

Transforming growth factor and intercellular communication in tubular epithelial cells: a role in diabetic nephropathy.

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Aims: Changes in cell-to-cell communication have been linked to several secondary complications of diabetes, including diabetic nephropathy. This study examines a role for glucose-evoked changes in the beta1 isoform of the pro-fibrotic cytokine transforming growth factor (TGF β 1), on connexin expression, gap-junction mediated intercellular communication and hemi-channel mediated ATP release from epithelial cells of the proximal tubule.

Methods: Connexin-26 and connexin-43 expression was assessed by immunoblot analysis in human kidney (HK2) tubular epithelial cells treated with TGF β 1 (2-10ng/mL) for 48hrs. Whole cell paired-patch electrophysiology assessed junctional conductance between TGF β 1 treated HK2 cells. Hemi-channel opening was determined by carboxyfluorescein uptake, whilst bio-sensing was used to determine real-time ATP release.

Results: Immunoblotting confirmed that TGF β 1 down-regulates connexin-26 to 72.7 \pm 13.3%, 71.6 \pm 4.8%, and 58.3 \pm 5.7% of control and connexin-43 to 61.2 \pm 10.4%, 49.5 \pm 6.1%, and 48.1 \pm 3.8% at 2, 4 and 10 ng/mL respectively. TGF β 1 significantly decreased junctional conductance at 48hrs (1.15 \pm 0.9nS compared to 4.5 \pm 1.3nS in control cells n=5; P <0.05), whilst carboxyfluorescein uptake increased 346 \pm 33% in TGF β 1-treated (10ng/mL) cells. A response inhibited by the hemi-channel blocker carbenoxolone (200 μ M, 30mins). Bio-sensing confirmed that increased channel opening was paralleled by elevated ATP release following 48hr TGF β 1 treatment (1.99 \pm 0.47 μ M compared to a control 0.29 \pm 0.06 μ M, P <0.01, n=3).

Conclusions: The current study suggests that acute 48hr application of the pro-fibrotic cytokine reduces connexin-mediated intercellular communication in proximal tubular epithelial cells in favour of hemi-channel mediated ATP release. The rise in intercellular ATP may contribute to tubular fibrosis in the diabetic kidney.

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