

FUNCTIONAL PROPERTIES OF TRPV1 EXPRESSED IN HUMAN UROTHELIAL CELLS

Hypothesis / aims of study

The traditional concept of the bladder urothelium as a barrier that prevents the back-flow of toxic contents of urine into the blood stream has changed. In rodents, urothelial cells express an impressive number of receptors usually present in neuronal cells including bradykinin, adrenergic and cholinergic receptors as well as numerous members of the TRP family (1). In addition, rodent urothelial cells release neuromodulators including nitric oxide, ATP, prostaglandins and acetylcholine (1). These findings raised the hypothesis that an intense cross talk occurs between urothelial cells and nerve fibres coursing the lamina propria of the urinary bladder. Such mechanism could be highly relevant to the generation of normal and abnormal bladder sensations, including pain, and to the control of bladder reflex activity.

Among the receptors identified in urothelial cells and bladder sensory fibres, TRPV1 seems to play an essential role for the genesis of bladder pain and bladder overactivity accompanying chronic cystitis (2). Its expression in the human urothelium was demonstrated by immunohistochemical studies (3). In the present study we investigate the expression of TRPV1 in urothelial cells using a molecular biology approach. In addition, the influence of inflammatory mediators in its expression and the response of the receptor to TRPV1 agonists in neuronal cells was also investigated.

Study design, materials and methods

Human urothelium was obtained with the permission of Ethical Committee of our Institution from patients submitted to open prostatectomy or radical cystectomy. Urothelium was detached from the lamina propria, dissociated with collagenase and cultured in keratinocyte serum free medium. When the cells reached 80% confluence, they were detached with trypsin and plated in poly-L-lysine and laminin coated glass coverslips. Experiments were performed 4 to 5 days after re-plating. Confirmation of the epithelial origin of the cultured cells was obtained by immunostaining against pan-cytokeratin. Culture media containing different concentration of nerve growth factor (NGF) and inflammatory mediators (bradykinin, histamine, prostaglandins and serotonin) were tested.

The amount of TRPV1 protein expressed by cultured cells was investigated by immunostaining with an antibody against human TRPV1 (Affinity) and by Western blotting.

To test the response of TRPV1 to capsaicin, protons and heat, the cobalt uptake method was used. In short, calcium in the culture medium was replaced by cobalt. Cells were challenged with capsaicin (100 nM and 1 μ M) in the presence and in the absence of the TRPV1 antagonist capsazepine, solutions with different pH values (7.4, 5.4 and 4.7) and temperature above 43°C. The cobalt up-taken by urothelial cells was then precipitated with sulphite and the grey gradient of the cells measured using Image J software.

Results

RT-PCR demonstrated the occurrence of TRPV1 mRNA in cultured cells. Its content was independent of the presence of NGF in the culture medium. However, the amount of TRPV1 mRNA more than triplicate if the inflammatory mediators (bradykinin, histamine, prostaglandins and serotonin) were added to the cell culture medium. TRPV1 mRNA expression was also present in freshly obtained urothelium cells.

Cultured human urothelial cells gave a positive reaction when immunostained against TRPV1. In addition, western blotting analysis of human cell homogenates identified a TRPV1 positive band with 97 kDa molecular weight, which corresponds to that of the human TRPV1 protein.

Capsaicin in concentrations of 100 nM or 1 μ M increased cobalt uptake in a dose dependent manner. Capsazepine completely inhibited the capsaicin-induced cobalt uptake. Likewise, pH increased cobalt uptake in a dose dependent way. Heating cells above 43 °C also increased cobalt uptake.

Interpretation of results

These data confirm that human urothelial cells express TRPV1 and that this receptor has functional properties similar to those previously recognised in sensory neurons, in particular the responses to capsaicin, protons and heat. Surprisingly, TRPV1 expression in urothelial cells was not dependent upon the presence of NGF in the culture medium. However, like in neuronal sensory cells, TRPV1 could be up-regulated by inflammatory mediators suggesting a positive influence of inflammation in the receptor expression.

Concluding message

TRPV1 expressed in human urothelial cells seems to play an important role in the cross-talk between the urothelium and bladder sensory fibres, in particular during inflammatory states in order to generate bladder sensations and regulate bladder reflex activity.

References

1. Am. J. Physiol. Renal Physiol. (2005) 289:F489-495
2. J. Neurosci. (2004) 24:11253-11263
3. Eur. Urol. (2004) 46:792-798

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