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EXPRESSION AND LOCALISATION OF CONNEXINS 43 AND 45 AND THEIR INVOLVEMENT IN MEDIATING ATP RELEASE IN PORCINE BLADDER

Hypothesis / aims of study

ATP released from urothelium in response to mechanical stretch of the bladder wall can modulate the sensation of fullness felt in the bladder through suburothelial afferent nerve fibres. Extracellular ATP signalling is altered substantially in bladder diseases, such as over active bladder and interstitial cystitis. Connexin (Cx) proteins form gap junctions between adjacent cells to regulate cell-to-cell communications. Cx hemichannels, in particular Cx43, have recently been demonstrated to be ATP release channels in many cell types, where they are functionally closely associated with purinergic receptors to regulate extracellular ATP signalling and inflammatory processes, however not many studies in regards to Cx hemichannels have been carried out in the bladder. We hypothesised that Cx43 and Cx45 form functional hemichannels to regulate ATP release in the bladder. In this study, we used porcine bladder, a well-recognised model to study human bladder function, to localise Cx43 and Cx45 cellular expression and to investigate their involvement in mediating ATP release in cultured urothelial, suburothelial and detrusor muscle cells.

Study design, materials and methods

Fresh porcine bladders were dissected into urothelial, suburothelial and detrusor muscle layers. Total RNA was extracted and quantitative RT-PCR was performed to confirm Cx43 and Cx45 mRNA expression. Immunohistochemistry was conducted on cross-sections using primary antibodies against Cx43 (AB1745 Millipore) and Cx45 (C6219 Sigma-Aldrich) and a secondary Alexa Fluor488 antibody (ab150077 Abcam) for fluorescent staining. Isolated urothelial, suburothelial and detrusor muscle cell were cultured and Cx43 and Cx45 mediated ATP release in response to hypotonic (~50%) solution induced stretch and extracellular Ca^{2+} depletion ($[Ca^{2+}]_0$, ~17nM) was measured by ATP Bioluminescence Assay (Sigma-Aldrich).

Results

PCR results confirmed the expression of Cx43 and Cx45 mRNA in urothelial, suburothelial and detrusor muscle layers. Immunohistochemistry experiments showed that in urothelium Cx43 expression spans from basal layer to terminally differentiated umbrella cells. Positive Cx43 signals were also seen on the surface of detrusor cell membranes, endothelial cells of the blood vessels as well as on some spindle shaped cells of suburothelial layer that likely correspond to suburothelial myofibroblasts. Cx45 showed similar cellular distribution pattern to Cx43, but was less prominent on endothelial cells of blood vessels compared to Cx43.

Functional studies on cultured urothelial, suburothelial and detrusor muscle cells have shown that stretch induced a significant rise in ATP release from all three cell types (Figure 1).

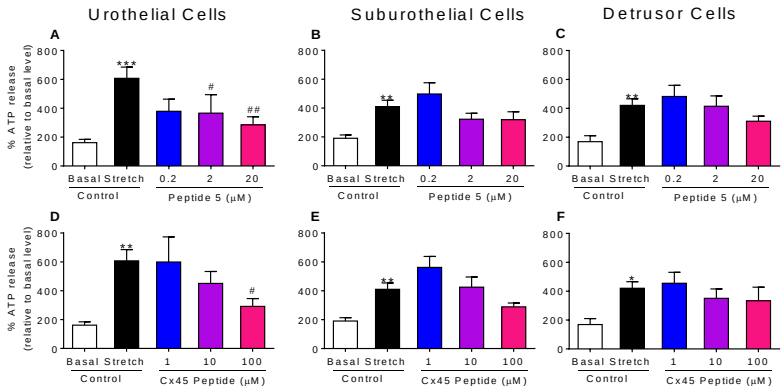

Figure 1. Stretch mediated ATP release. Data were analysed by one-way ANOVA followed by Fisher's LSD test. $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$ compared to basal control; $^{\#}P<0.05$, and $^{\#\#}P<0.01$ compared to stretch control.

Peptide 5, a Cx43 mimetic peptide that blocks Cx43 channels [1] significantly reduced this ATP release from cultured urothelial cells in a concentration dependent manner, showing 52.8% inhibition at 20 µM (Figure 1A). Similarly, Cx45 mimetic peptide (amino acids 202–217, QVHPFYVCSRLPCPHK) also reduced the stretch mediated ATP release from urothelial cell cultures by 51.7% at 100 µM (Figure 1D). For suburothelial and detrusor muscle cells, a trend of decrease of extracellular ATP release was observed in the presence of peptide 5 and Cx45 peptide, but it was not statistically significant.

$[Ca^{2+}]_0$ also stimulated ATP release from cultured urothelial, suburothelial and detrusor muscle cells which was inhibited by peptide 5 in all three cell populations by ~50% (Figure 2 upper panels). On the other hand, Cx45 peptide significantly reduced ATP release from urothelial cells by 52% (Figure 2D). The effect of Cx45 peptide on other two cell types was insignificant (Figure 2E, F).

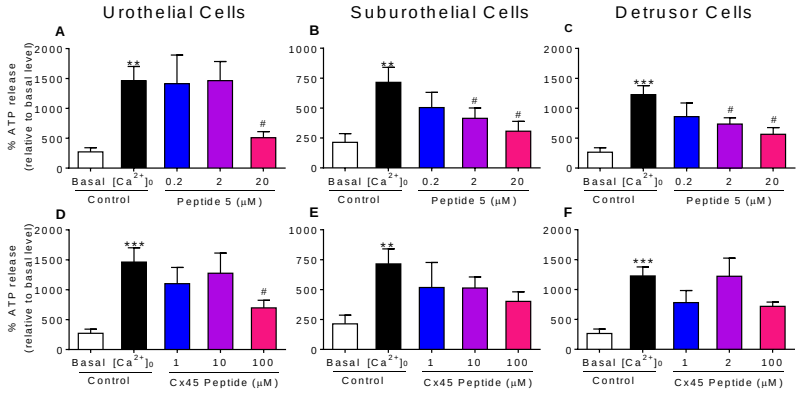

Figure 2. Ca^{2+} depletion mediated ATP release. Data were analysed by one-way ANOVA followed by Fisher's LSD test. $^{**}P<0.01$, $^{***}P<0.001$ compared to basal control; $^{\#}P<0.05$ compared to Ca^{2+} depletion control.

Interpretation of results

Our results demonstrated that in comparison to suburothelial and detrusor layers, strong immunoreactivity for both Cx43 and Cx45 was observed throughout the urothelium, including basal, intermediate and terminally differentiated umbrella cells. This suggests that these channels may function as gap junctions and hemichannels, important for intercellular communications and ATP release in urothelium. Inhibition of ATP release in the presence of peptide 5 and Cx45 mimetic peptide from cultured urothelial cells in response to hypotonic stretch and Ca^{2+} depletion conditions coincided with immunostaining data, indicating an essential role of Cx43 and Cx45 in urothelial mechanosensitive ATP release. Cx43 and Cx45 expression was also observed on some spindle shaped cells of suburothelial layer that are most likely to be suburothelial myofibroblasts. Myofibroblasts are in close contact with suburothelial sensory nerve terminals, and ATP released from these cells may therefore be associated with sensory nerve activation. Reducing extracellular Ca^{2+} from 2.5 mM to 17 nM increased Cx hemichannel activities, suggesting that the open/close switch of these channels are calcium dependent.

Concluding message

Here, we report for the first time that Cx43 and Cx45 are ATP release channels in response to physiological stretch in the bladder. This indicates that these channels are among several other channels, such as pannexin-1, that release ATP to initiate autocrine/paracrine signalling in response to bladder distension during the storage phase of micturition reflex.

A previous study has shown increased Cx43 and Cx45 expression in patients with urge symptoms such as chronic cystitis and high voiding frequencies [2]. Some studies have also demonstrated an augmented extracellular ATP signalling in urothelium of patients with interstitial cystitis, bladder overactivity and urge incontinence. Cx43 channels are implicated in the initiation of inflammation through the activation of purinergic signalling pathways. Randomised clinical trials have shown that the application of Cx43 mimetic peptide gel reduces inflammation and accelerates wound healing in conditions such as chronic diabetic foot ulcers, and acute corneal wounds [3]. Thus, Cx43 and Cx45 mimetic peptides may have the potential for the treatment of interstitial cystitis and other inflammatory bladder diseases.

References

1. O'Carroll SJ, Alkadhi M, Nicholson LF, Green CR. Connexin 43 mimetic peptides reduce swelling, astrogliosis, and neuronal cell death after spinal cord injury. Cell Commun Adhes. 2008,15: 27-42.
2. Neuhaus J, Pfeiffer F, Wolburg H, Horn LC, Dorschner W. Alterations in connexin expression in the bladder of patients with urge symptoms. BJU Int. 2005, 96: 670-6.
3. Grek CL, Prasad GM, Viswanathan V, Armstrong DG, Gourdie RG, Ghatnekar GS. Topical administration of a connexin43-based peptide augments healing of chronic neuropathic diabetic foot ulcers: A multicenter, randomized trial. Wound Repair Regen. 2015, 23: 203-12.

Disclosures

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