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Everaerts W[1], Owsianik G[1], Vriens J[1], Talavera K[1], Van Haute C[1], Nilius B[1], De Ridder D[1]
1. *KULeuven*

FUNCTIONAL CHARACTERISATION OF TRP CHANNELS IN MOUSE UROTHELIUM: A MAJOR ROLE FOR TRPV4

Hypothesis / aims of study

The urothelium is no longer considered as a passive barrier, but as a responsive structure with sensory characteristics. TRP (transient receptor potential) proteins form a large superfamily of cation channels functioning as cellular sensors (1). Recent studies have indicated that several TRP channels are present in the bladder, where they may play a crucial role as sensors of stretch, and chemical irritation (2). In this study we want to determine the functional expression of TRPA1, TRPV1, TRPV2, TRPV4 and TRPM8 as molecular sensors in mouse urothelial cells. This characterisation will increase our insights in the sensory function of the urothelium and may offer new targets for the treatment of sensory bladder dysfunction.

Study design, materials and methods

Isolation of urothelial cells: The preparation of primary urothelial cell cultures was carried out as previously described by others (3). The urothelial nature of the cultured cells was confirmed by a positive staining for cytokeratin 7. Urothelium was collected from 12 weeks old male C57BL/6J wild type and TRPV4$^{-/-}$mice. All animal experiments were carried out in accordance with the European Union Community Council guidelines and were approved by the local ethics committee. Quantitative PCR: Relative quantification of TRPA1, TRPV1, TRPV2, TRPV4 and TRPM8 mRNA was performed using the Taqman technology. mRNA was extracted from cultured urothelial cells, 48h after isolation. *Ca^{2+} imaging:* Ca^{2+} imaging experiments were performed on cultures urothelial cells within 24-48h after the isolation. Responsiveness to Mustard oil (100µM-1mM), capsaicin (1µM), 4αPDD (1µM) and menthol (100µM) was tested. 10µM ATP was used as a positive control at the end of the experiment.

Results

qPCR: Quantification of mRNA extracted from cultured urothelial cells showed very low levels of TRPA1 and TRPV1 mRNA (appearing after cycle 36), but high levels of TRPV4 and TRPV2 mRNA. TRPM8 mRNA was not detected (at cycle 40). Using TRPV1 as an internal calibrator, ΔΔCT analyses showed that TRPV2 mRNA and TRPV4 mRNA were respectively 238 and 3296 times more abundant than TRPV1 mRNA. To exclude that these low levels of TRPA1, TRPV1 and TRPM8 mRNA were due to dedifferentiation of the cells, we performed the same experiments on freshly dissected urothelial tissue. Freshly isolated tissue contained comparably low levels of TRPV1, TRPA1 and TRPM8 mRNA. *Ca^{2+} imaging:* In these experiments 82% of the cells (248/303 cells) responded to 1µM 4αPDD, a known TRPV4 agonist, with an increase in $[Ca^{2+}]_{in}$. These responses to 4αPDD were absent in the absence of extracellular Ca^{2+} and in urothelial cells obtained from TRPV4$^{-/-}$ mice. No responses were detected to mustard oil (0/108 cells), capsaicin (0/197 cells) or menthol (0/102 cells).

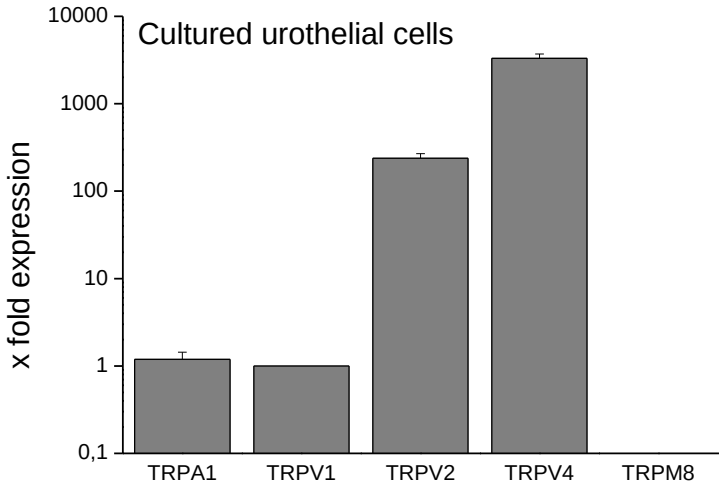

Fig 1. mRNA expression levels, expressed relative to TRPV1

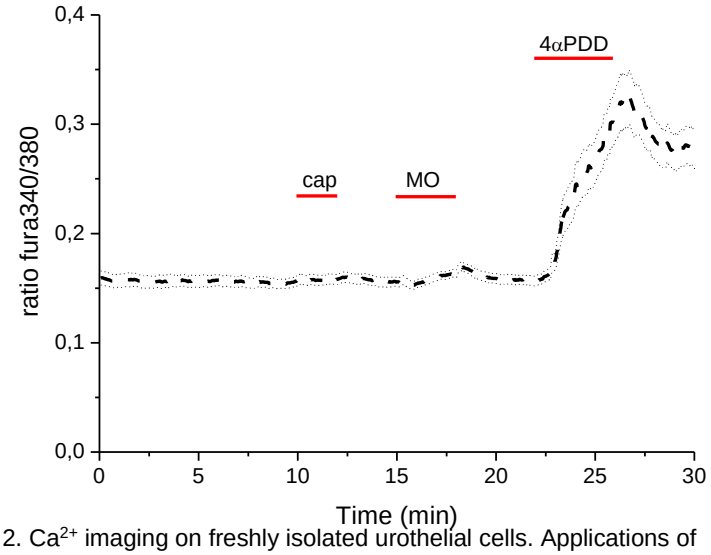

Fig 2. Ca^{2+} imaging on freshly isolated urothelial cells. Applications of 1µM capsaicin (cap), 100µM Mustard Oil (MO) and 1µM 4αPDD.

Interpretation of results

Analyses of mRNA levels of TRP channels in cultured urothelial cells showed that TRPV4 and TRPV2 are the most abundantly expressed TRP channels. TRPA1 and TRPV1 mRNA was detected in very low amounts and TRPM8 mRNA was hardly detected at all. These levels were comparable to mRNA obtained from freshly dissected urothelial tissue, indicating that there are no big fluctuations in TRP channels expression due to dedifferentiation of the cultured cells. Functional analysis using Ca^{2+} imaging showed that the majority (82%) of cultured urothelial cells responded to 4αPDD, indicating an important functional role for TRPV4 in these cells. Due to a lack of specific TRPV2 agonists, the functional expression of this protein could not be tested.

Concluding message

In mouse urothelium TRPV4 seems to be an important TRP channel, making it a possible target in the treatment over sensory bladder dysfunction. TRPV2 is also expressed by urothelial cells, but its function is not yet elucidated.

References

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Is this a clinical trial?	**No**
What were the subjects in the study?	**ANIMAL**
Were guidelines for care and use of laboratory animals followed or ethical committee approval obtained?	**Yes**
Name of ethics committee	**Animal Ethics Committee of KU Leuven**