Regulation of Insulin-like Growth Factor I Receptors in Diabetic Mesangial Cells*

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Mesangial cells are thought to play a central role in the renal complications of diabetes mellitus. Insulin-like growth factor I (IGF-I) has been found to promote mesangial cell proliferation and regulate normal mesangial cell function in an autocrine and/or paracrine fashion. To gain further insight into the potential regulatory role IGF-I may play in mesangial cell function in diabetes, IGF-I receptors were analyzed in mesangial cells isolated from diabetic mice (db/db) and their control littermates (db/m). Mesangial cells isolated from db/db mice exhibited higher levels of IGF-I receptors compared to cells from db/m mice. Insulin receptors were not detectable in either cell type by binding assays; however, immunoblot analysis revealed insulin receptor α-subunits in wheat germ agglutinin-Sepharose-purified membranes from db/db cells. Northern blot analysis further indicated a lack of detectable insulin receptor mRNA in db/m cells, whereas db/db cells expressed multiple insulin receptor mRNA transcripts. Both IGF-I and insulin receptor mRNA levels were increased in db/db cells grown in the presence of high glucose (28 mm), whereas the receptor protein levels remained relatively constant or increased, respectively. This increased expression of IGF-I and insulin receptors in diabetic mesangial cells may have an important role in the development of diabetic nephropathy.

The kidney glomerulus represents a highly organized vascular tuft which includes capillary endothelial cells, epithelial cells, and mesangial cells. Structural changes in the kidney glomerulus are the primary cause of the loss of kidney function in patients with diabetes mellitus. Diabetic nephropathy is characterized by thickening of the glomerular basement membrane, mesangial cell proliferation, and the accumulation of mesangial extracellular matrix. Progressive accumulation of mesangial matrix eventually leads to obstruction of the glomerular capillary lumen and loss of glomerular filtration and kidney function (1-3). Although the biochemical and molecular bases of the mesangial abnormalities in diabetes remain unknown, recent studies (2, 4-6) of mesangial cells in tissue culture have led to a better understanding of the role peptide growth factors may play in normal mesangial cell function. Normal human, rat, and mouse mesangial cells in primary culture possess high affinity receptors for insulin-like growth factor I (IGF-I)1 and few if any insulin receptors (3, 7, 8). IGF-I is mitogenic for mesangial cells; moreover, transgenic mice expressing increased levels of growth hormone and growth hormone-releasing factor exhibit high levels of circulating IGF-I and develop glomerulosclerosis, akin to diabetic nephropathy in the absence of hyperglycemia (9). Mesangial cells in culture also produce IGF-I and IGF-I-binding protein(s) (2). Thus, IGF-I may act as an autocrine or paracrine factor participating in the regulation of mesangial cell function.

In this study, we present the first evidence that mesangial cells derived from genetically diabetic mice (db/db) express higher levels of IGF-I receptors compared to mesangial cells from their normal littermates (db/m). High glucose was found to increase both IGF-I receptor and insulin receptor mRNA levels in diabetic cells. Under these conditions, the level of IGF-I receptor protein decreased, and the level of insulin receptor protein increased. These findings indicate that IGF-I receptor expression in diabetic mesangial cells is under dual regulation at the transcriptional and translational levels when grown in the presence of high glucose.

EXPERIMENTAL PROCEDURES

Materials—Diabetic mice (db/db, C57BL/KsJ) and their control nondiabetic littermates (db/m) were purchased from Jackson Laboratories (Bar Harbor, ME). cDNA probes (piGF-I-1.8 (IGF-I receptor) and pHR/P12-1 (insulin receptor)) were from the American Type Culture Collection (Rockville, MD).101-Labeled human insulin, human IGF-I, and the ECL (enhanced chemiluminescence) Western blotting detection system were from Amersham Corp. [γ-32P]ATP (6000 Ci/mmol) and [γ-32P]dCTP (3000 Ci/mmol) were from Du Pont-New England Nuclear. Crystalline porcine insulin was the generous gift of Dr. R. Frank ( Lilly). Recombinant human IGF-I was from U. S. Biochemical Corp. Alkaline phosphatase-conjugated goat anti-rabbit antibodies were from Vector Laboratories (Burlingame, CA). Peroxidase-conjugated goat anti-rabbit antibodies were from Promega Biotech (Madison, WI). PMSF, phenylmethylsulfonyl fluoride; HEPES, N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid; WGA, wheat germ agglutinin; SDS, sodium dodecyl sulfate.

1 The abbreviations used are: IGF-I, insulin-like growth factor I; PMSF, phenylmethylsulfonyl fluoride; HEPES, N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid; WGA, wheat germ agglutinin; SDS, sodium dodecyl sulfate.
**IGF-I Receptor Regulation**

Pharmacology, Yale University School of Medicine. PMSF, aprotinin, bovine serum albumin (radioimmunoassay-grade), N-acetyl-d-glucosamine, (HEPES), and bacitracin were from Sigma. Disuccinimidyl suberate was from Pierce Chemical Co. Parsinorin (standardized) was purchased from Calbiochem. Nicktroseulose paper was from MSI Sephardo, MA, and Zetabind nylon membrane was from AMF Cuno (Wallington, CF).

**Cell Culture**—Mouse mesangial cells were obtained from collagenase-treated renal glomerular remnants of 8-12-week-old diabetic mice (db/db, C57BL/Ka) and their non-diabetic littermates (db/m) as described (10) and maintained in Dulbecco's modified Eagle's medium with 0.1 M NaCl, 0.5 M NaCl, and finally 0.3 M GlcNAc in 0.5 M bovine serum albumin, 0.01% soybean trypsin inhibitor, 1 mM bacitracin, and 50 mM NaCl, 5 mM MgCl₂, 0.2% bovine serum albumin, 0.01% soybean trypsin inhibitor, 1 mM bacitracin, and finally 28 mM D-glucose for 5 days. 24 h prior to membrane preparation, the medium was changed to serum-free medium containing either 5.5 or 28 mM D-glucose. For the WGA-Sepharose-purified membranes was incubated in the absence or presence of various concentrations of IGF-I (10⁻⁶ to 10⁻¹¹ M) for 15 min at 22 °C. After addition of 50 μM ATP and 10 μCi of [γ³²P] ATP, the samples were further incubated for 10 min. The reaction was stopped by adding ice-cold immunoprecipitation buffer (50 mM HEPES (pH 7.4) containing 0.15 M NaCl, 5 mM MgCl₂, 0.2% bovine serum albumin, 0.01% soybean trypsin inhibitor, 1 mM bacitracin, and 50 mM NaCl, 0.5 mM MgCl₂, and finally 5 mM NaCl, all in 50 mM HEPES (pH 7.4) containing 0.1% Nonidet P-40) and the WGA-Sepharose beads. After incubation for 15 min at 25 °C with gentle rocking, the suspension was packed into a Bio-Rad Econo-Column. The flow-through was recycled three times, and the gel was sequentially washed with 0.1 M NaCl, 0.5 M NaCl, and finally 0.3 M GlcNAc in 0.5 M NaCl, all in 50 mM HEPES (pH 7.4) containing 0.1% Nonidet P-40.

**RESULTS AND DISCUSSION**

Mesangial cells isolated from diabetic mice (db/db, C57BL/Ka) and their non-diabetic littermates (db/m) were tested for their ability to bind [¹²⁵I]-IGF-I and [¹²⁵I]-insulin (Fig. 1). In experiments using intact cells (Fig. 1 A), no [¹²⁵I]-insulin binding could be detected in either diabetic or normal mesangial cells. In contrast, [¹²⁵I]-IGF-I (Fig. 1 A) bound with high affinity to both mesangial cell types with 50% inhibition attained with ~4 nM cold IGF-I. Cross-linking studies using membrane preparations from diabetic and normal mesangial cells revealed similar results (Fig. 1, B and D). These data indicate that diabetic mesangial cells possess high affinity IGF-I receptors and lack detectable surface receptors for insulin as previously reported for normal mouse mesangial cells (8).

To quantify the level of IGF-I and insulin receptor expression, WGA-purified membrane proteins were analyzed by immunoblotting using site-specific polyclonal antipeptide antibodies to the IGF-I and insulin receptors (14). The results revealed an ~5-fold higher level of IGF-I receptors in diabetic mesangial cells (Fig. 2A, lane 4) compared to normal mesangial cells (lane 5), which was consistent throughout early and late passages of mesangial cells derived from different animals. This finding is of particular significance with respect to the pathogenesis of diabetic nephropathy. Since IGF-I is known to be mitogenic for many cell types including mesangial cells (8) and overexpression of IGF-I receptors can induce a ligand-independent neoplastic transformation in mouse fibroblasts (16), it is likely that the increased expression of IGF-I receptors also increases the proliferation rate of mesangial cells. IGF-I has also been shown to upregulate the rate of synthesis of extracellular matrix components in chondrocytes, osteoblasts, and endothelial cells (2, 17, 18). Although such analyses have not as yet been performed with mesangial cells, it is likely that IGF-I also increases the...
production of extracellular matrix components in mesangial cells. Indeed, db/db mice develop the full features of diabetic nephropathy characterized by mesangial cell proliferation and increased accumulation of mesangial matrix (19). Along these lines, unilateral nephrectomy has been shown to accelerate the development of diabetic nephropathy in db/db mice, possibly due to an increase in tissue levels of IGF-I and IGF-I mRNA in the kidney (4). Further evidence implicating IGF-I receptor involvement in the pathogenesis of diabetic nephropathy is provided by Werner et al. (20), who recently reported that total IGF-I receptor mRNA and 125I-IGF-I binding are increased 2–3-fold in kidneys from streptozotocin-induced diabetic rats.

Interestingly, immunoblot analysis of WGA-purified membrane proteins revealed the presence of insulin receptors in diabetic (Fig. 2B, lane 4), but not normal (lane 6), mesangial cells. Although the binding studies of intact mesangial cells and cross-linking experiments on crude membrane preparations using 125I-insulin failed to detect insulin receptors on the surface of diabetic cells, the increased sensitivity of the immunoblot methodology coupled with the enrichment of membrane proteins clearly demonstrated that diabetic mesangial cells express insulin receptors.

When diabetic mesangial cells were cultured in the presence of high glucose (28 versus 5 mM in control medium) for 5 days, the IGF-I receptor level was essentially unchanged based on immunoblot analysis (Fig. 2A, lanes 3 and 4). Surprisingly, the expression of IGF-I receptors in normal mesangial cells decreased significantly (lanes 5 and 6). Finally, insulin receptor levels were increased in db/db grown in the presence of high glucose (Fig. 2B, lane 3).

To correlate the changes in receptor protein with receptor mRNA expression, we carried out Northern blot analyses of cells cultured under the conditions described. As illustrated in Fig. 3, IGF-I receptor mRNA levels increased 2-fold in db/db cells and ~1.5-fold in db/m cells after 5 days in high glucose (Fig. 3, A and C). The observed discrepancy in the protein and mRNA levels may, in part, be due to a translational block as recently shown for other membrane proteins (21) and in skeletal muscle of streptozotocin-induced diabetic rats (22). Alternatively, these cells may exhibit an increased turnover of receptors while maintaining a constant level of biosynthesis. This decrease in the net expression of IGF-I receptors under high glucose conditions is particularly pronounced in normal cells, perhaps representing a regulatory mechanism involved in preventing an increased proliferation rate and/or deposition of matrix components. This regulatory mechanism may be either attenuated or lost by diabetic cells since their levels of IGF-I receptor mRNA increased several-fold with a minimal change in receptor protein level.
cannot rule out the possibility that the elevated insulin receptor expression by diabetic cells under conditions of high glucose may also play a role in this phenomenon (Fig. 3, B and D). Indeed, diabetic mesangial cells proliferate much faster than normal in the presence of high glucose (data not shown).

Fig. 2. Immunoblot analysis of diabetic (db/db) and normal (db/m) mesangial cells. 50 μg of WGA-Sepharose-purified membrane proteins was subjected to electrophoresis on a 7.5% SDS-polyacrylamide gel and transferred to a nitrocellulose membrane. Immunoblotting was performed using site-specific antipeptide antibodies against the α-subunits of the IGF-I (A) and insulin (B) receptors. WGA-purified from Madin-Darby canine kidney (MDCK) and HepG2 cells were used as controls. Three representative results from five separate experiments using three different WGA-purified membrane preparations are shown for each receptor. The results of the scanning densitometry of these data are shown in C for the IGF-I receptor and in D for the insulin receptor. The values shown represent the mean ± S.E. of three experiments.

Fig. 3. Northern blot analysis of IGF-I receptor (A) and insulin receptor (B) mRNAs. 20 μg of total cellular RNA was subjected to electrophoresis on 1% formaldehyde-agarose gels and transferred to Zetabind filters. The filters were hybridized in 50% formamide at 42 °C with the indicated 32P-labeled cDNA probe prepared by random prime labeling and washed in 0.5 × SSC at 42 °C. Autoradiographic exposures with the IGF-I and insulin receptor
**FIG. 4.** Dose-dependent autophosphorylation of IGF-I receptors from diabetic mesangial cells. 10 μg of WGA-Sepharose-purified membranes was incubated in the absence (Control) or presence of 10^{-11} to 10^{-6} M IGF-I followed by addition of [γ-32P]ATP. The phosphorylation reaction was terminated after 10 min as described under “Experimental Procedures,” and the samples were immunoprecipitated with anti-phosphotyrosine antibody. Phosphotyrosine-containing proteins were eluted from the IgG with 10 mM O-phospho-L-tyrosine and then immunoprecipitated with site-specific antipseudotyrosine antibodies against the α-subunit of the IGF-I receptor. The samples were boiled in SDS sample buffer and resolved on a 7.5% SDS-polyacrylamide gel. The dried gel was exposed to Kodak XRP x-ray film for 3 days at -70°C.

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