

PRIMARY MOUSE UROTHELIAL CELL RESPONSE TO ATP IS MEDIATED BY P2X BUT NOT TRPV1 RECEPTORS

Hypothesis / aims of study

The urothelium is considered an important component in bladder function and it is capable of releasing and responding to various neurotransmitters, neuromodulators and peptides [1]. ATP is released from the urothelium upon stretch [2] during bladder filling and it activates afferent nerves and initiates the micturition reflex. Urothelial cells are also capable of responding to ATP, but the receptors to which ATP binds and the responses it causes, have not been characterised. The aim of this study was to characterise mouse urothelial cell responses to ATP and to determine if the TRPV1 receptor is important in modulating these responses.

Study design, materials and methods

Primary cultures of urothelial cells from the bladders of TRPV1 knockout and age matched wild type control mice were prepared. Cells were loaded with Fura-2 AM (2 μ M) and excited alternately with 340 and 380nm wavelengths of light to measure intracellular calcium levels. Responses were then obtained to ATP and the TRPV1 agonist capsaicin. Data are presented as mean \pm SEM. The number of cells examined (n) from 3 animals is indicated.

Results

Urothelial cells from control wild type mice responded to ATP with an increase in intracellular calcium (Figure 1), producing an EC₅₀ of 3.49 \pm 0.97 μ M (n=360). The EC₅₀ of ATP in TRPV1 knockout mice was not significantly different 4.57 \pm 0.92 μ M (n=478) to the controls, indicating that the TRPV1 receptor plays no part in modulating mouse urothelial responses to ATP. In addition, Urothelial cells did not respond to capsaicin (1 μ M) (figure 2), indicative of a lack of a functional TRPV1 receptor within these cells. Wild type cells showed a reduced efficacy for ATP when calcium responses were recorded in a nominal calcium solution EC₅₀ 9.53 \pm 1.12 μ M (n=310) (figure 3).

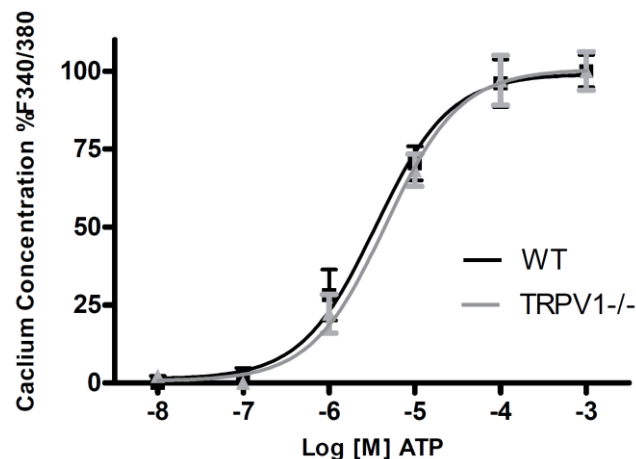


Figure 1: Dose response of urothelial cells from TRPV1 knockout mice and wild type controls to ATP. Knockout of the TRPV1 receptor has no influence on the ability of urothelial cells to increase intracellular calcium concentrations when ATP is bound.

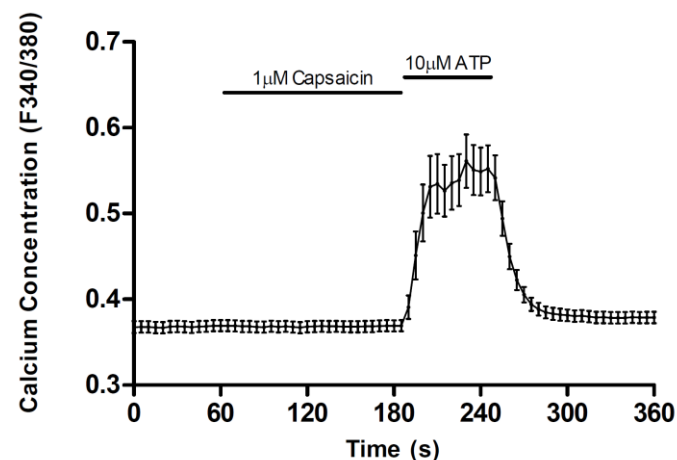


Figure 2: Urothelial cells do not respond to the TRPV1 agonist capsaicin (1 μ M) but respond robustly to ATP.

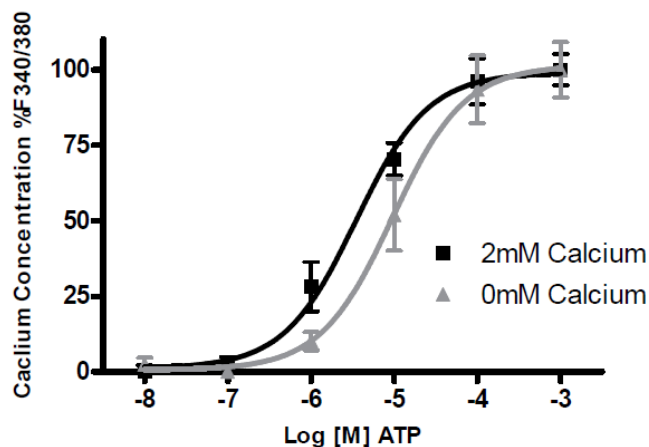


Figure 3: Urothelial cells respond to ATP with an increase in intracellular calcium which is significantly reduced in a nominal calcium solution.

Interpretation of results

These results show that there is a lack of a functional TRPV1 receptor in mouse urothelial cells and knockout of this receptor has no influence on their ability to respond to ATP. These results also indicate a significant role of the membrane bound P2X and P2Y receptors in mediating urothelial responses to ATP.

Concluding message

Urothelial cells release ATP when stretched, but these data show that these cells also possess receptors for ATP and the cells respond to ATP with an increase in intracellular calcium, indicating an autocrine effect. Unlike sensory nerves, the urothelial cell responses to ATP are not regulated by TRPV1 receptors and the cells do not possess functional TRPV1 receptors.

References

1. Birder, L.A., Urinary bladder urothelium: Molecular sensors of chemical/thermal/mechanical stimuli. *Vascular Pharmacology*, 2006. 45(4): p. 221-226.
2. Sadananda, P., et al., Release of ATP from rat urinary bladder mucosa: role of acid, vanilloids and stretch. *British Journal of Pharmacology*, 2009. 158: p. 1655-1662.

Disclosures

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