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Altered expression of insulin receptor types A and B in the skeletal muscle of non-insulin-dependent diabetes mellitus patients

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ABSTRACT **The human insulin receptor exists in two isoforms, HIR-A and HIR-B, which are generated by alternative splicing of a primary gene transcript and differ by a 12-amino acid insertion sequence in the α-subunit. The two receptor isoforms bind insulin with different affinities and are differentially expressed in human tissues. We report here a tissue-specific alteration of the insulin receptor RNA splice pattern in non-insulin-dependent diabetes mellitus (NIDDM) patients. Whereas skeletal muscle of healthy individuals contains exclusively high-affinity HIR-A-encoding RNA, we consistently find low-affinity HIR-B RNA expression in NIDDM muscle tissue at levels similar to HIR-A.**

Non-insulin-dependent diabetes mellitus (NIDDM) is characterized by abnormalities in insulin secretion and impaired insulin action in target tissues (1–6). The pathogenesis of NIDDM has been studied in various ethnic groups. It appears that insulin resistance can precede the clinical onset of NIDDM. Indeed, there is increasing evidence that an insulin resistance of the nonoxidative glucose disposal in the skeletal muscle is an early event in the development of the disease‡. The molecular basis for this insulin resistance is not understood; however, an alteration of insulin signal transmission at the level of the insulin receptor could contribute to an insulin-resistant state.

Insulin exerts its biological effects by binding to a transmembrane receptor consisting of disulfide-linked α- and β-subunits that are generated by proteolytic cleavage of a common precursor (8). cDNA cloning demonstrated the existence of two insulin receptor mRNAs, generated by differential splicing of a single gene transcript and differing by 36 nucleotides in the distal part of the α-subunit (9–13). Recently it was shown that the two different receptors, termed HIR-A and HIR-B (14), exhibit distinct binding characteristics for insulin and that the ratio of the two insulin receptor mRNAs varied in a tissue-specific manner (11–13). These data suggested the possibility that insulin responsiveness could be regulated in a cell-type-specific manner by alternative splicing.

In this paper we present an analysis of the expression pattern of the two insulin receptor forms in insulin target tissues of patients with NIDDM in comparison to healthy individuals. Using polymerase chain reaction (PCR) technology we were able to demonstrate an altered expression pattern of alternatively spliced insulin receptor mRNA forms in the skeletal muscle of diabetic individuals. In contrast, the ratio of HIR-A and HIR-B RNAs was unaltered in blood cells obtained from NIDDM patients. Our findings suggest a role for the differential and cell-specific expression of functionally different insulin receptors in the pathology of NIDDM.

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MATERIAL AND METHODS

Tissue Samples. Muscle tissue samples (musculus gastrocnemius) were obtained from diabetic ($n = 10$) and nondiabetic ($n = 6$) 60- to 80-year-old patients after leg amputation due to peripheral arteriovascular complications. Informed consent was obtained from all patients. All samples were taken immediately after operation, cut into small pieces of 0.3 g, frozen in liquid nitrogen, and stored at −80°C.

The muscle samples were processed for analysis after quality control, which included the following criteria: (*i*) light microscopic, electron microscopic, and histochemical quality of the tissue; (*ii*) enzymatic activity of lactate dehydrogenase (EC 1.1.1.27), phosphofructokinase (EC 2.7.1.11), phosphoglycerate kinase (EC 2.7.2.3), and phosphoglucomutase (EC 2.7.5.1); and (*iii*) noncollagen protein in the normal range for the muscle tissue. The methodology and the results of the morphological and enzymatic characterization have been reported elsewhere (15) together with a characterization of the insulin receptor kinase in these samples and the clinical characteristics of the patients.

Blood samples (5 ml) were taken from five NIDDM patients and five controls in the morning after a 12-hr fasting period, mixed with EDTA to prevent clotting, and immediately frozen in liquid nitrogen.

RNA Preparation. One gram of muscle tissue was pulverized in liquid nitrogen, dissolved in 1 ml of a 4 M guanidine thiocyanate solution, and subsequently homogenized. RNA was harvested after centrifugation through a CsCl cussion as described (16). Five milliliters of blood was diluted with a 2× guanidine thiocyanate solution to final concentration, homogenized, and precipitated with LiCl as described (17).

cDNA Synthesis and PCR. Five to 10 μg of total RNA was used for cDNA synthesis. The cDNA was specifically primed with an oligonucleotide spanning nucleotides 2858–2879 of the human insulin receptor A sequence (9). First strand cDNA synthesis was performed essentially as described (18) in a total volume of 20 μl of 50 mM KCl/10 mM Tris·HCl, pH 8.3/4 mM $MgCl_2$/1 mM dNTPs/10 μg of bovine serum albumin per ml/50 pmol of primer. The reaction product was directly subjected to 40 cycles of PCR amplification without further purification. PCR was performed as described earlier (13) using oligonucleotides flanking the 12-amino acid insertion site. The oligonucleotides used represented nucleotides 2136–2257 and 2327–2348 of the human insulin receptor sequence (9) and gave rise to specific fragments of 112 (HIR-A) and 148 (HIR-B) base pairs (bp), respectively. PCR products were analyzed after electrophoresis in 5% polyacrylamide gels.

Abbreviation: NIDDM, non-insulin-dependent diabetes mellitus.

‡Del Prato, S., DeFronzo, R. A., Bonora, E., Gulli, G., Solini, A. & Bonadonna, R. C., Fourth International Symposium on Insulin Receptor and Insulin Action, Sept. 4–7, 1990, Verona, Italy, pp. 60–61.

RESULTS

It has recently been shown that RNAs encoding the A and B forms of the insulin receptor are expressed in a tissue-specific manner (11–13). Importantly, HIR-A and HIR-B were shown to differ in their binding affinity for insulin (13), which suggested that alternative splicing of a primary insulin receptor gene transcript could be involved in tissue-specific modulation of insulin responsiveness. To investigate whether this mechanism could play a role in diabetes we determined the expression of HIR-A and HIR-B mRNA variants in tissues of patients with NIDDM and in nondiabetic controls. To study whether the ratio of HIR-A and HIR-B insulin receptor isoforms was altered in patients with NIDDM, we prepared RNA from whole blood and skeletal muscle tissue samples from normal individuals and patients suffering from NIDDM (Table 1). The healthy and diabetic groups had a comparable age distribution and body mass index. As reported earlier (15), no significant differences were observed between tissues from nondiabetic controls and NIDDM patients, based on the criteria described in *Material and Methods*. Using these criteria we concluded that the tissue samples used were of comparable quality. In all cases, the tissue was frozen in liquid nitrogen within minutes after surgery.

The ratio of HIR-A- and HIR-B-encoding RNA was determined by PCR amplification analysis, using oligonucleotide primers from locations flanking the 12-amino acid insertion that distinguishes the two insulin receptor isoforms. The electrophoretic separation of the end products of the PCR reaction yielded fragments comigrating with controls in which cloned HIR-A and HIR-B cDNAs were employed (Figs. 1 and 2). Analysis of blood samples from NIDDM patients and controls revealed exclusive expression of HIR-A receptor transcripts in both groups (Fig. 1). A strikingly different picture emerged when we analyzed RNA from different skeletal muscle samples. As expected from previous work, analysis of the PCR end products by electrophoresis showed that skeletal muscle samples from nondiabetic controls expressed only HIR-A sequences, as demonstrated by the exclusive detection of a 112-bp fragment (Fig. 2). This was consistent within the control group with the exception of one sample, where traces (2–5%) of the 148-bp band representing HIR-B were found. In contrast to the controls, all NIDDM samples showed amplification of cDNA sequences derived from RNAs of both receptor variants. The amount of the HIR-B receptor form varied between 50% and 100% of the corresponding HIR-A receptor form. As reported earlier (13), some additional bands appeared; however, Southern blotting and S1 analysis identified these bands as nonspecific background or single-stranded by-products of the PCR reaction (not shown).

DISCUSSION

Abnormal insulin receptor function has been reported in rare syndromes of extreme insulin resistance (19–27) as well as in NIDDM (28–31). Although in some recently reported cases receptor dysfunction in syndromes of extreme insulin resistance could be attributed to mutations in the insulin receptor gene (19, 20, 24, 32), the cause of the altered receptor kinase activity in NIDDM is thus far unclear. In a study of isolated adipocytes from Pima Indians, which show abnormal insulin receptor autophosphorylation, it was demonstrated that the primary insulin receptor gene sequence was unaltered (7). This finding suggested acquired consequences of the diabetic milieu as a reason for the receptor defect. Similarly, the insulin receptor kinase from the skeletal muscle of NIDDM patients has been shown to be less active, possibly due to an abnormality in the phosphorylation of tyrosine residues located in exon 20 of the insulin receptor gene.

Table 1. Characteristics of the proband population

Patient group	No.	Age, yr	BMI, kg/m^2	Glucose, mg/dl
Nondiabetic	6	67 ± 12	22 ± 3.0	114 ± 7.5
NIDDM	10	74.2 ± 9.2	22.7 ± 4.4	207 ± 69.2

The muscle type was gastrocnemius. BMI, body mass index.

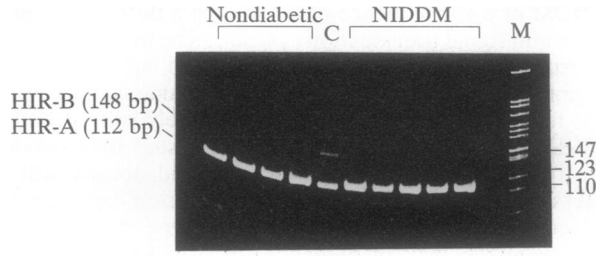

FIG. 1. PCR analysis of HIR-A and HIR-B sequences in human blood cells from nondiabetic and diabetic (NIDDM) subjects. The control lane (C) contains a mixture of PCR fragments derived from cloned HIR-A and HIR-B cDNA. M, size markers (in bp).

We present here experimental data that suggest an alternative mechanism that could lead to the generation of reduced insulin responsiveness in specific tissues. Recently we reported that the HIR-A and HIR-B isoforms of the insulin receptor exhibit distinct insulin-binding affinities. The discovery of high- and low-affinity receptors led us to speculate that disturbance of the alternative splicing mechanism that controls HIR-A versus HIR-B expression could result in pathophysiological conditions of the NIDDM phenotype. Examination of HIR-A and HIR-B RNA expression levels in blood and skeletal muscle of NIDDM patients and in normal controls yielded contrasting results. Whereas in normal individuals both of these tissues express almost exclusively RNA encoding the high-affinity HIR-A isoform, 100% of the skeletal muscle biopsies from diabetic patients display elevated expression of low-affinity HIR-B-encoding RNA. In diabetic muscle the levels of HIR-B RNA were roughly equal to that representing the HIR-A isoform for all (n = 10) samples analyzed; nevertheless, the overall amount of HIR sequences appeared to be in the range found in healthy control tissues. The consistency of our finding suggests a close linkage between the expression of sequences encoding the low-affinity insulin receptor isoform and the pathophysiological state of insulin resistance. Recently we confirmed the results presented here for a different skeletal muscle (vastus lateralis). However, a previous report (11) suggests that such clearcut differences of isotype expression might not be found in all muscle types.

Is alternative splicing in favor of low-affinity HIR-B expression a cause for lower insulin responsiveness in

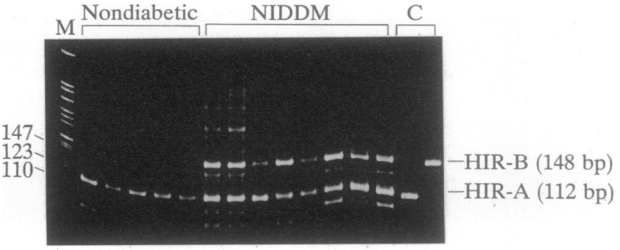

FIG. 2. PCR analysis of HIR-A and HIR-B sequences in muscle samples from nondiabetic and diabetic (NIDDM) subjects. The control lanes (C) contain PCR fragments derived from cloned HIR-A or HIR-B cDNA. Fragments not comigrating with the HIR-A or HIR-B controls represent nonspecific or single-stranded by-products of the PCR reaction. M, size markers (in bp).

NIDDM or a consequence of a defect at a different point of the insulin signal transduction cascade? The finding that HIR isoform expression is not altered in blood cells of NIDDM patients suggests that hyperinsulinemia alone is not responsible for the observed effect on alternatively spliced HIR-A and HIR-B RNAs. Further analysis of tissues from patients with related disorders such as IDDM and obesity will be necessary to further assess the connection between the molecular and pathophysiological phenotype. Nevertheless, in spite of the lack of evidence for a causal relationship between altered insulin receptor variant expression in skeletal muscle and the pathology of NIDDM, our findings suggest a potentially important role of RNA splicing in the pathogenesis of human disease.

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