1021

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

G.W. Price, E. Siamantouras, J.A. Potter, P.E. Squires, C.E. Hills;
School of Life Sciences, University of Lincoln, Lincoln, UK.

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.cm^{2}to 27.6±2.0Ω.cm^2 and 42.6±3.0Ω.cm^2 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 adhesion. 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.
Supported by: Diabetes UK (16/0005427 and 16/0005544) and an EFSD/Boehringer Ingelheim Award
Disclosure: **G.W. Price:** None.