Insulin Role in Pathogenesis of Nephropathy on Type 2 Diabetes

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Hyperglycemia and hyperinsulinemia are seen in the early phase of type 2 diabetes; it is unclear if hyperinsulinemia has a role in pathogenesis of renal disease in type 2 diabetes. In order to examine if insulin receptor activation occurred in renal tissue, renal cortical signaling pathways were examined in control and *db/db* mice with type 2 diabetes in the early phase (2-4 weeks of diabetes) associated with renal hypertrophy, onset of matrix expansion and albuminuria.

Tyrosine phosphorylation of proteins and PI 3-kinase activity were increased in renal cortex in diabetic mice. Renal cortical PI 3-kinase activity was partly due to insulin receptor activation as PI 3-kinase activity associated with β chain of the insulin receptor was increased nearly 4-fold.

Additionally, the kinase activity of the immunoprecipitated insulin receptor β chain was augmented in the diabetic renal cortex. Tyrosine phosphorylation of insulin receptor and IRS-2 was also increased. However, there was no change in the hepatic insulin receptor-associated PI 3-kinase activity. In vitro, insulin stimulated protein synthesis in proximal tubular epithelial (MCT) cells in association with increased activities of PI 3 kinase and Akt. We examined important events in the initiation phase of protein translation as it is the rate limiting step for protein synthesis. Initiation of mRNA translation is promoted by mRNA cap binding protein, eIF4E, which needs to be freed from a complex with its repressor, 4E BP1, by phosphorylation of the latter. Insulin augmented 4E-BP1phosphorylation in a PI 3-kinase-, mTOR- and Erk-dependent manner, suggesting regulation of protein translation by insulin in MCT cells and possible involvement of insulin in renal hypertrophy seen in early stage of type 2 diabetes.

As activation of insulin receptor signaling occurred at the time of initiation of matrix expansion including expression of laminin, regulation of synthesis of laminin was examined in MCT cells in media containing high glucose and high insulin levels. Immunoblotting showed that 30 mM glucose alone, but not equimolar mannitol, and, 1 nM insulin alone, and high glucose and high insulin together significantly increased laminin beta1 protein content in both cell lysates and medium within 5 min, the effect lasting for up to 30 min. Actinomycin-D did not affect laminin response to glucose or insulin, suggesting a non-transcriptional mechanism, including regulation of laminin translation. High glucose, high insulin and both together induced phosphorylation of 4E-BP1 within 5 min. Immunoblotting with phospho-specific antibodies showed that the three conditions stimulated activity of Akt and mTOR, the kinases that regulate 4E-BP1 phosphorylation. LY294002, a PI 3-kinase inhibitor, and rapamycin, an mTOR inhibitor, blocked laminin synthesis induced by high glucose, high insulin or both together. The three conditions also failed to promote laminin synthesis in cells stably expressing 4E-BP1 phosphorylation mutant. Phosphorylation of eIF4E and activation of its upstream kinase, Erk, were also seen with the three metabolic con-

ditions in the same time frame.

Our data show the following : (1) the renal cortex in type 2 diabetes is not insulin resistant, but demonstrates insulin sensitivity. (2) In proximal tubular epithelial cells, insulin induces protein synthesis and may contribute to renal hypertrophy in type 2 diabetes. (3) In proximal tubular epithelial cells, high glucose, high insulin or both together : (a) Rapidly increase laminin beta 1 chain synthesis by possibly regulating the initiation phase of its translation. (b) Recruit PI 3-kinase-Akt-mTOR pathway, perhaps, to regulate 4E-BP1 phosphorylation. (c) Recruit Erk pathway to possibly phosphorylate eIF4E. Insulin may have a role in pathogenesis of matrix expansion in type 2 diabetes by possibly regulating translation of laminin in proximal tubular epithelial cells.

References

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