Functional characterization of transient receptor potential channels in mouse urothelial cells

Wouter Everaerts,1,2 Joris Vriens,1 Grzegorz Owsianik,1 Giovanni Appendino,3 Thomas Voets,1 Dirk De Ridder,2 and Bernd Nilius1
1Department of Molecular Cell Biology, Laboratory Ion Channel Research, and 2Department of Surgery, Laboratory of Experimental Urology, Campus Gasthuisberg, Katholieke Universiteit Leuven, Leuven, Belgium; and 3Dipartimento di Scienze Chimiche, Alimentari, Farmaceutiche e Farmacologiche, Università del Piemonte Orientale, Novara, Italy

Submitted 19 October 2009; accepted in final form 10 December 2009

Everaerts W, Vriens J, Owsianik G, Appendino G, Voets T, De Ridder D, Nilius B. Functional characterization of transient receptor potential channels in mouse urothelial cells. Am J Physiol Renal Physiol 298: F692–F701, 2010. First published December 16, 2009; doi:10.1152/ajprenal.00599.2009.—The bladder urothelium is currently believed to be a sensory structure, contributing to mechanosensation in the bladder. Transient receptor potential (TRP) cation channels act as polymodal sensors and may underlie some of the receptive properties of urothelial cells. However, the exact TRP channel expression profile of urothelial cells is unclear. In this study, we have performed a systematic analysis of the molecular and functional expression of various TRP channels in mouse urothelium. Urothelial cells from control and trpv4−/− mice were isolated, cultured (12–48 h), and used for quantitative real-time PCR, immunocytochemistry, calcium imaging, and whole cell patch-clamp experiments. At the mRNA level, TRPV4, TRPV2, and TRPM7 were the most abundantly expressed TRP genes. Immunohistochemistry showed a clear expression of TRPV4 in the plasma membrane, whereas TRPV2 was more prominent in the cytoplasm. TRPM7 was detected in the plasma membrane as well as cytoplasmic vesicles. Calcium imaging and patch-clamp experiments using TRP channel agonists and antagonists provided evidence for the functional expression of TRPV4, TRPV2, and TRPM7 but not of TRPA1, TRPV1, and TRPM8. In conclusion, we have demonstrated functional expression of TRPV4, TRPV2, and TRPM7 in mouse urothelial cells. These channels may contribute to the (mechanosensory) function of the urothelial layer and represent potential targets for the treatment of bladder dysfunction.

vanilloid receptor; bladder; ion channel

The urothelium is a complex epithelial cell layer lining the inside of the urinary bladder wall. This specialized epithelium is able to maintain a highly impermeable barrier for water, solutes, and pathogens despite large variations in mucosal surface area during urinary storage and voiding (20, 31). Moreover, it is currently believed that the urothelial cells have a sensory role, contributing to mechanosensation in the bladder. Urothelial cells express a large variety of ion channels, including transient receptor potential (TRP) channels (14), as well as purinergic (8), muscarinic (22), and nicotinic receptors (1). In addition, urothelial cells are able to release a number of signaling molecules, such as ATP (13), nitric oxide (NO) (2), and acetylcholine (ACh) (16). During bladder filling, urothelial cells release ATP (13), thereby promoting apical membrane trafficking in neighboring cells (39) and activating nearby sensory nerve fibers (8, 20). Under pathological conditions, the urothelium may contribute to the development of functional bladder disorders such as interstitial cystitis (IC) or overactive bladder (OAB).

TRP channels are unique cellular sensors characterized by highly variable mechanisms of activation. Based on sequence homology, the 28 mammalian TRPs are classified into 6 subfamilies: TRPC (canonical), TRPM (melastatin), TRPV (vanilloid), TRPA (ankyrin), TRPP (polycystin), and TRPML (mucolipin). Importantly, mutations in different TRP genes are linked to human diseases (32, 33). Recent studies have indicated that several TRP channels, namely, TRPV1, TRPV2, TRPV4, TRPM8, and TRPA1, are expressed in the bladder, where they may act as sensors of stretch and/or chemical irritation (10). As such, TRP channels represent potential pharmacological targets for the treatment of bladder dysfunction. The exact distribution of these channels in urothelial cells and their particular role in the generation ofafferent signals is still a matter of debate. In this report we therefore focus on the functional expression of TRP receptors in freshly isolated mouse urothelial cells by using a combination of imaging and electrophysiological techniques.

MATERIALS AND METHODS

Animals. Wild-type (C57Bl/6) and trpv4−/− mice, backcrossed on a C57Bl/6 background, were used. Experiments were performed on bladders from 10- to 12-wk-old mice. All animal experiments were carried out in accordance with the European Union Council guidelines and were approved by the local ethics committee.

Primary urothelial cell culture. Isolation and culture of urothelial cells was performed as previously described by others (4, 37). After euthanasia, bladders were quickly removed, cut open, and stretched out on a Sylgard-coated dish containing MEM (Invitrogen) with 2.5 mg/ml dispase (Invitrogen) for 15 min, and resuspended in keratinocyte medium (Invitrogen). The cell suspension was plated on collagen (type IV; Sigma)-coated coverslips. Cells were used for experiments 12–48 h after isolation.

Quantitative real-time PCR. For quantitative real-time PCR (qPCR) experiments, RNA was isolated from cultured urothelial cells and freshly dissected urothelial tissue. Total RNA was extracted using the RNeasy mini kit (Qiagen). Subsequently, cDNA was synthesized using Ready-To-Go You-Prime first-strand beads (GE Healthcare), and qPCR was performed with the 7500 Fast Real-Time PCR system (Applied Biosystems) using specific TaqMan gene expression assays for TRPA1, TRPM6, TRPM7, TRPM8, TRPV1, TRPV2, and TRPV4 (Applied Biosystems). GAPDH and β-actin were used as endogenous controls (Applied Biosystems). The protocol consisted of 40 replica-
tion cycles. Data represent relative expression of detected mRNAs normalized to TRPV4 mRNA, which was used as a calibrator for comparative analysis.

**Immunocytochemistry.** Urothelial cells from wild-type and trpv4−/− mice were fixated with 3.7% formaldehyde for 10 min, permeabilized with 0.2% Triton X-100 for 10 min, and blocked with 3% BSA for 3 h. Primary antibodies for TRPV4 [1:200, rabbit anti-rat TRPV4 (14)], TRPV2 (2 μg/ml, PC421; Calbiochem), TRPM7 (1:400, ab85016; Abcam), and cytokeratin 7 (CK7; 1:50, clone OV-TL 12/3; Dako) were incubated overnight. The secondary antibodies (1:1,000, Alexa594-conjugated anti-mouse IgG and AlexaFluor488-conjugated anti-rabbit IgG) were applied for 1 h at room temperature. Triple washing with PBS was performed between each step. Finally, the coverslips were mounted in a medium containing 4’,6-diamidino-2-phenylindole. Since we had no access to trpv2−/− mice for negative controls, we used a blocking peptide against the TRPV2 primary antibody (COOH terminal, KNSASEEDHLPLQVQSP; Calbiochem) to check primary antibody specificity. The primary antibody and a 10× excess of the blocking peptide were incubated for 2 h at 4°C before the above-described immunohistochemistry was performed. Images were taken using a confocal microscope imaging system (Zeiss).

**Measurement of intracellular Ca²⁺ concentration.** Cells were loaded with 2 μM fura-2 acetoxyethyl ester for 30 min at 37°C. Intracellular Ca²⁺ concentration ([Ca²⁺]i) was monitored through the ratio of fluorescence measured by alternating illumination at 340 and 380 nm, as described in detail elsewhere (38), using an MT-10 illumination system and cell™ software (Olympus). Calibration was performed as described previously (38). The temperature of the perfusate was controlled using an SC-20 dual in-line heater/cooler (Warner Instruments).

**Patch-clamp experiments.** Membrane currents were measured in the whole cell mode of the patch-clamp technique using an EPC-10 amplifier (HEKA Elektronik, Lambrecht, Germany). Patch electrodes had a direct current (DC) resistance between 2 and 4 MΩ when filled with intracellular solution. An AgCl wire was used as a reference electrode. Currents were sampled at 20 kHz and digitally filtered at 2.9 kHz. Capacitance and access resistance were monitored continuously. Between 50 and 70% of the series resistance was electronically compensated to minimize voltage errors. A ramp protocol was applied consisting of a voltage step from the holding potential of 0 mV to −150 mV, followed by a 400-ms linear ramp to +150 mV applied every 2 s. The voltage-step protocol consisted of 40-ms steps from −80 to +200 mV. The standard extracellular solution for electrophysiological measurements contained (in mM) 150 NaCl, 6 KCl, 1 MgCl₂, 1.5 CaCl₂, 10 glucose, and 10 HEPES, buffered at pH 7.4 with NaOH. The osmolality of this solution, measured with a vapor pressure osmometer (Wescor 5500; Schlag, Gladbach, Germany), was 320 mosmol/kgH₂O. The pipette solution was composed of (in mM) 20 CsCl, 100 Cs-aspartate, 1 MgCl₂, 10 HEPES, 4 Na₂ATP, 10 BAPTA, and 2.93 CaCl₂, adjusted to pH 7.2 with CsOH. For TRPM7 measurements, the pipette solution contained either 0 or 8 mM MgCl₂. The free Ca²⁺ concentration of this solution was 50 nM. This Ca²⁺-containing, K⁺-free intracellular solution efficiently prevented contamination of outward TRPM7 currents by K⁺ currents, such as the recently described ATP-sensitive K⁺ channels (46). For measuring TRPV1 and TRPM8 currents, the extracellular solution contained (in mM) 150 NaCl, 1 MgCl₂, and 10 HEPES, buffered at pH 7.4 with NaOH. All measurements were carried out at room temperature, 22–25°C. Cell membrane capacitance values were used to calculate current densities.

**Reagents.** Capsaicin, icilin, ruthenium red (RR), and mustard oil (MO; allyl isothiocyanate) were purchased from Sigma-Aldrich (Bornem, Belgium). Menthol was acquired from Merck (Darmstadt, Germany), AM251 and AM630 from Cayman Chemical (Ann Arbor, MI), and 4α-phorbol 12,13-didecanoate (4α-PDD) from Alexis Biochemicals (Lausen, Switzerland). Tetrahydrocannabinol (THC) was provided by G. Appendino, and 4α-phorbol 12,13-dihexanoate (4α-PDH) was synthesized as described previously (21). HC067047 was a kind gift from Hydro (Cambridge, MA). Stock solutions were dissolved in DMSO or ethanol.

**Data analyses.** Electrophysiological data were analyzed using PATCHMASTER and FITMASTER programs (HEKA Elektronik, Lambrecht, Germany). For statistical analysis and data display, the Origin 7.1 software package was used (OriginLab, Northampton, MA). Data are means ± SE. The Student’s unpaired, two-tailed t-test was used for statistical comparison, and P < 0.05 was considered statistically significant.

**RESULTS**

**TRP expression in urothelial cells.** Using the qPCR technique, we tested the expression of seven different TRP genes and compared their expression level relative to the TRPV4 mRNA level. We measured the expression of TRPV1, TRPV2, TRPV4, TRPM6, and TRPM7 and compared their expression level relative to the mRNA level of TRPV4. The results showed that TRPV1 and TRPM7 were the most prominent TRP channels. A: the cultured cells were positive for the intermediate filament cytokeratin 7 (CK7; red), confirming their epithelial nature. Blue, 4’,6-diamidino-2-phenylindole (DAPI). Scale bars, 10 μM.
gene. We tested the expression of TRPA1, TRPM8, TRPV1, TRPV2, and TRPV4, which are known to be expressed in the bladder. In addition, we tested the expression of TRPM7, an ubiquitously expressed TRP that was recently suggested to have mechanosensitive properties (35). Finally, TRPM6 was tested to differentiate whether the Mg^{2+}-inhibited currents, observed in our cultured urothelial cells (described below), were mediated by TRPM7 or TRPM6.

The use of cDNA generated from total RNAs isolated from urothelial cells in culture for 48 h revealed that TRPV4 is the most abundantly expressed TRP gene, followed by TRPM7 and TRPV2 (Fig. 1A). Messenger RNAs of three other TRPs were detected (TRPA1, TRPV1, and TRPM6), but their expression levels were more than 1,000 times lower than that of TRPV4 mRNA. TRPM8 mRNA was below the detection level. TRPM8 mRNA was below the detection level. Similar expression patterns of TRP genes were obtained in samples prepared from freshly dissected urothelial tissue (Fig. 1A), indicating that the cultured urothelial cells are a relevant model to study urothelial TRP channel function. Cultured cells were stained for CK7, an intermediate filament protein that is used as a marker for urothelial cells. The majority of cells (~90%) in our culture showed a positive staining for CK7, confirming their urothelial nature (Fig. 1B).

Immunocytochemistry revealed a clear staining of TRPV4 in the membrane of the urothelial cells, with only a weak staining of TRPV4 in the cytoplasm (Fig. 2, A and C). In single urothelial cells, expression of TRPV4 could be detected at the plasma membrane and in the filopodia of the migrating cells (Fig. 2C). These stainings were absent in cells obtained from trpv4^{−/−} mice (Fig. 2B).

TRPM7 expression was detected in the plasma membrane, as well as cytoplasmic vesicles (Fig. 2D), as previously described by others (5, 36). Urothelial cells also stained positive for TRPV2, showing a predominant expression in cytoplasmic compartments (Fig. 2E). The cytoplasmic staining was no longer observed after preincubation of the antibody with a blocking peptide (Fig. 2F).

Fig. 2. Immunocytochemistry on cultured urothelial cells. A and C: TRPV4 (green) is stained in the plasma membrane of confluent (A) and single urothelial cells (C). B: no TRPV4 immunoreactivity was detected in cells from trpv4^{−/−} mice. D: TRPM7 (green) is present in the plasma membrane as well as cytoplasmic vesicles. E: TRPV2 (green) staining shows the presence of TRPV2 in the cytoplasm. F: no TRPV2 staining was detected after incubation of the blocking peptide. Blue, DAPI. Scale bars, 10 μM.
Unfortunately, *trpm7*<sup>−/−</sup> and *trpv2*<sup>−/−</sup> mice were not available to confirm the specificity of these stainings.

**Ca<sup>2+</sup> imaging.** To address the functional expression of TRP channels in urothelial cells, we tested whether these cells responded to known TRP agonists. ATP (10 μM) was used as a positive control.

We recently described the phorbol ester 4α-PDH as a new, very potent TRPV4 agonist (21). At a concentration of 1 μM, this compound produced Ca<sup>2+</sup> influx in most of the wild-type cells (Fig. 3, A, G, and H) but not in urothelial cells from *trpv4*<sup>−/−/−</sup> mice (Fig. 3, B and G). Responses to 4α-PDH were absent when Ca<sup>2+</sup> was omitted from the extracellular solution (Fig. 3C). The responses were blocked by the nonspecific TRPV inhibitor RR (10 μM; Fig. 3D) and the TRPV4-specific inhibitor HC067047 (1 μM; Fig. 3E) (12). When the basal Ca<sup>2+</sup> levels in wild-type and *trpv4*<sup>−/−/−</sup> cells were compared, no differences were observed at room temperature (25°C). However, at 37°C, basal Ca<sup>2+</sup> levels in wild-type cells were significantly higher than in *trpv4*<sup>−/−/−</sup> cells (Fig. 3F), in accordance with the known heat activation of TRPV4 (15, 43).

In line with previously published data, most of the urothelial cells responded to 4α-PDD (1 μM; Fig. 3, G and H), a well-described selective agonist of TRPV4 (41). These responses were not observed in cells derived from *trpv4*<sup>−/−/−</sup> mice (Fig. 3G; see also Ref. 14).

To test for the functional expression of TRPV2, we applied 30 μM THC, a known TRPV2 agonist. Single application of THC produced highly variable responses in urothelial cells from different coverslips. However, when cells were preexposed to a short pulse of ATP (10 μM), THC induced a fast Ca<sup>2+</sup> influx in ~30% of the cells (Fig. 4, A, E, and F). These responses were not observed in the absence of extracellular Ca<sup>2+</sup> (Fig. 4B) and could be blocked by preapplication of RR (30 μM; Fig. 4C). The responses to THC were not influenced by application of the cannabinoid receptor blockers AM251 (80 nM) and AM630 (800 nM; Fig. 4, D–F).

**Fig. 3.** Ca<sup>2+</sup> imaging on wild-type (WT) and *trpv4*<sup>−/−/−</sup> urothelial cells. A–E: examples of 4α-phorbol 12,13-dihexanoate (4α-PDH)-induced changes in intracellular Ca<sup>2+</sup> concentrations ([Ca<sup>2+</sup>]<sub>i</sub>), with each line representing a cell. 4α-PDH (1 μM) induced a Ca<sup>2+</sup> influx in WT (A) but not in *trpv4*<sup>−/−/−</sup> cells (B). Responses were dependent on extracellular Ca<sup>2+</sup> (C) and could be blocked by ruthenium red (RR; D) and HC067047 (E) (12). When the basal Ca<sup>2+</sup> levels in wild-type and *trpv4*<sup>−/−/−</sup> cells were compared, no differences were observed at room temperature (25°C). However, at 37°C, basal Ca<sup>2+</sup> levels in wild-type cells were significantly higher than in *trpv4*<sup>−/−/−</sup> cells (Fig. 3F), in accordance with the known heat activation of TRPV4 (15, 43).

In line with previously published data, most of the urothelial cells responded to 4α-PDD (1 μM; Fig. 3, G and H), a well-described selective agonist of TRPV4 (41). These responses were not observed in cells derived from *trpv4*<sup>−/−/−</sup> mice (Fig. 3G; see also Ref. 14).

To test for the functional expression of TRPV2, we applied 30 μM THC, a known TRPV2 agonist. Single application of THC produced highly variable responses in urothelial cells from different coverslips. However, when cells were preexposed to a short pulse of ATP (10 μM), THC induced a fast Ca<sup>2+</sup> influx in ~30% of the cells (Fig. 4, A, E, and F). These responses were not observed in the absence of extracellular Ca<sup>2+</sup> (Fig. 4B) and could be blocked by preapplication of RR (30 μM; Fig. 4C). The responses to THC were not influenced by application of the cannabinoid receptor blockers AM251 (80 nM) and AM630 (800 nM; Fig. 4, D–F).
As described above, the qPCR assay indicated that the expression levels of TRPV1, TRPA1, and TRPM8 are low. In line with these findings, we did not measure any significant rises in $[\text{Ca}^{2+}]_i$ in response to $1-10\,\mu M$ capsaicin (0/248 cells; Fig. 5A), 100 $\mu M$-1 mM MO (0/144 cells; Fig. 5B), 100 $\mu M$ menthol (0/121 cells; Fig. 5C), or 1 $\mu M$ icilin (0/22 cells; Fig. 5D). From this, we conclude that TRPV1, TRPA1, and TRPM8 are not functional in cultured urothelial cells.

**Patch-clamp experiments.** To further establish the functional expression of the different TRP channels, we directly measured channel activation using the whole cell patch-clamp technique. In urothelial cells from wild-type mice, we measured robust current activation on application of the TRPV4 agonists 4-$\alpha$PDD (5 $\mu M$; $-87 \pm 15$ pA/pF at $-150\, mV$ and $+161 \pm 29.3$ pA/pF at $+150\, mV$) and 4-$\alpha$PDH (2 $\mu M$; $-95 \pm 27$ pA/pF at $-150\, mV$ and $+165 \pm 34$ pA/pF at $+150\, mV$) (Fig. 6, A–F). In contrast, 4-$\alpha$PDD or 4-$\alpha$PDH did not evoke any significant current response in urothelial cells derived from the trpv4$^{-/-}$ mice ($<2$ pA/pF; $n = 4$; Fig. 6, G and H).

In urothelial cells from wild-type mice, application of 30 $\mu M$ THC evoked an outwardly rectifying current ($-17 \pm 3$ pA/pF at $-150\, mV$ and $+30 \pm 3$ pA/pF at $+150\, mV$) with an average reversal potential of $+6$ mV (Fig. 7, A and B). The current-voltage relationship is comparable with earlier described TRPV2 currents (30). A similar current was also observed in urothelial cells derived from trpv4$^{-/-}$ mice ($-13 \pm 4$ pA/pF at $-150\, mV$ and $+27 \pm 6$ pA/pF at $+150\, mV$) (data not shown).

TRPM7 is a ubiquitously expressed TRP channel that is activated when intracellular Mg$^{2+}$ is lowered (29). In cultured urothelial cells, we observed a clear, gradual run-up in current density on dialyzing urothelial cells with a Mg$^{2+}$-free intracellular solution (Fig. 8, A–C). These currents were partially suppressed by high external Mg$^{2+}$ (10 mM; Fig. 8, A and B), indicating involvement of TRPM7 channels. No current density run-up was observed with 8 mM intracellular Mg$^{2+}$ (Fig. 8, D–F). Similar TRPM7-like currents were observed in trpv4$^{-/-}$ cells (data not shown).

In line with the Ca$^{2+}$ imaging experiments, we never observed any increase in current density after applications of capsaicin (10–100 $\mu M$; Fig. 9A), menthol (100–200 $\mu M$; Fig. 9B), and MO (1 mM; Fig. 9C). In these experiments, subsequent application of 4-$\alpha$-PDD or 4-$\alpha$-PDH consistently evoked robust TRPV4 currents.
DISCUSSION

The urothelium is the epithelial layer that lines the urinary tract from the renal pelvis to the urethra. Urothelium is a very specialized epithelium that acts as a permeability barrier, protecting underlying tissues against noxious urine components (20, 25, 31). Recently, an important sensory role was attributed to the urothelium of the bladder. The urothelial cells have mechano- and chemosensory properties and are able to communicate to the underlying afferent nerve fibers (20). In the present report, we provide evidence for the functional expression of three important TRP channels, TRPV4, TRPV2, and TRPM7, in mouse urothelial cells.

TRPV4 was described in detail as a channel activated by hypotonic cell swelling (34) but can be activated by other physical stimuli (shear stress and innocuous warmth, ~27–35°C) and chemical ligands [endogenous (e.g., anandamide and arachidonic acid metabolites; Ref. 42) and synthetic ligands (e.g., phorbol esters; Ref. 33)]. Quantitative RT-PCR experiments revealed that TRPV4 is highly expressed in cultured urothelial cells as well as freshly dissected urothelial tissue. Immunohistochemistry demonstrated a clear expression of TRPV4 in the plasma membrane. In addition, Ca2+ imaging in isolated urothelial cells showed robust Ca2+ influxes in response to the TRPV4 agonists 4α-PDD and 4α-PDH, but only in the presence of external calcium. At higher temperatures (37°C), a lower basal Ca2+ level was observed in trpv4−/− urothelial cells compared with wild type. The abundance of TRPV4 was confirmed in patch-clamp experiments, in which robust increases in current densities were observed after application of TRPV4 activators, whereas no responses were measured in trpv4−/− urothelial cells. These data are consistent with our previous report showing the expression of TRPV4 in the urothelium (14). A functional role of TRPV4 in the bladder was previously demonstrated, based on the observation that trpv4−/− mice exhibit an increased micturition threshold and reduced ATP release in response to bladder stretch (14). These data suggest that TRPV4 can function as an important urothelial mechanosensor, mediating stretch-induced Ca2+ influx and subsequent ATP release.

Our present data provide evidence for the functional expression of TRPV2 in urothelial cells. TRPV2 can be activated by noxious heat (>52°C) (7), 2-aminoethoxydiphenyl borate (2-APB) (30), and THC (30). In serum free conditions, TRPV2 resides in the cytoplasm and is translocated to the plasma membrane by the addition of growth factors such as IGF-1 (19). The exact activation mechanisms and physiological roles of TRPV2 are still controversial and hampered by the lack of specific pharmacology or trpv2−/− mice. RT-PCR and immunocytochemistry revealed the expression of TRPV2 in urothelial cells. During Ca2+ imaging experiments, the onset of responses to THC was very slow and highly variable between cells. Moreover, Ca2+ influx was often observed on washout of the drug. However, when the cells were pretreated with ATP, fast and consistent responses to THC were observed. These responses were dependent on extracellular Ca2+ and could be blocked by preapplication of RR (30 μM), in line with the properties of TRPV2. Possibly, stimulation of urothelial cells with ATP facilitates translocation of TRPV2 from the cytoplasm to the plasma membrane, thereby priming the cells for subsequent THC-induced TRPV2 activation. Similarly, we could measure THC-induced currents in urothelial cells, with properties reminiscent of TRPV2. The Ca2+ influxes observed in response to THC were not influenced by coapplication of AM251 (24) and AM360 (17), antagonists of cannabinoid receptors CB1 and CB2, respectively, indicating these responses are not mediated by urothelial cannabinoid receptors. Although THC can also activate another TRP channel, TRPA1 (18), we could exclude a contribution of TRPA1 to the THC responses in urothelial cells, because no TRPA1 expression was detected and stimulation by MO did not evoke a measur-
able response. Expression of TRPV2 in the urothelium has already been described at the mRNA and protein level in human (6) and rat (3), but no functional data about the expression of TRPV2 in the urothelium have been published yet. Importantly, a role for TRPV2 as a mechanosensor in vascular smooth muscle cells has been described (28), suggesting that TRPV2 may contribute to the mechanosensitive properties of the urothelium.

Furthermore, we have described the functional expression of TRPM7. TRPM7 is ubiquitously expressed and essential for cellular viability. The channel is constitutively open, permeable to Ca\(^{2+}\), Mg\(^{2+}\), and other divalent cations, and can be inhibited by intracellular Mg\(^{2+}\) and Mg\(^{2+}\) nucleotides (29).

Our expression experiments showed a high expression of TRPM7 in urothelial cells, whereas almost no mRNA was detected of TRPM6, a close homolog with similar functional properties. TRPM7 was detected in the plasma membrane as well as cytoplasmic vesicles. Patch-clamp experiments revealed the presence of TRPM7-like, outwardly rectifying currents, with a reversal potential around 0 mV. The currents developed gradually on dialysis of the cell with a pipette solution containing low Mg\(^{2+}\) and were rapidly inhibited by the application of extracellular Mg\(^{2+}\). Interestingly, some reports have reported TRPM7 activation by shear stress and other mechanostimuli (35), raising the possibility that the channel could contribute to sensing urothelial stretch.

Finally, we were unable to detect any functional expression of TRPV1, TRPA1, and TRPM8 in isolated mouse urothelial cells. Our qPCR experiments detected mRNA for TRPV1 and TRPA1, but these amounts were extremely low, suggesting
these channels have limited functional significance. TRPM8 mRNA was, at least in our hands, not detectable at all. Moreover, we were unable to detect any responses to agonists of TRPV1 (capsaicin), TRPA1 (MO), or TRPM8 (menthol) during Ca^{2+} imaging or patch-clamp experiments.

With the use of trpv1−/− mice, it has been shown that TRPV1 plays a major role in regulation of the micturition reflex (4) and mechanical hypersensitivity during cystitis (40). Moreover, as the receptor for intravesical vanilloid therapy, TRPV1 is an important therapeutical target to treat detrusor overactivity (9). It is generally accepted that the therapeutic effect of vanilloid compounds is the result of TRPV1-mediated desensitization of afferent C-fibers (9). However, in addition to its expression on afferent nerve fibers, some authors suggested that TRPV1 is functionally expressed in urothelial cells in human (26), rat (3, 23), and mouse bladder (3), introducing the idea of TRPV1 as a urothelial chemosensor. In our expression and functional data, however, we were unable to confirm a functional role for TRPV1 in urothelial cells. At the mRNA level, TRPV1 could hardly be detected in both cultured urothelial cells and freshly dissected urothelial tissue, and no functional responses could be detected in Ca^{2+} imaging and electrophysiological experiments. These data are in agreement with recent work of other groups that failed to detect TRPV1 expression in urothelial cells at the mRNA, protein or functional level (27, 44, 45). Similarly, TRPM8 and TRPA1 have been described in urothelial cells (23), but we (this work) and others (27) were unable to confirm this.

Several factors may contribute to the discrepancies in the literature concerning the urothelial TRP channel expression profile. First, the urothelial cell isolation and culture technique itself may introduce essential differences. The urothelium is a complex epithelium that consists of three different cell layers: a basal layer, an intermediate layer, and a layer of highly differentiated umbrella cells. Many urothelial cell culture systems fail to induce terminal differentiation of the umbrella cells (37). Moreover, culturing native cells can significantly alter their gene expression. We worked with cells that were only cultured for a short period (12–48 h) and found similar TRP gene expression profiles in cultured cells and freshly dissected urothelial tissue. In both types of tissue, TRPV4, TRPM7, and TRPV2 were most abundantly expressed, whereas TRPV1, TRPA1, TRPM6, and TRPM8 were expressed at very low levels. This suggests that the urothelial culture model we used is suitable to test the functional expression of these TRP channels. Second, nonspecificity of TRP channel antibodies can account for some of the observed differences. For example, it has been well documented that commercially available anti-TRPV1 antibodies show clear immunoreactivity in bladder.
tissue from *trpv1*−/− animals, causing nonspecific signals in immunohistochemistry (11, 45) and Western blot analysis (45). Finally, it has to be noted that studies have been conducted on urothelial cells from various mammals, including mice, rats, guinea pigs, and humans. Species differences and even the genetic background of model animals may potentially influence the TRP gene expression profile.

In conclusion, we have analyzed the functional expression of TRP channels in freshly cultured urothelial cells and found evidence for the functional expression of TRPV4, TRPV2, and TRPM7. These channels may act as mechano- and/or chemosensors in the bladder urothelium. In addition, our work provides evidence that TRPV1, TRPA1, and TRPM8 are not functionally expressed in the urothelial cell layer, suggesting that their contribution to the sensory properties of the bladder is mainly confined to the afferent nerve fibers.

**ACKNOWLEDGMENTS**

We thank all members of the Laboratory of Ion Channel Research for helpful discussions. We thank Feniq Xue for technical assistance. We thank the Cell Imaging Core facility of the Katholieke Universiteit Leuven (KULeuven) for the use of the confocal microscope. We kindly thank Hydra for supplying the HC067047.

**GRANTS**

This work was supported by Belgian Federal Government Grant IUAP PS05, Research Foundation-Flanders (FWO) Grants G.0172.03, G.0149.03, G.0172.03, G.0149.03, GRANTS HC067047.

**REFERENCES**


