.5×10^{-2}% (wt/wt) (*lanes 3,4,11,12*), 2.5×10^{-2}% (*lanes 5,6,13,14*), and 25×10^{-2}% (*lanes 7,8,15,16*).

idues (Lys-Arg) to produce an active hormone (pheromone, called α-factor). We used these prohormone-processing enzymes to cleave the basic residues of proreceptor and found that they failed to increase insulin binding, indicating that insulin proreceptors did not appear to be substrates of these enzymes.

DISCUSSION

Most of the peptide hormones and neurotransmitters in eukaryotic cells are excised from their precursors by processing enzymes at pairs of basic residues (12). However, the nature of these processing enzymes remains elusive. One of the better-studied examples of such a system is proteolytic processing of precursors of α-factor pheromone in the yeast *Saccharomyces cervisiae*. The mutants of the yeast (Kex 2) produce biologically inactive precursors of α-factor, which is due to a deficiency of the endopeptidase cleaving at pairs of basic residues (13). Familial hyperproinsulinemia (4,14,15) and proalbuminemia (16,17) are also disease states in humans showing uncleaved propeptides (proinsulin or proalbumin) in the circulation, and the defects were localized to the conversion site. Peptide hormone receptors are also excised from their precursors (3,18), and the uncleavage can produce proreceptors with decreased affinity to cause decreased signal transmission.

Our case is the first to show such a phenomenon. The causes for this uncleavage remain to be clarified and may include *1*) deletion or mutation at the cleavage site, e.g., the cases in hyperproinsulinemia syndrome (14,15) and familial proalbuminemia (16,17) or *2*) defects in processing enzymes for the conversion, e.g., a case in the mutant yeast Kex 2 (13). Cloning of the insulin-receptor gene of the patients and clarification of its sequence is under way to prove these possibilities.

NOTE ADDED IN PROOF

After submitting this article for publication, we were successful in sequencing cDNA of the interconnecting region and found an AGG (Arg) to AG*T* (Ser) point mutation, re-

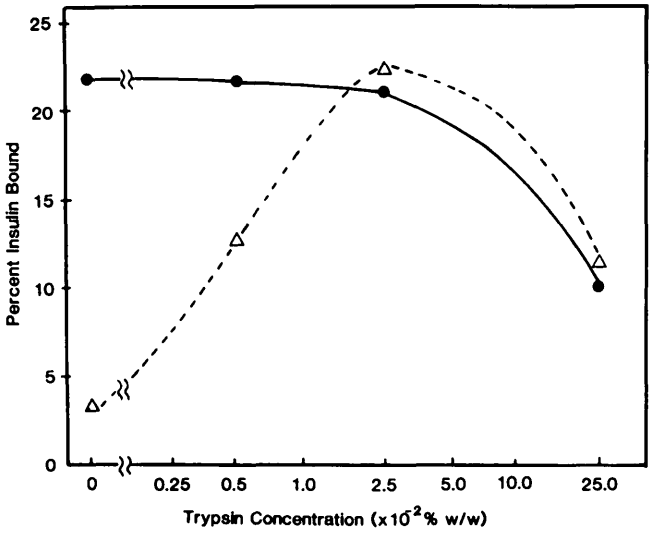

FIG. 3. Insulin binding to trypsin-treated transformed lymphocytes. Transformed lymphocytes from control subject (●) and patient Y.T. (△) were treated with trypsin at indicated concentration for 5 min at 25°C, and binding studies were performed. Results are expressed in terms of binding of 5 × 10^6 cells/ml.

sulting in the structural change of the cleavage site from Arg-Lys-Arg-*Arg* to Arg-Lys-Arg-*Ser*.

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