Purinergic receptor (P2X7) activation contributes to disassembly of adherens and tight junctions in tubular epithelial cells of the diabetic kidney

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Background and aims: Preceded by a loss of cell-cell adhesion, glucose-evoked changes in connexin (Cx) expression/function are linked to increased hemichannel-mediated release of adenosine triphosphate (ATP) in tubular epithelial cells of the diabetic kidney. Elevated ATP is associated with inflammation and fibrosis, and this study investigates a role for Cx43 hemichannel-mediated ATP release in regulating adherens- and tight-junction proteins, effects that initiate the morphological and phenotypic changes of tubular damage.

Materials and methods: Human primary proximal tubule epithelial cells (HPTECs) and clonal tubular epithelial cells (HK2) were cultured in TGFβ1 (10ng/mL) ± apyrase (5U/ml), or non-hydrolysable ATPγS (100μM) at 48h. Immunoblotting assessed protein expression. Trans-epithelial electrical resistance assessed paracellular tight junction formation and atomic force microscopy force spectroscopy measured cell-cell adhesion. Carboxyfluorescein uptake and ATP biosensing measured hemichannel activity and nucleotide release. Co-incubation of cells with TGFβ1 ± Peptide5 (25μM) or A438079 (50μM) assessed effect of Cx43 hemichannel and purinergic receptor (P2X7) blockade respectively.

Results: Immunoblotting confirmed that TGFβ1 downregulated E-cadherin (ECAD), claudin-2 and ZO-1 to 38.5±4.1%, 60.5±4.4% and 64.8±4.4% respectively, whilst N-cadherin (NCAD) expression increased to 213.3±28.0% compared to control (P<0.01; n=4). The effect was replicated by ATPγS, which decreased expression of ECAD, claudin-2 and ZO-1 to 43.4±6.1%, 42.0±2.6% and 45.9±1.4% respectively. NCAD increased to 181.3±6.3% (P<0.01; n=3). In a separate series of experiments, co-incubation with the ectonucleotidase apyrase partially restored ECAD expression to 51.2±3.2%, and NCAD to 133.3±9.1%, compared to control (P<0.001; n=3). Trans-epithelial resistance decreased in TGFβ1 and ATPγS treated cells from 67.7±5.5Ω.cm² to 27.6±2.0Ω.cm² and 42.6±3.0Ω.cm² respectively (P<0.05; n=3). Mean unbinding forces between ATPγS treated cells also decreased from 2.17±0.64nN in control cells to 1.60±0.48nN (P<0.001; n=3) confirming a loss of cell-cell adhesions. Increased carboxyfluorescein uptake (609.4±46.0%) and ATP release (6.10±0.36μM from 0.43±0.03μM) confirmed increased hemichannel mediated ATP release in TGFβ1 treated cells, an effect blocked by Cx43 mimetic, Peptide5 (163.0±10.2% and 0.60±0.20μM) (P<0.001; n=3). Co-incubation of HPTECs with TGFβ1 and Peptide5 restored expression of ECAD (108.9±17.1% from 31.5±9.2%), NCAD (154.7±10.6% from 280.5±16.7%), claudin-2 (100.9±10% from 65.3±5.4%) and ZO-1 (91.6±12.8% from 59.6±3.1%) compared to control (P<0.01; n=3). Blocking P2X7 with A438079 restored ECAD expression from 22.2±5.5% to 52.8±5.4% in TGFβ1 treated cells (P<0.001; n=3), with unbinding forces restored from 2.17±0.64nN to 2.45±0.89nN (P<0.001; n=3).

Conclusion: Hemichannel mediated ATP release is downstream of TGFβ1-evoked changes to adherens- and tight-junction proteins, effects blocked by inhibiting P2X7 receptors or Cx43 hemichannel activity. Disassembly of these junctions is a pivotal event in progression of tubular injury in diabetic nephropathy and data suggests a potential role for Cx-mediated hemichannel activity as a future therapeutic target in diabetic kidney disease.

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