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Mechanisms of Reactive Oxygen Species Generation in Renal Tubular Epithelial Cells Cultured under High Glucose

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Background: High glucose may increase reactive oxygen species (ROS) generation not only through glucose metabolism but also through induction of growth factors including TGF-β1 and angiotensin II (Ang II) and their receptor binding. While NADPH oxidase is presently accepted as the most important mechanism for receptor-stimulated ROS generation in both phagocytic and nonphagocytic cells, high glucose-induced mitochondrial superoxide generation has been proposed as an unifying mechanism for high glucose-induced vascular complications (Nishikawa T et al. Nature 404:787-90, 2000). Thus, the present study examined the importance of NADPH oxidase and mitochondrial electron transfer complex in high glucose-, TGF-β1-, and Ang II-induced ROS generation and fibronectin secretion by cultured proximal tubular cells (LLC-PK1).

Methods: Growth arrested and synchronized LLC-PK1 cells were stimulated by high glucose (30 mM D-glucose), TGF-β1 (2 ng/ml), or Ang II (100 nM) for 48 hours in the presence or absence of inhibitors of NADPH oxidase, diphenyleneiodonium (DPI: 100 nM) and apocynin (100 μM), or an inhibitor of mitochondrial electron transfer complex I, rotenone (10 nM). Dichlorofluorescein (DCF)-sensitive intracellular ROS was measured by FACS, fibronectin secreted by Western blot analysis, and p22phox mRNA expression by RT-PCR.

Results: High glucose, TGF-β1, and Ang II all significantly increased intracellular ROS. High glucose upregulated fibronectin secretion by 1.4-fold, TGF-β1 by 1.4-fold, and Ang II by 1.6-fold that of control. Both inhibitors of NADPH oxidase and an inhibitor of mitochondrial electron transfer complex I effectively inhibited high glucose-, TGF-β1, and Ang II-induced, but not basal, fibronectin secretion by LLC-PK1. In addition, high glucose upregulated p22phox mRNA expression by 2.5-fold, TGF-β1 by 2.7-fold, and Ang II by 2.7-fold that of control.

Conclusion: The present data suggest that high glucose, TGF-β1, and Ang II generate intracellular ROS through both NADPH oxidase and mitochondrial metabolism, that ROS, thus generated, upregulate fibronectin secretion by LLC-PK1 cells, and that ROS generated by TGF-β1 and Ang II may amplify high glucose signaling in diabetic kidney.