Connexins 43 and 45 hemichannels and CALHM1 mediate ATP release in human urothelial RT4 cells

[1]Hafiz Sana-ur-rehman, [2]Kylie J. Mansfield, [3]Kate H Moore, [1]Lu Liu

[1]School of Medical Sciences, UNSW Sydney, NSW, Australia; [2]Graduate School of Medicine, University of Wollongong, NSW, Australia and [3]St. George Hospital, UNSW Sydney, NSW Australia

INTRODUCTION

Over the last two decades, a prominent role of ATP in the urinary bladder sensation has been established. We have previously demonstrated that ATP release from the porcine bladder could occur via many different mechanisms, including connexin (Cx) 43 and Cx45 hemichannels, pannexin-1 and calcium homeostasis modulator 1 (CALHM1) channels (1, 2). However, the cellular pathways responsible for ATP release from human bladder are still under-investigated.

AIM

This study aimed to reveal pannexin-1, CALHM1, Cx43 and Cx45 expression in human urothelial RT4 cells and to investigate whether they function as ATP releasing channels.

METHODS

1. Immunocytochemistry: Immunocytochemistry was conducted in human urothelial RT4 cells using antibodies against pannexin-1 (ab124131 Abcam), CALHM1 (HPA018317 Sigma), Cx43 (C6219 Sigma-Aldrich) and Cx45 (AB1745 Millipore), respectively. Alexa Fluor 488 (ab150077 Abcam) and Alexa Fluor 594 (ab150116) secondary antibodies were used for fluorescent staining. Double labelling has also been performed with the cytokeratin marker AE1/AE3 (M3515 Dako) to confirm the identity of urothelial RT4 cells.

2. Cell culture and ATP release assay: RT4 cells were cultured in McCoy's 5A culture media (Invitrogen 16600-108), supplemented with 10% FBS, 1% glutamax (Invitrogen 35050061), and 1% antibiotic-antimycotic. Pannexin-1, CALHM1, Cx43 and Cx45 mediated ATP release in response to hypotonic (~50% NaCl) induced stretch and extracellular Ca^{2+} depletion ($[Ca^{2+}]_0$~17nM) was measured by ATP Bioluminescence Assay (Sigma-Aldrich) (1).

RESULTS

1. Pannexin-1, CALHM1, CX43 and Cx45 Immunoreactivity:

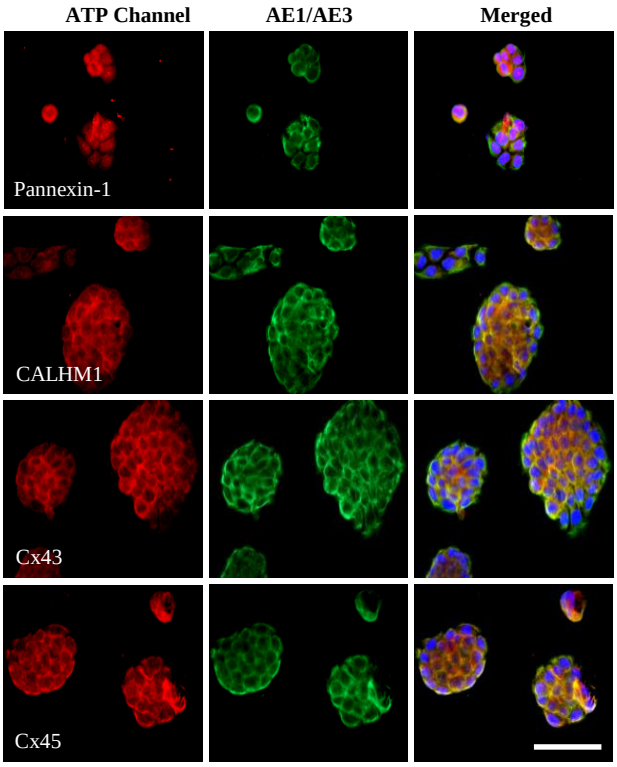

Figure 1. Double labelling of pannexin-1, CALHM1, Cx43 and Cx45 antibodies (red) with cytokeratin marker AE1/AE3 antibody (green) in urothelial RT4 cells. The blue color represents the nuclear staining by DAPI.
All panels are shown at the same magnification, and the scale bars represent 100 µm.

REFERENCES

1. Sana-Ur-Rehman, H., et al., Expression and localization of pannexin-1 and CALHM1 in porcine bladder and their involvement in modulating ATP release. Am J Physiol Regul Integr Comp Physiol, 2017: doi: 10.1152/ajpregu.00039.2016.
2. Sana-Ur-Rehman, H., et al. " Expression and localisation of connexins 43 and 45 and their involvement in mediating ATP release in porcine bladder." Neurourology and Urodynamics Vol. 35. pp.S60-S61. 111 River st, Hoboken 07030-5774, NJ USA: Wiley-Blackwell, 2016

2. ATP release from RT4 cells:

(A) Hypotonic stretch-induced ATP release

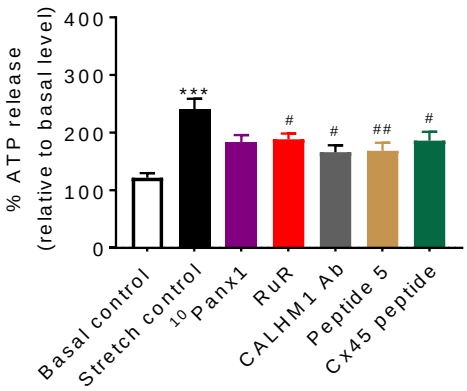

Figure 2. Effects of ^{10}Panx1, a pannexin-1 blocking peptide, RuR, a non selective CALHM1 channel blocker, CALHM1 antibody, peptide 5, a Cx43 mimetic peptide that blocks Cx43 channels and the Cx45 mimetic peptide on hypotonic stretch induced ATP release from cultured urothelial RT4 cells. Significance by one way ANOVA followed by Sidak's multiple comparisons is indicated with a single symbol for $P < 0.05$, two for $P < 0.01$ and three for $P < 0.001$. (* for basal control vs. stretch control; # for stretch control vs. stretch + inhibitor).

B. Ca^{2+} depletion stimulated ATP release

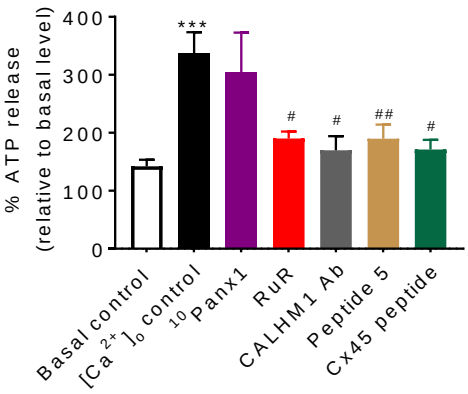

Figure 3. Effects of ^{10}Panx1, a pannexin-1 blocking peptide, RuR, a non selective CALHM1 channel blocker, CALHM1 antibody, peptide 5, a Cx43 mimetic peptide that blocks Cx43 channels and the Cx45 mimetic peptide under control (2 mM) and depleted (~17 nM) Ca^{2+} conditions. Significance by one way ANOVA followed by Sidak's multiple comparisons is indicated with a single symbol for $P < 0.05$, two for $P < 0.01$ and three for $P < 0.001$. (* for basal control vs. Ca^{2+} depletion; # for Ca^{2+} depletion vs. Ca^{2+} depletion + inhibitor).

SUMMARY

- Intensive Cx43, Cx45, pannexin-1 and CALHM1 immunoreactive signals were observed on urothelial RT4 cells. Their cellular expression appeared to be mainly on cell membranes (Figure 1). Double labelling of pannexin-1 CALHM1, Cx43 and Cx45 with the epithelial cell marker AE1/AE3 has confirmed the identity of urothelial cells.
- The blockage of Cx43, Cx45 and CALHM1 channels by their blocking peptides significantly reduced ATP release from urothelial RT4 cells by ~30%. On the other hand, a trend of decrease of extracellular ATP release was observed in the presence of ^{10}Panx1, a selective pannexin-1 channel blocker, but the result was not statistically significant.
- $[Ca^{2+}]_0$ also stimulated ATP release from RT4 cells which was significantly inhibited by Peptide 5, Cx45 mimetic peptide, ruthenium red and CALHM1 antibody. ^{10}Panx1 showed no effect on $[Ca^{2+}]_0$ potentiated ATP release (Figure 2).

CONCLUSIONS

Here, we report for the first time that Cx43, Cx45 and CALHM1 are ATP release channels in response to stretch and $[Ca^{2+}]_0$ in the human bladder urothelial cells, suggesting that these channels may play an important role in the initiation of ATP signalling in response to bladder distension during the storage phase of micturition reflex. Furthermore, modulation of extracellular Ca^{2+} may also regulate ATP release in the bladder through Ca^{2+} sensitive Cx43 and Cx45 hemichannels and CALHM1.