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Combined acute hyperglycemic and hyperinsulinemic clamp induced profibrotic and proinflammatory responses in the kidney

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Mariappan MM, DeSilva K, Sorice GP, Muscogiuri G, Jimenez F, Ahuja S, Barnes JL, Choudhury GG, Musi N, DeFronzo R, Kasinath BS. Combined acute hyperglycemic and hyperinsulinemic clamp induced profibrotic and proinflammatory responses in the kidney. *Am J Physiol Cell Physiol* 306: C202–C211, 2014. First published October 9, 2013; doi:10.1152/ajpcell.00144.2013.—Increase in matrix protein content in the kidney is a cardinal feature of diabetic kidney disease. While renal matrix protein content is increased by chronic hyperglycemia, whether it is regulated by acute elevation of glucose and insulin has not been addressed. In this study, we aimed to evaluate whether short duration of combined hyperglycemia and hyperinsulinemia, mimicking the metabolic environment of prediabetes and early type 2 diabetes, induces kidney injury. Normal rats were subjected to either saline infusion (control, $n = 4$) or 7 h of combined hyperglycemic-hyperinsulinemic clamp (HG+HI clamp; $n = 6$). During the clamp, plasma glucose and plasma insulin were maintained at about 350 mg/dl and 16 ng/ml, respectively. HG+HI clamp increased the expression of renal cortical transforming growth factor-β (TGF-β) and renal matrix proteins, laminin and fibronectin. This was associated with the activation of SMAD3, Akt, mammalian target of rapamycin (mTOR) complexes, and ERK signaling pathways and their downstream target events in the initiation and elongation phases of mRNA translation, an important step in protein synthesis. Additionally, HG+HI clamp provoked renal inflammation as shown by the activation of Toll-like receptor 4 (TLR4) and infiltration of CD68-positive monocytes. Urinary F2t isoprostane excretion, an index of renal oxidant stress, was increased in the HG+HI clamp rats. We conclude that even a short duration of hyperglycemia and hyperinsulinemia contributes to activation of pathways that regulate matrix protein synthesis, inflammation, and oxidative stress in the kidney. This finding could have implications for the control of short-term rises in blood glucose in diabetic individuals at risk of developing kidney disease.

hyperglycemia; hyperinsulinemia; renal fibrosis; TGF-β; mTOR; laminin

CHRONIC ELEVATION of blood glucose levels is known to be the primary initiating factor in the gradual development of diabetic complications of organ systems, including the kidney (44, 45). It is not known whether metabolic defects associated with prediabetic and early diabetic states set the stage for these diabetic complications (4, 16, 25). Acute elevation of plasma glucose concentration triggers an array of tissue responses that may contribute to the development of diabetic complications. Growing evidence suggests that postprandial hyperglycemia is important in the development of cardiovascular disease (1, 5, 36). Atherosclerotic changes start to develop in the prediabetic state when postprandial blood glucose levels are only moderately and briefly elevated (19). In addition, hyperinsulinemia is provoked by acute hyperglycemia and its role in pathogenesis of diabetic target tissue injury is of great interest. Elevated circulating insulin levels have been identified as an important risk factor for atherosclerotic lesions (48).

In normal humans, acute combined hyperglycemic and hyperinsulinemic clamp modulates proinflammatory gene expression and cytokine production following LPS stimulation (47); it also stimulates accumulation of myocardial lipids and leads to alterations in myocardial function (51). Acute hyperglycemia has been reported to elicit an injurious response in the kidney. A 2-h hyperglycemic insult is sufficient in normal humans to induce an increase in urinary excretion of transforming growth factor-β1 (TGF-β1) and isoprostane (33). However, whether such acute transient changes are sufficient for stimulating regulatory mechanisms of protein synthesis resulting in increased extracellular matrix (ECM) proteins in the kidney tissue is not known; this is a matter of significance considering the central role of ECM accumulation in renal pathology in diabetes.

Since chronic hyperglycemia-induced progressive accumulation of extracellular matrix is strongly implicated in failure of clearance function of the kidney in diabetes (11), it is important to know whether acute elevation in plasma glucose and insulin levels contributes to this process. Even a brief exposure of proximal tubular epithelial cells and glomerular epithelial cells, in vitro, to high glucose or high insulin for 5–15 min stimulates synthesis of laminin. Laminins, a major component of kidney ECM (29, 32), are glycoprotein chains expressed primarily in basement membrane, which is important in maintaining the integrity of the glomerular filtration barrier and normal renal function. This rapid stimulation occurs by a nontranscriptional mechanism that involves activation of mRNA translation under the control of mammalian target of rapamycin (mTOR) complex 1 (mTORC1). The purpose of the present study was to determine whether acute hyperglycemia in combination with

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