ATP reduces functional cell-to-cell tethering between renal tubular epithelial cells.

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Background: Loss of epithelial (E)-cadherin mediated cell-cell adhesion impairs gap junction formation and facilitates hemichannel-mediated ATP release in the diabetic kidney. Linked to inflammation and fibrosis, we hypothesize that local increases in inter-cellular ATP activate P2X7 receptors on neighbouring epithelial cells of the proximal tubule, to further impair cell-cell adhesion and exacerbate tubular injury.
Methods: Atomic force microscopy (AFM)-single cell force spectroscopy (SCFS) was used to quantify the unbinding forces needed to separate clonal tubular epithelial cells of the proximal kidney (HK2) following treatment with non-hydrolysable ATPγS (100μM) ± the P2X7 purinergic receptor antagonist A438079 (50μM) over 48hr. Densitometry was used to semi-quantify Western blot protein expression. Biophysical measurements quantified maximum unbinding force (F_{max}), tether (or ligation cluster) rupture events (*TREs*) and work of detachment (W_d). Three force-displacement curves were acquired per cell. Data from SCFS are expressed as mean ± SEM. Sample numbers refer to separate cell passages (n) from multiple individual cells. Data was evaluated using univariate anova followed by Tukey's multiple comparisons post-test. $P<0.05$ was taken to indicate statistical significance.
Results: The P2-purinergic receptor agonist ATPγS (100μM), decreased E-cadherin expression by 54.4±4% compared to control ($P<0.01$, n=3), an effect negated by the P2X7 antagonist A438079 (50μM; 113.6±9.7% of control). The agonist (100μM) weakened intercellular ligation forces (F_{max}) by 26.8% ($P<0.001$; n=3) and affected the formation of ligation clusters by reducing tether rupture events (*TREs*) by 22.3% ($P<0.001$; n=3), effects largely reversed by A438079 (50μM). Total energy consumed during the pulling process prior to complete cell separation, was calculated by the integration of the retraction *Force-Displacement* curve and is referred as the work of adhesion W_d. Our results showed that control cells exhibited a W_d of 21.85fJ±1.1, whilst the total energy consumed in separating ATPγS (50μM) treated cells was reduced to 13.32fJ±1.13, indicating a reduction of 39% ($P<0.001$; n=3). Co-incubation with A438079 (50μM) increased W_d back to control (23.7fJ±1.22, n=3) and negated the response to ATP.
Conclusion: Determining strength of adhesion in disease is challenging due to the underlying molecular assembly that regulates formation of the adherens junction. Force-displacement measurements during cell-cell separation can provide valuable information about the response to ligands and their antagonists. Nanoscale force-displacement measurements capture the molecular activity underlying ATPγS evoked changes in cell adhesion, loss of which appears to be mediated by downstream P2X7 receptor activation and supports a role for P2X7 as a potential therapeutic target in managing progression of diabetic nephropathy.

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