Transient Receptor Potential channels in sensory mechanisms of the lower urinary tract

Matthias Vanneste¹, Andrei Segal¹, Thomas Voets¹ and Wouter Everaerts^{2,†}

¹Laboratory of Ion Channel Research, VIB Center for Brain & Disease Research, Leuven, Belgium; Department of Cellular and Molecular Medicine, KU Leuven, Leuven, Belgium.

²Laboratory of Experimental Urology, Department of Development and Regeneration, KU Leuven, Leuven, Belgium

[†]e-mail: wouter.everaerts@kuleuven.be

Abstract

Disruptions to sensory pathways in the lower urinary tract commonly occur and can give rise to lower urinary tract symptoms (LUTS). The unmet clinical need for treatment of LUTS has stimulated research into the molecular mechanisms that underlie neuronal control of the bladder and transient receptor potential (TRP) channels have emerged as key regulators of the sensory processes that regulate bladder function. TRP channels function as molecular sensors in urothelial cells and afferent nerve fibres and can be considered the origin of bladder sensations. TRP channels in the lower urinary tract contribute to the generation of normal and abnormal bladder sensations through a variety of mechanisms, and have demonstrated potential as targets for the treatment of LUTS in functional disorders of the lower urinary tract.

Introduction

Healthy adults are largely unaware that their bladder is constantly filling. Only when urine has accumulated beyond a certain volume do they perceive a sense of bladder fullness and seek a socially acceptable time and place to void. If required, voiding can be postponed for an extended period of time until an urgent desire to void makes further delay impossible. This process repeats itself several times per day, seemingly automatically and without problems¹.

These apparently simple functions of storage and voiding are regulated by complex communication pathways between the lower urinary tract and central nervous system (CNS), which synchronize the activity of the bladder muscle and urinary sphincter. Disruptions in these communication pathways commonly occur and can cause lower urinary tract symptoms (LUTS). Storage LUTS occur during the filling phase of the micturition cycle and include increased urinary frequency, urgency, incontinence and nocturia. Difficulties in passing urine cause voiding LUTS such as hesitancy, intermittency and slow stream, whereas incomplete emptying and postmicturition dribble comprise postvoiding symptoms². LUTS can substantially affect patients' quality of life and are associated with increased rates of stress and depression³. Almost half of adults aged over 40 years report experiencing at least one of these symptoms regularly (a few times per week)⁴. In 2008, 1.9 billion individuals worldwide were estimated to experience LUTS⁵; this high prevalence places a substantial burden on patients and societies. The total direct and indirect costs of care for patients with overactive bladder (OAB) symptoms (a subset of LUTS) in six European countries amounts to €9.7 billion per year⁶.

A high unmet clinical need for treatment of LUTS has fueled research into the molecular mechanisms that underlie neuronal control of the bladder⁷. In the past two decades, members of the transient receptor potential (TRP) channel family have emerged as key regulators of the sensory processes that regulate bladder function. In this Review, we provide a comprehensive overview of the current knowledge of TRP channels in the lower urinary tract (LUT), how they contribute to the generation of normal and abnormal bladder sensations, and how they might be targeted for the treatment of LUTS.

[H1] Transient Receptor Potential channels

TRP channels constitute a superfamily of cation-permeable ion channels present in organisms from green algae to humans^{8,9}. The TRP superfamily comprises a diverse group of cation channels related to the product of the *Drosophila trp* (transient receptor potential) gene¹⁰. Based on structural homology, the 27 human TRP channels are classified into six subfamilies: TRPC (Canonical), TRPV (Vanilloid), TRPM (Melastatin), TRPP (Polycystin), TRPML (Mucolipin) and TRPA (Ankyrin)¹¹⁻¹³. Typically, TRP proteins comprise four subunits forming homomeric or heteromeric channels. Each subunit is composed of six transmembrane-spanning helices (S1-S6) and a cytoplasmatic amino-terminus and carboxy-terminus (Fig. 1). The putative pore region is formed by the loop between S5 and S6 that consists of a pore helix and a selectivity filter, which determines the ion permeation properties of the channel¹¹. A common characteristic of TRP channels is that a single channel can respond to a wide variety of physical stimuli, including changes in temperature^{14,15}, pH¹⁶, osmolarity¹⁷ and mechanical stress¹⁸ as well as to a broad range of chemical ligands^{14,15,19}, enabling channels to function as polymodal cellular sensors.. Notably, the use of a specific threshold temperature to describe the activation threshold for thermosensitive ion channels is being abandoned. Opening of ion channels is a gradual process during temperature changes and the previously used threshold is merely the temperature at which the temperature-activated currents are strong enough to be discriminated from the background noise. Furthermore, this threshold temperature highly depends on the specific experimental settings⁷⁵. Therefore, in this Review no threshold temperatures are described for thermosensitive TRP channels.

Upon activation, the channel's central pore opens and allows cations to flow in or out of the cell, depending on their electrochemical gradient across the plasma membrane. TRP channels are non-selective cation channels, conducting mono- and bivalent cations including Na⁺ and Ca²⁺, with the exception of TRPM4 and TRPM5, which are only permeable to monovalent cations^{20,21}. TRP channels in the urinary bladder are expressed on both excitable cells^{22,23} (sensory neurons and smooth muscle cells) and non-excitable cells²⁴ (for example, urothelial cells).

[H1] Mechanisms of sensing in the bladder

The bladder is able to sense the degree of filling (mechanosensation) and the presence of noxious stimuli, including bacterial toxins, inflammation or overdistension (nociception). This sensory capability enables appropriate timing of storage and evacuation of urine, and relies on a dense network of sensory neurons whose nerve endings are distributed throughout the bladder wall — from the serosa to a dense sub-urothelial layer — and a small number of which penetrate into the urothelium (Fig. 2). The majority of sensory nerve endings are located in the detrusor smooth muscle (~80%) and a smaller subset (~20%) are located in the urothelium and lamina propria^{25,26}. In mice, but not in rats, urothelial and non-urothelial bladder afferent nerve[G] fibres are anatomically distinct populations with very little overlap and different electrophysiological properties^{27,28}. Whether a similar innervation pattern is present in humans remains unknown. Bladder afferent nerve fibres travel through the hypogastric and pelvic nerves to the thoracic (T11-T12) or sacral (S2-S4) dorsal root ganglia (DRG), respectively, where the cell bodies of bladder sensory neurons are located. Hypogastric nerve endings are clustered at the base of the bladder, and pelvic nerve endings are distributed across the bladder²⁶. TRP channels expressed on afferent nerve fibres, urothelial cells and/or smooth muscle cells can contribute to bladder sensation. **(Fig. 2).**

In afferent nerve fibres, cation influx through activated TRP channels induces cell depolarization which in turn can trigger an action potential and neuropeptide release. This action potential can trigger spinal reflexes and/or propagate to the brain to evoke conscious perception of bladder sensations. Most bladder afferents are polymodal and respond to different chemical, thermal and mechanical stimuli depending on the specific receptor subtypes that they express²⁹. Approximately three-quarters of pelvic nerve fibres are mechanosensitive and respond to bladder stretch²⁶.

In urothelial cells, Ca^{2+} influx through TRP channels initiates a myriad of Ca^{2+} -dependent signaling pathways. Accumulating evidence suggests that, although the majority of afferent nerve fibres are located in detrusor muscle, urothelial cells also contribute to mechanosensation and chemosensation in the bladder. When stimulated, urothelial cells release messengers such as purines (ATP, ADP and AMP) and nitric oxide (NO) that stimulate

the neighboring urothelial cells, sensory nerve fibres and potentially the underlying smooth muscle cells, constituting a complex sensory network^{30–33} **(Fig. 2)**.

Detrusor smooth muscle cells are densely innervated by spinal afferent nerve endings and contribute to bladder (mechano)sensation²⁵. During bladder filling, transient contractions of muscle cells occur that generate bursts of afferent nerve activity proportional to the filling pressure of the bladder³⁴. This demonstrates a potential mechanism of signalling bladder distension to the CNS. The relative contribution of smooth muscle cells versus interstitial cells to these sensory pathways and the signalling molecules involved remain elusive.

[H1] TRP channel expression in the bladder

Understanding the specific role of TRP channels in bladder physiology first requires identifying where each ion channel is expressed. Although this objective seems straightforward, no consensus yet exists regarding the exact expression patterns of TRP channels in the bladder wall^{35,36}.

TRPV1 is arguably the most intensively studied TRP channel and is notable for the lack of agreement regarding its expression pattern in the bladder. Expression of TRPV1 in nociceptive nerve fibres in the bladder is generally accepted and intravesical vanilloids have been used to desensitize afferent C-fibres by targeting TRPV1 in patients with neurogenic detrusor overactivity (NDO)³⁷. By contrast, expression of TRPV1 in urothelial cells is not widely accepted. *Trpv1* expression and functional TRPV1-like responses were first reported in isolated urothelial cells from mice³⁸. Subsequently, several groups reported urothelial TRPV1 expression in human^{39–43} and rat⁴⁴ bladder samples, based on Western blot and immunohistochemical data. By contrast, other groups reported no functional TRPV1 expression using calcium imaging of isolated urothelial cells from mice^{24,45,46}, guinea pigs⁴⁷, rats⁴⁸ and humans⁴⁹. Differences in urothelial cell culture between the studies could explain the conflicting results⁵⁰. However, a separate study investigating two *Trpv1*-reporter mouse lines identified TRPV1 expression only in primary afferent neurons and not in urothelial cells⁵¹. Although the reporter mouse lines might have limitations — for example, in reporting low levels of *Trpv1* expression^{52,53} — the absence of *Trpv1* expression in non-neuronal cells has been confirmed using single-cell transcriptomics and bulk transcriptomics^{54,55}. Non-specific immunoreactivity of TRP channel antibodies³⁶, the specific physical and chemical properties of the urothelium and glycosaminoglycan layer^{35,36} and a lack of appropriate controls seem to have contributed to the conflicting conclusions regarding urothelial TRPV1 expression.

Urothelial expression of other TRP channels with established roles in sensory neurons, including TRPM8 and TRPA1, has also been debated^{46,48,56–60}. Another source of data on expression of TRP channel, which could provide further insight, are single-cell transcriptomic datasets from mouse bladder and DRG neurons.

[H2] Single-cell TRP channel transcriptomics

In the past decade, single-cell RNA sequencing has emerged as a powerful method to study gene regulation and has been used as a strategy for unbiased cell type discovery and classification⁶¹. In a landmark study from the Tabula Muris Consortium, single-cell transcriptomic data from 20 different mouse organs and tissues, including the urinary bladder, were collected from >100,000 cells⁵⁵; the data are publicly available from the Tabula Muris Consortium⁵⁴. The cellular expression pattern of TRP channel-encoding mRNA in the bladder can be evaluated using this dataset, which contains single-cell preparations from epithelial and stromal cells from bladder tissue above the bladder neck⁵⁵. However, this dataset lacks specific information on TRP channel expression in bladder smooth muscle cells and in the region of the bladder neck.

Based on their expression profile, urothelial cells (expressing *Epcam* and *uroplakins*) can be separated from mesenchymal cells (expressing *Dcn* and *Vim*) (Fig. 3). Urothelial cells can be further subdivided into cells that express higher levels of uroplakins (for example, *Upk3a* (Fig. 3D)) and *Grhl3*, likely representing intermediate and umbrella cells, and cells that express basal markers such as *Krt14*, which suggests a basal urothelial cell identity.

Trpc1, *Trpc2*, *Trpv2*, *Trpv4*, *Trpv6*, *Trpm4*, *Trpm7*, *Trpml1*, *Trpml2*, *Trpp2* and *Trpp3* transcripts are present in isolated bladder cells (Fig. 3E).

In urothelial cells, *Trpv4* is the most abundantly expressed TRP channel. *Trpv4* mRNA is present in >75% of urothelial cells and <5% of non-urothelial bladder cells⁵⁴. Immunostaining for *Trpv4* in mouse urothelium shows more intense staining in basal urothelial cells than in intermediate and umbrella cells⁶². High levels of *Trpv4* mRNA are in line with functional studies that show robust TRPV4-mediated currents and calcium signals in isolated mouse urothelial cells, as well as clear effects of TRPV4 modulation on bladder function^{24,62–64}. This high urothelial expression makes TRPV4 as an interesting target when studying the specific function of urothelial cells.

Notably, the single-cell transcriptomic study⁵⁴ did not reveal any expression of *Trpv1*, *Trpa1* or *Trpm8* mRNA in mouse urothelium. This lack of expression might seem at odds with the well-documented role of these three TRP channels in bladder physiology and pathophysiology; however, despite early studies reporting functional expression of these TRP channels in urothelial cells^{38,40,57–60}, subsequent molecular and functional studies indicate that these channels are not expressed in urothelial or mesenchymal cells of the

bladder mucosa^{24,46,48,65,66}. Instead, these studies report expression in the sensory nerves that project from the neuronal cell bodies in the lumbosacral DRG neurons into the suburothelial space⁶⁷.

A similar urothelial transcription profile of TRP channel genes can be found in the GenitoUrinary Development Molecular Anatomy Project (GUDMAP) database⁶⁸. In a bulk RNA sequence analysis on FACS-sorted urothelial cells from adult mice, high expression levels of *Trpv4*, *Trpm4*, *Trpm7* and *Mcoln1* (*TRPML1*) have been described, in contrast with very low to undetectable levels of *Trpv1*, *Trpa1* and *Trpa1*⁶⁹.

DRG neurons have also been investigated using unbiased single-cell RNA sequencing^{44,54}. Based on a database containing transcriptomic information from mouse lumbar DRG neurons, one study classified sensory neurons into 11 neuronal types⁷⁰. *Trpv1*, *Trpa1* and *Trpm8* are expressed at various levels in the neural subtypes, whereas expression of *Trpv4* in sensory neurons is limited. Importantly, the neuron population in this database comprises primarily somatic sensory neurons and relatively few visceral sensory neurons. Moreover, the exact spinal level of the neurons is not specified, making it impossible to determine which of these sensory neurons are innervating the urinary bladder. Although analysis of single-cell RNA sequencing data from retrogradely labelled DRG neurons has enabled identification of seven classes of colonic sensory neurons⁷¹, a similar transcriptomic analysis of bladder innervating DRG neurons is not yet available.

These analyses, as well as previously published studies, have helped develop our understanding of TRP channel expression in the urinary bladder (Fig. 4; Table 1).

[H1] TRP channels in bladder urothelium

The specialized cells of the urothelium form an impermeable barrier to water, solutes and pathogens despite large variations in mucosal surface area during bladder filling and voiding. Moreover, urothelial cells contribute to mechanosensation and chemosensation by transmitting information about the mucosal environment to the underlying sensory nerve fibres, interstitial cells and detrusor smooth muscle cells. Several TRP channels enable urothelial cells to respond to changes in their environment^{30–32}.

[H2] TRPV4

TRPV4 is a polymodal ion channel that can be activated by diverse stimuli including hypotonic cell swelling, deflection of the plasma membrane, warm temperatures and endogenous activators including anandamide-derived and arachidonic acid-derived epoxyeicosatrienoic acids^{18,72–74}.

The study of TRPV4 has been facilitated by the description of specific agonists[G] (phorbol esters and GSK1016790A) and antagonists[G] (HC-067047, RN-1734 and the orally bioavailable GSK2193874)^{63,64,76,77}. In addition to bladder urothelium, *Trpv4* expression has been detected in kidney, bone, pancreas, skin, vascular endothelium, neuronal tissue and epithelia of the respiratory tract in mice and rats^{24,62,78–85}.

In the mouse and human bladder, *TRPV4* is expressed in the various cell layers of the urothelium^{24,45,54,62,64,86,87}. The functional expression of TRPV4 channels has been confirmed using calcium imaging and patch-clamp recordings on cultured urothelial cells from mice, rats, guinea pigs and humans; robust calcium influx and ionic currents upon activation of the cells with specific TRPV4 agonists have been observed^{24,45,46,49,62}. Furthermore, mechanical stimuli such as hypotonic cell swelling and urothelial stretch induce TRPV4-mediated calcium influx and subsequent release of ATP^{46,62,88}. TRPV4-mediated ATP release is dependent on Ca^{2+} influx and is mediated by the pannexin–connexin pathway, but not by vesicular transport⁸⁸. TRPV4 activation is responsible for basal ATP release in unstimulated urothelial cells and for approximately half of the ATP release in response to urothelial stretch⁸⁸. TRPV4-mediated ATP release can activate ionotropic purinergic receptors such as P2X₂ and P2X₃ on bladder afferents that project to the spinal cord^{89–91}. Extracellular nerve recordings from mechanosensitive bladder afferents suggest that intravesical activation of TRPV4 increases firing of capsaicin-insensitive C-fibres rather than capsaicin-sensitive C-fibres or Aδ fibres⁹².

Three distinct *Trpv4*-knockout mouse strains have been used to study TRPV4 in vivo^{64,93,94}. Urodynamic investigations in all three knockout strains confirmed a phenotype of decreased voiding frequency, increased voided volume and increased number of non-voiding contractions^{62,64,95}. Similarly, intraperitoneal injection of the TRPV4 antagonist HC-067047 increases the bladder capacity and voided volumes in wild-type mice but not in *Trpv4*-knockout mice⁶³. In combination with the observations that TRPV4-mediated urothelial ATP release is decreased in *Trpv4*-knockout mice, these data support the role of TRPV4 as a urothelial stretch sensor that contributes to bladder mechanosensation in vivo^{46,62}. Unanesthetized, decerebrate *Trpv4*-knockout mice also demonstrated increased non-voiding contractions but without changes in bladder capacity or intercontractile interval, suggesting a role for the forebrain in regulating voided volume⁹⁵.

Our group has recently described the urinary phenotype of the first *Trpv4*-knockout rat strain⁹⁶. Unlike in mice, no changes in baseline cystometric parameters were detected in these rats. Nevertheless, the TRPV4 antagonist HC067047 reduces voiding frequency in rats, similar to the effects observed in mice, emphasizing the importance of species differences and potential compensatory mechanisms when using transgenic animal models. Conversely, intravesical infusion of the TRPV4 agonist GSK1016790A increases the voiding frequency in wild-type rats but not in *Trpv4* KO rats, and has been used successfully to improve bladder function in rats with detrusor underactivity (DUA)⁹⁶.

Variations in *Trpv4* expression levels have been linked to circadian variations in urinary frequency. Expression of *Trpv4* and the mechanosensitive ion channel *Piezo1* follow circadian variations that are regulated by the *Clock* gene⁹⁷. *Clock*^{Δ19/Δ19}-mutant mice with a homozygous deletion in exon 19 of the *Clock* gene lose all circadian rhythms. These mice demonstrate nocturia characterized by frequent urinations during the sleep phase⁹⁸. This phenotype resulted from increased urine production during the sleep phase as well as the loss of circadian changes in urine volume per void. In wild-type mice, but not in *Clock*^{Δ19/Δ19} mice, urine volume per void was significantly higher ($P < 0.05$) in the light than in the dark⁹⁹.

In cultured murine urothelial cells treated with horse serum to induce circadian gene expression, the amplitude of Ca^{2+} influx and urothelial ATP release in response to mechanical stretch varies throughout the day–night cycle^{98,100}. A maximal response was observed 24h after the serum shock, corresponding to the beginning of the dark period^{97,98}. These circadian changes are absent in *Clock*^{Δ19/Δ19} mice and are inhibited in wild-type mice by

blockers of Piezo1 and TRPV4 (GsMTx4 and ruthenium red, respectively)^{97,98}. Thus, circadian changes in expression of TRPV4 and Piezo1 might account for the variable response of urothelial cells to stretch stimuli throughout the day-night cycle⁹⁸.

A role for TRPV4 in innate defence mechanisms against bacterial infections has also been described¹⁰¹. In human and mouse urothelial cells, TRPV4 can be directly activated by bacterial lipopolysaccharide (LPS). Intravesical instillation of LPS leads to an increased voiding frequency in wild-type mice. These acute responses are absent in *Trpv4*-knockout mice but preserved in Toll-like receptor 4 (TLR4)-knockout mice, which suggests a direct role of TRPV4 in rapid detection and subsequent expulsion of LPS-producing bacteria, before an immune response is initiated.

Together, these data support a pivotal role of TRPV4 in urothelial mechanosensation and potentially as a detector of bacterial toxins.

[H2] TRPV2

TRPV2 was initially characterized as an ion channel activated by noxious heat and insulin-like growth factor 1 (IGF1)^{102,103}. Subsequent research revealed that TRPV2 is a polymodal sensor that also responds to hypo-osmolarity and mechanical cell stretch, as well as to chemical ligands including 2-aminoethoxydiphenyl borate (2-APB), probenecid and cannabinoids^{19,104–107}. *Trpv2* is expressed in mouse neuronal tissues, including a subset of DRG neurons and specific brain regions (such as the hypothalamus and supraoptic nucleus), and in non-neuronal cells such as macrophages, endometrial cells, urothelial cells, vascular smooth muscle cells and cardiomyocytes^{24,102,104,108–110}. *Trpv2*-knockout mice lack efficient bacterial phagocytosis, resulting in shorter survival after intraperitoneal injection with *Listeria monocytogenes* bacteria¹¹⁰. However, these mice have normal thermal and mechanical nociception¹¹¹.

In the mouse urinary bladder, *Trpv2* mRNA and protein are present in urothelial cells, albeit at lower mRNA levels than *Trpv4*^{24,54}. TRPV2-like currents occur in cultured urothelial cells upon application of the TRPV2 agonist tetrahydrocannabinol and are unaffected by cannabinoid receptor antagonists²⁴. Similarly, TRPV2 mRNA and protein expression has been reported in the human urothelium¹¹². Interestingly, TRPV2 expression is proportional to disease stage in urothelial cell carcinoma, resulting in a 60-fold increase in TRPV2 expression in pT4 bladder tumours^{112,113}. Moreover, activation of TRPV2 using cannabidiol results in

TRPV2-dependent apoptosis of T24 cells, a poorly differentiated urothelial carcinoma cell line¹¹³. At present, the exact role of TRPV2 in bladder (patho)physiology remains elusive, owing to the lack of specific tools to study its expression and function.

[H2] TRPM7

TRPM7 is a ubiquitously expressed non-selective cation channel that comprises an ion channel fused to a protein kinase, and that acts as a key regulator of Mg^{2+} homeostasis in mammals¹¹⁴. TRPM7 is expressed in virtually all tissues in mammals and is vital for embryonic development¹¹⁵. Non-selective deletion of *Trpm7* in mice results in prenatal death of all embryos before day 7.5 of embryogenesis^{114,116}.

Trpm7 is present at high levels in cultured mouse urothelial cells, and Mg^{2+} -inhibited TRPM7-like currents have been described using whole-cell patch clamping²⁴. The urothelium of mice with urothelium-specific deletion of *Trpm7* is characterized by immature intercellular junctions, increased width between urothelial cells, submucosal oedema and increased inflammatory cytokine secretion, suggesting involvement of TRPM7 in intracellular junction formation¹¹⁷. These urothelium-specific *Trpm7*-knockout mice display reduced bladder capacity and reduced voided volumes¹¹⁷. Abnormal intercellular junctions disrupt urothelial barrier function and result in increased permeability and submucosal inflammation that cause nerve hyperexcitability and bladder overactivity¹¹⁷. Thus, although TRPM7 might not have an intrinsic role in sensory signaling, it is essential for urothelial cell homeostasis and urothelial barrier function.

[H1] TRP channels in bladder nerve endings

Sensory nerve fibres project from primary afferent neurons in thoracic and sacral DRG into the different layers of the bladder wall. TRP channels and other sensory proteins enable these bladder afferents to convert sensory stimuli into action potentials and, therefore, relay the sensory information to the CNS²⁹.

[H2] TRPV1

TRPV1 is best known as the receptor for vanilloids such as capsaicin, the pungent ingredient in chili peppers¹⁴. TRPV1 also responds to heat, changes in pH (extracellular acidification and intracellular alkalization), endogenous ligands such as anandamide and 2-arachidonoylglycerol, and reactive oxygen species, and is, therefore, a molecular integrator of various noxious stimuli^{14,16,118,119}.

Expression of TRPV1 in primary afferent nociceptors with unmyelinated, small diameter sensory nerve fibres (C-fibres) that innervate the skin, muscles and visceral organs is well established in humans^{120,121} and animal models^{14,16,118}. Although wider tissue distribution of TRPV1 — both in the CNS and in non-neuronal tissues — has been claimed in a multitude of studies^{122–124}, generation of *Trpv1* reporter mice indicated that *Trpv1* expression is limited to primary afferent neurons, specific brain regions (for example, the caudal hypothalamus) and arteriolar smooth muscle cells in thermoregulatory tissues^{11,51}. Single-cell RNA sequencing has also confirmed this restricted pattern of expression^{54,55,125}.

Activation of TRPV1-expressing C-fibres generates an acute painful burning sensation. TRPV1-containing C-fibres not only transmit sensory signals to the CNS but also exert efferent functions by releasing neuropeptides such as substance P and calcitonin gene-related peptide^{126–128}. These neuropeptides influence afferent nerve excitability, induce smooth muscle contraction and initiate neurogenic inflammation[G]^{129,130}.

In the lower urinary tract, TRPV1 is robustly expressed in C-fibres that innervate the urethra and the bladder, a subpopulation of which are mechanosensitive^{22,38,39,92,131}. *Trpv1* reporter mice demonstrate TRPV1 expression in sensory nerve fibres and in arteriolar smooth muscle cells in the suburothelium^{51,65}. TRPV1 is highly expressed in smooth muscle cells of small-diameter (15-40 µm) arterioles and application of capsaicin in isolated bladder arterioles causes contraction. This suggests a role for TRPV1 in regulating the perfusion through these bladder arterioles. Arteriolar TRPV1 expression is absent in newborn mice and increases with

age. TRPV1-positive smooth muscle cells per unit length of vesical artery is sixfold higher in female mice than in male mice ($P < 0.001$), however, the functional implications of this expression pattern remain elusive^{51,65}.

Trpv1-knockout mice have been extensively used to study the functional role of TRPV1 in normal voiding behavior^{123,132}. Although expression of TRPV1 in bladder afferents is firmly established, the specific functional alterations in mice with genetic disruption of *Trpv1* differ between studies. In the initial report describing the urinary phenotype of *Trpv1*-knockout mice, urodynamic analysis revealed an increased number of non-voiding contractions and larger bladder capacity in awake *Trpv1*-knockout mice than in wild-type controls, whereas reflex bladder contractions were absent in 45% of anesthetized *Trpv1*-knockout mice, resulting in overflow incontinence¹²³. Subsequent studies in anesthetized¹³³ and decerebrated⁹⁵ mice did not report changes in bladder capacity but confirmed an increased number of non-voiding contractions in *Trpv1*-knockout animals. Extracellular nerve recordings of sensory fibres in ex vivo bladder preparations have demonstrated attenuated responses to bladder distension¹³⁴ and to ab-Methylene-ATP (an agonist of purinergic receptors) in *Trpv1*-knockout mice as well as in wild-type animals treated with the TRPV1 antagonist capsazepine. Moreover, bladder distension with saline containing 300 μ M histamine in ex vivo preparations increased responses in a subpopulation of mechanosensory afferents compared with distension with saline and recruited so-called silent afferents that were previously unresponsive to bladder filling with saline. These responses to histamine were not observed in *Trpv1*-knockout mice, suggesting that histamine sensitizes bladder afferents to distension through interaction of the histamine H1 receptor and TRPV1¹³⁵.

Together, these data suggest that TRPV1 in sensory afferents of the bladder modulates P2X-mediated afferent responses rather than having a direct role in mechanosensation¹³⁶. Moreover, TRPV1 acts as a sensor for noxious stimuli and modulates the excitability of silent C-fibres.

[H2] TRPA1

TRPA1 is also a polymodal sensor of noxious stimuli, including irritant compounds (for example, allyl-isothiocyanate, allicin and cinnamaldehyde), bacterial endotoxins^{137–139} and

endogenous activators of inflammation and oxidative stress (for example, H₂O₂, nitrooleic acid)^{140–142}. In addition, both cold and hot temperatures can activate TRPA1^{143–145}.

TRPA1 is mainly expressed in nociceptive neurons^{138,146–149} and plays a pivotal role in pain perception, including in osteoarthritis, gout and pancreatitis as well as in neuropathic pain (such as in diabetic neuropathy and chemotherapy-induced neuropathy) and migraine^{150–152}. TRPA1 is involved in the acute hyperalgesic phase of inflammatory pain as well as the maintenance of nociceptor hypersensitivity leading to chronic pain¹⁵⁰.

In the urinary tract, TRPA1 expression has been confirmed in unmyelinated C-fibres in the urothelium, suburothelium and muscle layer of rats, where it co-localizes with TRPV1 and the neuropeptides CGRP and substance P⁵⁷. Retrograde labelling of bladder afferent neurons has demonstrated expression of TRPA1 in DRG neurons at the level L6-S1¹⁵³.

Intravesical instillation of the TRPA1 agonists cinnamaldehyde and allyl isothiocyanate dose dependently increases voiding frequency in rats and mice^{57,153,154}. Similarly, intravesical allyl isothiocyanate increases the firing rate of A δ -fibre and C-fibre bladder afferents during bladder filling¹⁵⁵. However, in *Trpv1*-knockout, *Trpa1*-knockout and *Trpv1/Trpa1* double-knockout mice, the effects of allyl isothiocyanate are not only mediated via TRPA1, but also via TRPV1¹⁵⁴. Intravenous injection of HC-030031, a selective TRPA1 antagonist, does not alter cystometric parameters; however, it decreases the firing rate of C-fibres and A δ fibres during bladder filling^{155–157}. *Trpa1*-knockout mice have a normal water intake, normal urodynamic characteristics and normal voiding behavior under physiological circumstances^{154,158,159}. These data suggest that TRPA1 does not play a substantial role in normal bladder function but is a detector of noxious stimuli that can contribute to bladder pain perception and nociceptive behavior.

[H2] TRPM8

TRPM8 is a cold-activated channel that is sensitive to cool temperatures and cooling agents such as menthol, eucalyptol or icilin^{15,160}. TRPM8 is highly expressed in pain-sensitive and cold sensitive A δ -fibres and C-fibres¹⁶¹ that innervate the skin and visceral organs, and in non-neuronal tissues including the prostate, brown adipose tissue and skin^{15,160,162–164}. In the somatosensory system, TRPM8 contributes to cold avoidance, cold-induced pain behavior and cooling-induced analgesia^{165–167}. TRPM8 is also involved in brown adipose tissue thermogenesis¹⁶⁸ and body temperature homeostasis¹⁶⁹. Several selective synthetic

antagonists such as AMBT, PMBC, PF-05105679, KPR-2579 and RQ-00434739 have been used to study the function of TRPM8^{170–174}.

In the genito-urinary tract, *TRPM8* (originally *TRP-p8*) was first cloned from a human prostate-specific library and found to be expressed in epithelial cells and upregulated in prostate carcinomas¹⁶³. TRPM8 is present in a subset of bladder-innervating thoracolumbar and lumbosacral DRG neurons in guinea pigs and rats^{174,175}, and the majority of these neurons are small-diameter A-fibre and C-fibre neurons (cross-sectional area <600µm²)¹⁷⁵.

Intravesical infusion of menthol or cold saline (3°C) reduces the threshold volume for triggering micturition^{174,176,177}. These effects of intravesical cold or menthol are overcome by administration of PMBC, a selective TRPM8 antagonist¹⁷⁴. Moreover, administration of RQ-00203078, another selective TRPM8 antagonist, increases bladder capacity and reduces the activity of mechanosensitive bladder afferents¹⁷⁶.

Mice with genetic disruption of the *Trpm8* gene have normal bladder function with intravesical pressures and voiding frequency similar to wild-type controls¹⁵⁹. Nevertheless, TRPM8 seems essential for bladder responses to intravesical or external cold application¹⁵⁹. Although TRPM8 is considered the cold sensor that mediates the pathological ice-water reflex and cold-induced LUTS, the physiological role of TRPM8 in the lower urinary tract remains unclear.

[H2] TRPC1/TRPC4

TRPC1 is expressed broadly in mammalian cells, but whether TRPC1 monomers are capable of forming functional homomeric channels or whether TRPC1 has a regulatory function on other TRP channels is not yet clear¹⁷⁸. TRPC1 forms heteromeric TRPC1–TRPC4 and TRPC1–TRPC5 channels¹⁷⁹. TRPC1–TRPC4 channels are activated by stimulation of G_{q/11}-coupled receptors such as the muscarinic acetylcholine receptors M₁ and M₃ and are non-selective cation channels permeable to Ca²⁺, Na⁺, Li⁺ and Cs⁺^{179,180}. Similarly, TRPC1–TRPC5 channels are non-selective cation channels activated by stimulation of G_{q/11}-coupled receptors^{180,181}. TRPC4 and TRPC5 can also assemble as homomeric or heteromeric channels with other TRPC monomers such as TRPC3 and TRPC6^{182,183}. TRPC4 proteins are widely expressed in vascular endothelial cells, gastro-intestinal smooth muscle cells and neuronal cells in the hippocampus and olfactory bulb^{182,184–187}. TRPC5 proteins are predominantly expressed in the brain^{181,183,188}.

Using retrograde labelling, TRPC1 and TRPC4 expression has been demonstrated in L6-S1 DRG neurons innervating the urinary bladder in rats. Moreover, mRNA levels of *Trpc1* ($P < 0.01$) and *Trpc4* ($P < 0.05$) are significantly increased in a rat model of cyclophosphamide-induced cystitis¹⁸⁷. In the same study, *Trpc1/Trpc4* double-knockout mice treated with cyclophosphamide, demonstrated reduced neural sprouting of sensory afferents compared with wild-type controls. Moreover, knockout mice did not develop cyclophosphamide-induced cystitis. These data indicate that that TRPC1 and TRPC4 are crucial mediators for nerve outgrowth and detrusor hyperreflexia in mice with chemical cystitis¹⁸⁷.

[H1] TRP Channels in detrusor smooth muscle

Detrusor smooth muscle cells relax during bladder filling and contract to expel urine during voiding. Several TRP channels are expressed in smooth muscle cells but their exact role remains elusive.

[H2] TRPM4

TRPM4 is expressed in immune cells (lymphocytes, mast cells and dendritic cells), neurons, cardiomyocytes, keratinocytes and many other cell types^{20,189–192}. *Trpm4*-knockout mice present with increased mast cell activation and anaphylactic responses after IgE-receptor stimulation¹⁹¹. Furthermore, *Trpm4*-knockout mice are protected against formation of secondary hemorrhage after spinal cord injury and are less susceptible to neuronal injury in autoimmune encephalomyelitis than wild-type mice^{193,194}. In cardiomyocytes, TRPM4 contributes to normal cardiac rhythmicity and conduction. Despite atrial myocytes from *Trpm4*-knockout mice having a shortened action potential, cardiac parameters of *Trpm4*-knockout mice are similar to wild-type mice^{195–197}. In humans, mutations in *TRPM4* are associated with cardiac conduction disorders and erythrokeratoderma^{198–200}. In spite of these well-established roles of TRPM4 in both murine and human disease, the function of TRPM4 in the bladder remains unclear.

In the rodent urinary bladder, *Trpm4* mRNA and protein have been detected in the urothelium and in detrusor smooth muscle cells^{23,54,66,201}. TRPM4 mRNA has also been detected in human detrusor smooth muscle cells²⁰². In freshly isolated rat²³, guinea pig²⁰¹ and human²⁰² detrusor smooth muscle cells, application of the TRPM4 antagonist 9-phenantrol reduces TRPM4-like currents and intracellular Ca^{2+} levels. Moreover, 9-phenantrol inhibits contractions of isolated rat, guinea pig and human bladder muscle strips in response to carbachol and electrical-field stimulation, suggesting a role for TRPM4 in regulating detrusor smooth muscle cell excitability^{23,201,202}. Application of the TRPM4 antagonist 9-phenantrol reduces the amplitude and frequency of spontaneous phasic detrusor smooth muscle contractions more in human than in rat ($P < 0.01$) or guinea pig ($P < 0.01$) isolated muscle strips²⁰². This suggests a possible difference in TRPM4 function in humans versus rodents despite a similar expression pattern. In mice with spinal cord injury, isolated bladder strips display increased spontaneous activity associated with TRPM4 upregulation ; increased spontaneous activity was reduced by application of the TRPM4

antagonist 9-phenantrol²⁰³. However, 9-phenantrol also affects several other ion channels in smooth muscle cells, including the Ca²⁺-activated chloride channel TMEM16A (also known as ANO1), L-type Ca²⁺ channels, delayed outward rectifier K⁺ channels and Ca²⁺-activated K⁺ channels^{204–207}. Thus, the effects of 9-phenantrol on smooth muscle contractility cannot be solely attributed to TRPM4. Stricter controls and more specific agonists are required to uncover the precise role of TRPM4 in detrusor smooth muscle and urothelial cells.

[H2] TRPV4

The function of TRPV4 in detrusor smooth muscle cells is debated. Although *Trpv4* mRNA and protein have been detected in mouse and guinea pig smooth muscle tissue, these are present at lower levels than in the urothelium^{64,88}. TRPV4 immunoreactivity in detrusor muscle and contractile responses to the TRPV4 agonist GSK1016790A have been demonstrated in one study; however, other studies have failed to provide evidence for functional TRPV4 expression in detrusor smooth muscle^{62,64,84,96,208}. This discrepancy in the literature could be caused by differences in the experimental set-up, such as the composition of organ bath solutions, or the technique that was used to separate the urothelial cells from the smooth muscle cells.

Platelet-derived growth factor α -positive (PDGF α^+) interstitial cells also express TRPV4²⁰⁸. PDGF α^+ cells are present in the lamina propria and detrusor muscle between smooth muscle bundles, often in close association with nerve fibres²⁰⁹. Activation of TRPV4 using GSK1016790A, cell stretch or hypo-osmotic solutions induces Ca²⁺-influx into PDGF α^+ cells, activating Ca²⁺-activated K⁺ (SK) channels and causing hyperpolarization of the cell membrane. Applying GSK1016790A to isolated bladder strips decreases spontaneous activity; this decrease in activity can be inhibited by the SK channel inhibitor apamin²⁰⁸. Similarly, in a whole-bladder ex vivo preparation, contractions during bladder filling are inhibited by GSK1016790A and inhibition of SK channels using apamin increases spontaneous detrusor activity. This increase is more pronounced in the presence of GSK1016790A. Overall, these data suggest that TRPV4 activation during bladder filling inhibits spontaneous bladder contractions through activation of SK channels in PDGF α^+ interstitial cells, which enables the filling bladder to accommodate large rises in volume

without a rise in pressure. The mechanism through which hyperpolarization of PDGF α^+ interstitial cells could lead to detrusor relaxation requires further elucidation.

[H2] TRPC4

Trpc4 mRNA has been detected in isolated mouse detrusor smooth muscle cells²¹⁰. Carbachol, a muscarinic acetylcholine receptor agonist, stimulates an influx of Ca²⁺ into mouse detrusor myocytes which is blocked by application of the TRPC4 antagonist ML204²¹⁰. Moreover, ML204 inhibits isometric contractions of isolated mouse detrusor muscle strips in response to pharmacological and electrical stimuli^{210,211}. Knockout of *Trpc4* abolishes spontaneous activity in isolated detrusor strips and reduces the amplitude of muscle contractions in response to cholinergic stimulation²¹¹. Surprisingly, contractions in response to muscarinic stimuli are also reduced in *Trpc4*-knockout mice on application of ML-204. These effects might be explained by the inhibitory effects of ML204 on TRPC5, which is not expressed in normal detrusor smooth muscle cells but is upregulated in detrusor smooth muscle cells of *Trpc4*-knockout mice^{210,211}. More specific TRPC4 agonists and antagonists are therefore essential to study the effect of TRPC4 activation or inhibition in the bladder without causing off-target effects on other TRP channels such as TRPC5. Ideally, *Trpc4*-knockout mice should always be used to verify TRPC4-specificity of any observed response. The current lack of specific TRPC4 (agonists and antagonists) has hampered a better understanding of the role of TRPC4 in bladder (patho)physiology.

[H1] Prostatic and urethral TRP channels

The urethra and prostate (in men) form the outflow tract of the bladder. In these organs, TRP channels are expressed on urothelial cells, stromal cells and afferent nerves.

[H2] Prostate

TRP channels have been described in the prostatic stroma and the prostatic urethra, as well as in the afferent nerve fibres that innervate both these structures.

Notably, many studies have described various roles for TRPV1, TRPV2, TRPV6, TRPC6, TRPM4, TRPM7 and TRPM8 in prostate cancer^{212–218}. However, this topic is beyond the scope of this Review.

In rat homogenized prostate tissue, mRNA expression of *Trpm2*, *Trpm3*, *Trpm4*, *Trpm6*, *Trpm7*, *Trpm8*, *Trpv2* and *Trpv4* has been detected²¹⁹. Although *Trpv1* mRNA was detected at very low levels, fluorescence immunohistochemistry reported high expression levels of TRPV1 in epithelial cells and smooth muscle cells. This discrepancy in expression patterns likely reflects poor antibody specificity of TRPV1 antibodies, which causes aspecific staining, whereas in reality TRPV1 is only expressed at low levels as indicated by the mRNA expression³⁶. Based on the human protein atlas (a collection of three RNA sequencing databases), *TRPM8*, *TRPM4*, *TRPP1*, *TRPV6* and *TRPC1* are the TRP channels with highest mRNA expression in the human prostate^{220,221}. Expression of *TRPV6* in the prostate is debated as some studies have detected *TRPV6* expression in prostate cancer cells but not in healthy prostatic tissue^{213,219}. The lack of validated antibodies has resulted in very few immunohistochemistry studies that have examined the cellular and subcellular localization of these TRP channels expressed at the RNA level in the prostate (such as *TRPM8*, *TRPM4*, *TRPP1*, *TRPV6* and *TRPC1*).

For the past ten years, functional research has focussed primarily on the roles of TRPV1 and TRPA1 in models of prostate inflammation. In humans, TRPV1-immunoreactive nerve fibres are present in the lamina propria of the prostatic urethra and in the prostatic stroma²²². In a model of experimental autoimmune prostatitis (EAP), wild-type mice sensitized with rat prostate antigen developed pelvic allodynia that was reversed by intra-urethral application of the TRPV1 antagonist heptapeptide L-R4-W₂²²³. By contrast, *Trpv1*-knockout mice do not exhibit pelvic allodynia despite having a similar degree of prostatic inflammation²²³. In rats

with EAP, expression of TRPV1 was increased not only in the prostate, but also in L5-S1 DRG neurons as well as in bladder tissue²²⁴. Additionally, nerve growth factor (NGF) and tropomyosin receptor kinase A (TrkA) were upregulated in DRG neurons from EAP rats²²⁴. Similarly, a separate study showed that retrogradely labelled rat DRG neurons innervating the bladder and prostate demonstrate increased *Trpv1*, *Trpa1* and *P2rx2* mRNA levels and become hyperexcitable after induction of chemical prostatitis²²⁵. These data support a pathway of cross-sensitization from the prostate to the bladder that could induce bladder overactivity during prostatitis²²⁵.

TRPA1 is expressed on sensory nerve fibres between stromal smooth muscle bundles and around the glandular structures⁵⁹. TRPA1 agonists do not affect tension of human prostatic smooth muscle strips under baseline conditions; however, these agonists dose-dependently relax muscle strips precontracted by phenylephrine or electrical stimulation⁵⁹. To date, experiments have lacked adequate controls (such as antagonists) to determine whether the observed effects are truly the results of TRPA1 activation⁵⁹.

Taken together, these data support a functional role of nerves expressing TRPV1 and TRPA1 in nociceptive responses during prostatitis. Whether TRPV1 or TRPA1 merely act as signal amplifiers or initiate these responses requires further elucidation. The TRP channels with the highest prostatic RNA expression, TRPM8 and TRPM4, have no known function in the prostate besides upregulation in prostate cancer cells, which is beyond the scope of this Review. Many studies have focused on the role of TRP channels in prostate cancer but only few have investigated their role in normal prostatic tissue. This lack of research represents an important knowledge gap that should drive future studies.

[H2] Urethra

In contrast to the bladder, relatively few studies have investigated urethral sensation and afferent nerve signalling. Urethral afferents that respond to urethral distension or flow are located in the pelvic and pundental nerves²²⁶. Urine entering the urethra at low bladder filling activates the guarding reflex — a spinal reflex that contracts the external urethral sphincter and relaxes the detrusor muscle to maintain urinary continence. By contrast, urine

flow in the urethra at high bladder volumes will stimulate detrusor contraction (micturition reflex) and relax the external urethral sphincter (augmenting reflex)²²⁷, allowing urination.

Trpv4 mRNA and protein expression has been described in the urothelium lining the urethra of rats and mice^{62,228,229}. The specific role of TRPV4 in the urethra remains elusive. As TRPV4 is a sensor for shear stress and flow in blood vessels and renal tubules²³⁰, a similar role as a urethral flow sensor has been suggested.

TRPV1 and TRPA1 are expressed in sensory nerve fibres that innervate the muscular wall and suburothelium of the human urethra⁵⁹. In rats, TRPV1-expressing peptidergic afferent fibres innervate the mucosal and muscular layers of the urethra²². Capsaicin, a TRPV1 agonist, induces relaxation of canine urethral smooth muscle strips precontracted with noradrenaline and of rat external urethral sphincter muscle²³¹. Similarly, human urethral muscle strips precontracted with phenylephrine are dose-dependently relaxed by TRPA1 agonists²³². These data suggest that activation of nociceptive afferents expressing TRPV1 and/or TRPA1 stimulates urine expulsion by contracting the bladder^{123,154} and relaxing its outlet. The endogenous stimuli for activating TRPV1 and TRPA1 have not yet been determined.

Similar to airway epithelium, the inner lining of the urethra contains specialized polymodal chemosensory brush cells²³³. In the respiratory tract, these cells detect potentially harmful bitter substances, such as acyl-homoserine lactones produced by Gram-negative bacteria, and trigger protective reflexes^{234,235}. Similarly, the bitter compound denatonium and heat-inactivated *Escherichia Coli* both trigger intracellular calcium rises in urethral brush cells²³³. When triggered, these cells release acetylcholine, which stimulates neighboring urothelial cells and sensory nerve fibres that penetrate the urothelium and make close contact with brush cells²³³. Furthermore, intraurethral instillation of denatonium in rats induces bladder contractions, causing washout of the urethral content²³³.

TRPM5 is a calcium-activated monovalent cation-permeable channel that is an important component of the taste transduction cascade in taste buds and is expressed in pancreatic β -cells²³⁶. TRPM5 expression has also been demonstrated in urothelial brush cells and pharmacological inhibition of TRPM5 reduces the response of urethral brush cells to

denatonium, suggesting that, similar to taste buds, TRPM5 regulates the response of urethral brush cells to hazardous stimuli such as bacterial toxins.

Compared with the urinary bladder, knowledge of TRP channels in the prostate and urethra remains very limited. An improved understanding of the expression and function of TRP channels in the prostate and urethra is essential to develop a detailed and comprehensive overview of the role of TRP channels throughout the entire LUT.

[H1] TRP channels as targets in LUTS

Several TRP channels have a pivotal role in the generation of bladder sensations and are, therefore, interesting targets for pharmacotherapies in patients with LUTS. Animal models have been developed to study various LUTS. These models, although rightfully criticized for their discrepancies with the clinical setting, are currently the best available preclinical models to study alterations in the storage phase of the micturition circle.

[H2] Detrusor Overactivity

Patients with OAB typically complain of urgency with or without incontinence and other storage symptoms, such as frequency and nocturia. As these subjective symptoms cannot be assessed in animal models, preclinical models focus on urodynamic alterations that indicate detrusor overactivity and on increased voiding frequency as surrogate models for OAB.

[H3] Cystitis-induced detrusor overactivity

Inflammation of the bladder is usually accompanied by storage symptoms (urgency and frequency) and suprapubic painful or burning sensations. Several models of cystitis have been developed that induce hypersensitivity of bladder afferents and detrusor hyperreflexia²³⁷.

LPS, an important component of the outer membrane of gram-negative bacteria, triggers the innate immune response via activation of TLR4^{238,239}. Intravesical instillation of LPS in mice induces bladder inflammation typified by increased mucosal thickness, vascular congestion and cellular infiltration²⁴⁰. LPS-treated mice demonstrate increased reflex voiding frequency and increased spinal *c-Fos* expression^{240,241}. *c-Fos* is an immediate early gene that is activated rapidly after cell stimulation and is a transcription factor that modifies the long-term expression of target genes²⁴². Triggers for *c-Fos* expression include noxious stimulation (such as electrical stimulation of the paw or intraperitoneal acetic acid injection) as well as osmotic stimulation, stress, amphetamine administration and others^{242,243}. *Trpv1*-knockout mice treated with LPS developed a similar degree of histological bladder inflammation as LPS-treated wild-type controls, but do not exhibit changes in voiding frequency or spinal *c-Fos* expression²⁴⁰. Similarly, 24–48 hours after intravesical instillation of LPS voiding frequency as well as pain behavior (licking and biting of the skin at the lower abdomen) is increased in wild-type mice but not in *Trpa1*-knockout, whereas histological signs of cystitis were similar between both groups¹⁵⁸.

Interestingly, instillation of LPS during cystometry immediately (within minutes) increases voiding frequency in wild-type mice but not in *Trpv4*-knockout mice. These rapid effects are not mediated by the TLR pathways, but by the direct effect of LPS on TRPV4¹⁰¹.

Overall, these studies suggest a role for TRPV4 in the acute (within minutes) reaction to bacterial exposure, and that TRPV1 and TRPA1 mediate the subsequent (after several hours) increase in afferent signalling during cystitis.

In the frequently used model of cyclophosphamide-induced cystitis, hemorrhagic cystitis is induced by injections of cyclophosphamide — an alkylating agent used as a chemotherapeutic drug as well as an immunosuppressant²⁴⁴. Following systemic administration, cyclophosphamide is metabolized to acrolein in the liver and excreted through the kidneys²⁴⁴. Contact between acrolein and the urothelium causes irritation and inflammation that results in pain behavior, increased *c-Fos* expression and increased bladder reactivity, such as non-voiding contraction and increased voiding frequency^{63,244}. In mice with cyclophosphamide-induced cystitis, wild-type and *Trpv4*-knockout mice developed similar bladder inflammation. However, in *Trpv4*-knockout mice, alterations in bladder function and *c-Fos* expression in response to cystitis were reduced. Furthermore, the TRPV4 antagonist HC-067047 significantly reduced voiding frequency in wild-type mice (from 2.8 to 1.9 voids per 5 minutes, $P < 0.01$) and wild-type rats (from 2.76 to 1.90 voids per 5 minutes, $P < 0.01$) with cyclophosphamide-induced cystitis. These effects were absent in *Trpv4*-knockout mice, supporting the specific role of TRPV4 in the development of detrusor hyperreflexia during cystitis⁶³.

One study used different models of chemically-induced detrusor overactivity to demonstrate that TRPM8 antagonists reduce detrusor overactivity and afferent activity in mechanosensitive C-fibres¹⁷³. Intravenous pretreatment with RQ-00434739, a potent and selective TRPM8 antagonist, reduced C-fibre afferent nerve firing in response to intravesical infusion of menthol or prostaglandin E2¹⁷³. Similarly, pretreatment with the TRPM8 antagonist KPR-2579 inhibited detrusor overactivity and C-fibre hyperactivity during intravesical acetic acid instillation²⁴⁵ supporting a function of TRPM8 as a mediator of C-fibre hyperactivity and the resulting detrusor overactivity.

Taken together, these data indicate that TRPV1, TRPA1, TRPV4 and/or TRPM8 mediate the effects of bladder inflammation on detrusor overactivity in rodents. However, whether these channels can be targeted to treat detrusor overactivity in humans with inflammatory bladder disorders is still unknown.

[H3] Stress-induced bladder dysfunction

Psychological and social stress can influence bladder function. In addition to exacerbating symptoms of existing bladder diseases such as bladder pain syndrome (BPS), social stress can also induce dysfunctional voiding —typically in children but sometimes continuing into adulthood^{246–249}.

Depending on the duration of the stress stimulus, mice exposed to social stress have been observed to develop either detrusor overactivity or underactivity²⁵⁰. After 1-hour daily exposure to a breeder aggressor mouse for 2 weeks, submissive mice developed bladder overactivity with decreased intermicturition intervals and increased afferent nerve activity²⁵¹. This stress-induced bladder dysfunction can be reversed by treatment with the TRPV1 antagonist capsazepine^{251,252}. Conversely, in an intensified social stress model submissive mice exposed to aggressor mice for 23 hours per day for 2 weeks developed prolonged intermicturition intervals, larger bladder volumes and increased collagen deposition²⁵². *Trpv1*-knockout mice do not develop functional or structural bladder changes in response to mild or intensified stress²⁵², suggesting that TRPV1 has an essential role in the generation of social stress-induced bladder dysfunction.

In rats, similar models of social stress have been shown to induce functional bladder disorders^{253,254}. Rats exposed to different stressors for 5–60 minutes per day for 7 days developed bladder dysfunction characterized by decreased bladder capacity and increased voiding frequency. In these rats, *Trpv4* mRNA and protein levels were increased in the urothelium but not in detrusor smooth muscle tissue²⁵⁴. Intravesical infusion of the TRPV4 antagonist HC-067047 reversed the reduction in intercontractile intervals and voided volume in animals with stress-induced bladder dysfunction, suggesting TRPV4 inhibition as a potential treatment for stress-induced detrusor overactivity²⁵⁴.

[H3] Neurogenic detrusor overactivity

Electrophysiological experiments in cats and rats have demonstrated that TRPV1-expressing C-fibres, which are silent under physiological conditions, are activated after the induction of spinal cord injury and constitute the afferent arc of a spinal reflex that causes NDO²⁵⁵. Desensitization of these C-fibres by systemic capsaicin treatment successfully suppressed NDO in spinal cord-injured animals by interrupting the pathological reflex²⁵⁶. In humans, exposure to high concentrations of capsaicin has been shown to activate TRPV1 channels and generate a strong influx of calcium that produces long-lasting functional desensitization of C-fibres and even morphological evidence of neuronal damage²⁵⁷. A meta-analysis of three randomized controlled trials involving a total of 65 patients reported that treatment of patients with neurogenic detrusor overactivity using intravesical capsaicin improved symptoms, including incontinence episodes (mean difference in incontinence episodes per 24h -2.16 , 95% CI -2.87 to -1.45) and voiding frequency (mean difference in voids per 24h -0.54 , 95% CI -1.03 to -0.05), compared with placebo²⁵⁸. Response to intravesical capsaicin is biphasic: an initial excitation of capsaicin-responsive C-fibres leads to temporary worsening of symptoms, followed by long-term desensitization²⁵⁹. Typical side effects occur in >50% of the patients immediately after instillation and include suprapubic burning pain and transient worsening of OAB symptoms during the first 2 weeks^{258,260}. This poor clinical tolerance in combination with the availability of new drugs, such as antimuscarinic agents and botulinum toxin, has led to a decline in the use of intravesical capsaicin instillations. In a rat spinal cord injury model, spinal cord compression at the T10 induced NDO characterized by detrusor–sphincter dyssynergia[G] and an increased number of non-voiding contractions. In these rats, following spinal cord injury *Trpa1* mRNA levels and TRPA1 immunoreactivity progressively increased in L6–S1 DRG neurons but not in the spinal cord. Intravenous administration of HC-030031, a TRPA1 antagonist, significantly decreased the number (from 15.4 to 6.5, $P < 0.05$) and amplitude (from 21.5 to 12.4 mmHg; $P < 0.05$) of non-voiding contractions in animals with NDO, suggesting that TRPA1 is another interesting candidate for targeting the afferent limb of the spinal reflex in NDO²⁶¹.

[H2] Detrusor underactivity

DUA is characterized by a reduction in the strength and/or duration of a contraction that results in prolonged bladder emptying or failure to achieve complete bladder emptying^{262,263}. Patients with DUA experience symptoms including weak flow, hesitancy and intermittency, as well as frequency, nocturia, and (overflow) incontinence²⁶⁴. Ageing, bladder outlet obstruction, diabetes mellitus, and iatrogenic nerve injury are known aetiological factors²⁶². Pharmacological strategies to improve detrusor contractility, including muscarinic receptor agonists and cholinesterase inhibitors, have been unsuccessful and current treatment options are limited to intermittent self-catheterization and sacral neuromodulation²⁶⁵.

In 2018, TRPV4 was identified as a potential pharmacological target for DUA. In a rat model of DUA, bilateral pelvic nerve crush resulted in a significant reduction in voiding frequency (intercontractile interval reduced from 143 s to 461 s; $P < 0.001$) and the amplitude of voiding contractions (from 61 to 20 cmH₂O, $P < 0.001$), and a significant increase in the post-void residual volume (from 0.05 ml to 0.32 ml; $P < 0.001$)⁹⁶. Intravesical instillation of the TRPV4 agonist GSK1016790A significantly improved bladder function, resulting in increased voiding frequency (intercontractile interval from 461 seconds to 291 seconds; $p = 0.002$) and reduced postvoid residual volume (from 0.32 ml to 0.21 ml; $p = 0.02$)⁹⁶. These TRPV4-mediated effects are considered the result of increased afferent signalling as they affect the filling phase of the micturition cycle and GSK1016790A has only minor effects on bladder contractility in isolated bladder strips. The results of this study indicate that stimulating afferent signalling, for example, by activation of TRPV4, is a potential new strategy for treating DUA. The effects of intravesical instillation of a TRPV4 agonist on voiding frequency and postvoid residual volume in rats with underactive bladder were confirmed by another group in similar experiments, strengthening the potential for TRPV4 targeting in DUA²⁶⁶.

[H2] Bladder pain syndrome (BPS)

Bladder pain syndrome is a chronic pelvic pain syndrome characterized by suprapubic pain and storage symptoms such as frequency, urgency and nocturia. The aetiology of this disease is unknown, but increasing evidence suggests that TRP channels (including TRPM8, TRPV1 and TRPA1) expressed in nociceptive afferents are involved in the pathophysiology of BPS.

Patients with BPS experience pain during the 'ice water test' (instillation of cold saline (0–4°C) in the bladder), unlike control patients or patients with detrusor overactivity²⁶⁷. A five-fold increase ($P < 0.0001$) in TRPM8-immunoreactive nerve fibres per area has been observed in patients with BPS, and a higher density of these fibres correlates with increased frequency and pain scores²⁶⁸. Similarly, BPS patients have a 3–4-fold increase in suburothelial TRPV1-expressing nerve fibre density²⁶⁹.

The cyclophosphamide-induced cystitis model is also one of the most frequently used animal models for BPS. Mice with cyclophosphamide-induced cystitis typically develop bladder dysfunction as well as nociceptive behavior and increased mechanical sensitivity of the abdominal region and the lower limbs²⁷⁰.

Wild-type and *Trpv1*-knockout mice treated with cyclophosphamide displayed increased levels of nerve growth factor, endothelial nitric oxide synthase, cyclooxygenase-2 and bradykinin receptors. Nevertheless, increases in urinary frequency and mechanical sensitivity of the hind paws are detected in cyclophosphamide-treated WT but not in *Trpv1* KO mice¹³³.

In rats with cyclophosphamide-induced cystitis, intravesical instillation of the TRPV1 agonist resiniferatoxin (RTX) reduces the number of c-Fos expressing neurons in the spinal cord segments innervating the bladder and increases the volume threshold for reflex micturition²⁷¹. Based on these findings and the successful use of intravesical TRPV1 desensitization using capsaicin to treat a small cohort of 20 patients with neurogenic detrusor overactivity²⁷², several clinical trials were conducted to test intravesical RTX instillations as a therapy for BPS. The results from two small trials (both enrolled 18 patients) demonstrated an immediate reduction in pain scores (both trials) and frequency (one trial)²⁷³ that lasted for 1–3 months in patients receiving RTX instillation compared with patients receiving saline instillation²⁷³ or saline instillation with hydrodistension²⁷⁴. One trial also showed a reduction in pain score in patients receiving saline instillation with hydrodistension; however, this reduction was significantly smaller than in patients receiving RTX with hydrodistension (difference in pain score –0.2 versus –0.5, $P = 0.024$)²⁷⁴. The other trial showed significant decreases in frequency (from 12.44 to 7.11 voids, $P < 0.01$), nocturia (from 3.77 to 1.66 voids, $P < 0.01$) and pain score (from 5.55 to 2.66, $P < 0.01$) in RTX-treated patients that were absent in saline-treated patients²⁷³. By contrast, a larger randomized double blind, placebo-controlled study involving 163 patients found no improvement of overall symptoms, pain, urgency, frequency or nocturia during a follow-up period of 12

weeks²⁷⁵. Owing to the side effect profile (burning pain), the difficulties in preparation and handling of RTX instillations and the limited clinical benefit, the use of intravesical TRPV1 agonists such as RTX has been abandoned³⁷.

Several TRP channel antagonists have been tested in preclinical models of BPS. In rats with cyclophosphamide-induced cystitis, the TRPV1 antagonist SB366791 was shown to reduce nociceptive behavior and mechanical hypersensitivity to von Frey hairs in the paw and the abdominal region²⁷⁰.

Similarly, the TRPA1 antagonist HC-030031 reduced cutaneous hypersensitivity of the hind paws and the number of non-voiding contractions in rats with cyclophosphamide-induced cystitis²⁷⁶, whereas visceromotor reactions to bladder distension were completely blocked by pretreatment with a high dose (300mg/kg) of HC-030031²⁷⁷ in mice with cyclophosphamide-induced cystitis. In mice, the proportion of bladder-innervating DRG neurons that express TRPA1 increased from 20% to >50% after induction of cyclophosphamide-induced cystitis²⁷⁷. Although TRPV1 and TRPA1 mRNA levels were not increased in lumbosacral DRG neurons, the number of DRG neurons that responded to the TRPA1 agonist mustard oil significantly increased from <20% in the control group to >50% in rats with cyclophosphamide induced cystitis ($P < 0.01$)²⁷⁷.

Bladder pain syndrome is often accompanied by other clinical conditions such as fibromyalgia and irritable bowel syndrome²⁷⁸. Patients with BPS are 100-fold more likely to have irritable bowel syndrome than the general population. However, despite the striking clinical and statistical overlap between these syndromes, little research has focused on mechanisms of multi-organ disease and pelvic crosstalk^{278–280}. In rats, intracolonic application allyl isothiocyanate, a TRPA1 activator, induces bladder hyperactivity that is abolished after pretreatment with the TRPA1 antagonist HC-030031²⁸¹.

These results demonstrate that TRPV1 and TRPA1 can be targeted in treatment of BPS in preclinical models. Nevertheless, whether these TRP are responsible for the initiation of the painful sensation or merely affect the excitability of nociceptor neurons is not yet established.

[H2] Cold-induced LUTS

Bladder reflexes (both bladder-to-bladder and urethra-to-bladder) are essential in the LUT function and particularly in initiating and potentiating bladder emptying. Many of these reflexes have a temperature-dependent component that relies on thermosensitive TRP channels.

[H3] Bladder cooling reflex

In patients with neurogenic detrusor overactivity and in children until the age of 4, instillation of ice-cold fluid in the bladder triggers an involuntary detrusor contraction^{282,283}. The presence of this reflex has long been used in the ice water test to differentiate between upper motor neuron lesions (reflex present) and lower motor neuron lesions (reflex absent) before detailed neuro-imaging was available²⁸⁴. This bladder cooling reflex is a C-fibre-dependent segmental reflex pathway that, under normal conditions, is suppressed by descending neural control and only emerges when this inhibition is lost (neurological disease) or not yet present (infants)²⁸⁵. A positive bladder cooling reflex has also been described in 20–50% of patients with non-neurogenic detrusor overactivity and in 20–70% of patients with bladder outlet obstruction^{267,285}. The threshold temperature of the reflex varies between 20°C and 33°C, and intravesical application of menthol shifts the temperature response curve towards higher temperatures in cats and humans²⁸⁵. The bladder cooling reflex can be inhibited by desensitization of C-fibres using capsaicin pretreatment.

In guinea pigs, infusion of cold (3°C) saline in the bladder significantly reduces the volume threshold for micturition by 0.99–1.01 ml ($P < 0.0001$) compared with warm saline (38°C) infusion¹⁷⁴. Similar effects can be obtained by intravesical infusion of the cooling agent L-menthol, a TRPM8 agonist, which dose-dependently reduces the volume threshold for micturition¹⁷⁴. In this model, infusion of cold saline results in increased duration of the voiding contraction and intermittent bursts in external sphincter activity, as measured by electromyography (EMG)^{174,286}. The urodynamic changes induced by cold saline and L-menthol are inhibited by intravenous infusion of the selective TRPM8 antagonists PBMC and PF-05105679^{174,286}. Cumulatively, these results suggest that TRPM8 in submucosal nerve endings in the bladder functions as an intravesical cold sensor responsible for initiating reflex bladder contraction during the ice-water test^{174,286,287}.

[H3] *Acute cold induced urinary urgency*

Urinary urgency can be elicited by external cues such as entering home (latchkey urgency) or the sound of running water²⁸⁸. External cold is also a provocative stimulus, and OAB symptoms are generally aggravated by cold weather²⁸⁹. Moreover, patients have often reported a sudden urge to void in response to brief exposure of certain body parts, such as the hands or feet, to a mildly cold stimulus^{290–292}. In 2015, the term acute cold-induced urgency (ACIU) was introduced to describe this phenomenon¹⁵⁹. Patients with OAB frequently report ACIU, often in response to everyday cold stimuli (for example, washing hands, entering a swimming pool, stepping on a cold floor or experiencing a cold breeze).

Until 5 years ago, ACIU was considered a form of Pavlovian conditioning, as is the case of typical latchkey urgency^{292,293}. However, a study demonstrated that ACIU can be induced in unconscious animals, representing a conserved reflex rather than conditioning¹⁵⁹. Application of a stream of cold air over the hind paw or acetone on the skin caused an immediate detrusor contraction and associated voiding in ~80% of mice and rats. Pharmacological inhibition of TRPM8 by pretreatment with the selective antagonist AMTB inhibited the ACUI responses in mice and rats¹⁵⁹. Similarly, *Trpm8*-knockout mice, but not *Trpa1*-knockout mice, lacked ACIU in response to cold stimuli, demonstrating that the cold sensor TRPM8 is required for ACIU¹⁵⁹. Interestingly, ACIU could also be induced by brief pulses of cold air applied to rat ears, suggesting that cold-induced bladder responses are not limited to stimulation of dermatomes innervated by neurons from the DRG that also project to the bladder (mainly L6–S1), but are instead a central mechanism involving TRPM8¹⁵⁹. Nevertheless, these data indicate that TRPM8 is an exciting therapeutic target for treating OAB patients with typical ACIU symptoms. However, to the best of our knowledge, no clinical trials are yet registered to investigate TRPM8 as a therapeutic target for OAB.

[H1] Future perspectives

Emerging techniques provide exciting tools to study TRP function in the LUT. Several groups have used optogenetic tools to study the neural pathways of the LUT. Optogenetic applications use archaeae-derived or algae-derived light-sensitive proteins to non-invasively alter neural processing in a millisecond time-scale resolution²⁹⁴. Light-gated ion channels such as channelrhodopsins or photosensitive proton pumps such as archaerhodopsins are inserted into target cells (for example, neurons or smooth muscle cells) using a viral vector or the Cre-Lox system^{294,295}. When a localized pulse of light opens the ion channel, cations flow into the cell resulting in depolarization of the cell membrane. Alternatively, light-activated proton pumps pump cations out of the cell, causing hyperpolarization of the cell membrane^{294,295}. The possible applications of this technology in the LUT are diverse, ranging from silencing nociceptive afferent bladder nerves²⁹⁶ to activating smooth muscle cells or cortical motor neurons to cause light-induced bladder contraction and urination in vivo^{297,298}. Implantable, wireless optoelectronic systems have been shown to enable precise, non-invasive neuromodulation in freely moving awake animals²⁹⁹. Optogenetic techniques could further dissect the net effect of TRP channel activation in an organ as the sum of (sometimes opposite) effects on different cell types by selectively switching cell populations on or off. Selectively silencing or activating TRP-channel expressing cells by coupling opsin expression to TRP channel expression using TRP-Cre animals is yet another exciting new possibility³⁰⁰.

Trp-knockout mice and rats have been used extensively as negative controls for TRP-targeting antibodies or drugs. Moreover, comparing wild-type and *Trp*-knockout animals has enabled researchers to study the physiological role of these channels in vivo. Nevertheless, whether the observed changes in voiding behaviour (for example, in *Trpv4*-knockout mice) are a result of altered TRP channels function in the urothelium, smooth muscle cells or CNS is not always clear. Furthermore, other pathways might compensate for the alterations caused by the genetic modification. The use of tissue-specific *Trp*-knockout mice that lack *Trp* expression only in specific bladder cell types (for example, urothelial specific KOs)¹¹⁷ or inducible knockout models, can help to overcome the limitations of full-knockout models.

Despite the advances in understanding how TRP channels influence bladder function, many questions remain unanswered. The functional roles of channels such as TRPV2, TRPM3 and TRPP2 remain unexplored. Moreover, the specific function of TRP channels in the urethra and prostate as well as sex-related differences in TRP channel expression and function remain largely unknown. A transcriptomic analysis of lumbar (L4-L5) and Lumbosacral (L6-S1) DRG neurons has demonstrated differential expression of several genes in DRG ganglia between male and female mice. Interestingly, the number of genes differentially expressed was markedly increased in *Trpv1*-expressing neurons innervating the pelvic viscera³⁰¹. Whether and how this variation translates to functional differences remains elusive. The role of TRP channels in any potential functional variation is also not clear, and might be an interesting focus for future research to improve our understanding of the LUT.

[H1] Conclusions

During the past decade, improved understanding of the role of TRP channels as molecular sensors in urothelial cells and afferent nerve fibres has increased our understanding of the origin of bladder sensation as well as LUTS. TRPV4 is an essential urothelial mechanosensor that might be targeted in detrusor overactivity and underactivity, and its specific expression in urothelial cells can be used to test the functional role of these cells in the bladder. TRP channels in sensory nerve endings, such as the nociceptive channels TRPV1 and TRPA1, are essential in hypersensitivity and pain behavior, whereas TRPM8 is essential in cold-related lower urinary tract symptoms. Despite increasing interest from the urological scientific community as well as industry, their exact localization, structure and function are not yet fully established. Furthermore, the development of pharmacotherapies targeting TRP channels is hampered by their ubiquitous expression pattern and pivotal roles in multiple organs.

Despite these inherent difficulties, the potential of TRP-targeting therapies to treat LUT dysfunction remains promising. Targeted inhibition or activation of well-chosen TRP channels in the LUT could result in disease modification rather than merely treating symptoms.

References

1. Griffiths, D. Neural control of micturition in humans: A working model. *Nat. Rev. Urol.* **12**, 695–705 (2015).
2. Abrams, P. *et al.* Reviewing the ICS 2002 terminology report: The ongoing debate. in *Neurourology and Urodynamics* **25**, 293 (2006).
3. Irwin, D. E., Milsom, I., Kopp, Z., Abrams, P. & Cardozo, L. Impact of overactive bladder symptoms on employment, social interactions and emotional well-being in six European countries. *BJU International* **97**, 96–100 (2006).
4. Coyne, K. S. *et al.* The prevalence of lower urinary tract symptoms (LUTS) in the USA, the UK and Sweden: Results from the epidemiology of LUTS (EpiLUTS) study. *BJU Int.* **104**, 352–360 (2009).
5. Irwin, D. E., Kopp, Z. S., Agatep, B., Milsom, I. & Abrams, P. Worldwide prevalence estimates of lower urinary tract symptoms, overactive bladder, urinary incontinence and bladder outlet obstruction. *BJU Int.* **108**, 1132–1138 (2011).
6. Irwin, D. E. *et al.* The economic impact of overactive bladder syndrome in six Western countries. *BJU Int.* **103**, 202–209 (2009).
7. Irwin, D. E. *et al.* Population-Based Survey of Urinary Incontinence, Overactive Bladder, and Other Lower Urinary Tract Symptoms in Five Countries: Results of the EPIC Study. *Eur. Urol.* **50**, 1306–1315 (2006).
8. Fujii, K., Nakayama, Y., Iida, H., Sokabe, M. & Yoshimura, K. Mechanoreception in motile flagella of *Chlamydomonas*. *Nat. Cell Biol.* **13**, 630–632 (2011).
9. Arias-Darraz, L. *et al.* A transient receptor potential ion channel in *chlamydomonas* shares key features with sensory transduction-associated trp channels in mammals. *Plant Cell* **27**, 177–188 (2015).
10. Petersen, C. C. H., Berridge, M. J., Borge, M. F. & Bennett, D. L. Putative capacitative calcium entry channels: Expression of *Drosophila* trp and evidence for the existence of vertebrate homologues. *Biochem. J.* **311**, 41–44 (1995).
11. Flockerzi, V. & Nilius, B. TRPs: Truly Remarkable Proteins. in *Mammalian Transient Receptor Potential (TRP) Cation Channels, Handbook of Experimental Pharmacology* 222 1–12 (Springer, Berlin, Heidelberg, 2014). doi:10.1007/978-3-642-54215-2_1
12. Montell, C. *et al.* A unified nomenclature for the superfamily of TRP cation channels. *Mol. Cell* **9**, 229–231 (2002).

13. Montell, C. The TRP superfamily of cation channels. *Sci. STKE* **2005**, re3–re3 (2005).
14. Caterina, M. J. *et al.* The capsaicin receptor: a heat-activated ion channel in the pain pathway. *Nature* **389**, 816–824 (1997).
15. Peier, A. M. *et al.* A TRP Channel that Senses Cold Stimuli and Menthol. *Cell* **108**, 705–715 (2002).
16. Dhaka, A. *et al.* TRPV1 is activated by both acidic and basic pH. *J. Neurosci.* **29**, 153–8 (2009).
17. Liedtke, W. *et al.* Vanilloid receptor-related osmotically activated channel (VR-OAC), a candidate vertebrate osmoreceptor. *Cell* **103**, 525–535 (2000).
18. Servin-Vences, M. R., Moroni, M., Lewin, G. R. & Poole, K. Direct measurement of TRPV4 and PIEZO1 activity reveals multiple mechanotransduction pathways in chondrocytes. *Elife* **6**, e21074 (2017).
19. Hu, H.-Z. *et al.* 2-aminoethoxydiphenyl borate is a common activator of TRPV1, TRPV2, and TRPV3. *J. Biol. Chem.* **279**, 35741–8 (2004).
20. Launay, P. *et al.* TRPM4 Is a Ca²⁺-Activated Nonselective Cation Channel Mediating Cell Membrane Depolarization. *Cell* **109**, 397–407 (2002).
21. Hofmann, T., Chubanov, V., Gudermann, T. & Montell, C. TRPM5 is a voltage-modulated and Ca²⁺-activated monovalent selective cation channel. *Curr. Biol.* **13**, 1153–1158 (2003).
22. Avelino, A., Cruz, C., Nagy, I. & Cruz Ayb, F. Vanilloid Receptor 1 Expression In The Rat Urinary Tract. *Neuroscience* **109**, 787–798 (2002).
23. Smith, A. C. *et al.* TRPM4 channel: a new player in urinary bladder smooth muscle function in rats. *Am J Physiol Ren. Physiol* **304**, 918–929 (2013).
24. Everaerts, W. *et al.* Functional characterization of transient receptor potential channels in mouse urothelial cells. *Am. J. Physiol. Physiol.* **298**, F692–F701 (2010).
25. Spencer, N. J. *et al.* Identifying unique subtypes of spinal afferent nerve endings within the urinary bladder of mice. *J. Comp. Neurol.* **526**, 707–720 (2018).
26. Xu, L. & Gebhart, G. F. Characterization of Mouse Lumbar Splanchnic and Pelvic Nerve Urinary Bladder Mechanosensory Afferents. *J Neuro-physiol* **99**, 244–253 (2008).
27. Kanda, H., Clodfelder-Miller, B. J., Gu, J. G., Ness, T. J. & Deberry, J. J. Electrophysiological properties of lumbosacral primary afferent neurons innervating urothelial and non-urothelial layers of mouse urinary bladder. *Brain Res* **1648**, 81–89

(2016).

28. Clodfelder-Miller, B. J. *et al.* Urothelial bladder afferent neurons in the rat are anatomically and neurochemically distinct from non-urothelial afferents. *Brain Res.* **1689**, 45–53 (2018).
29. De Groat, W. C. & Yoshimura, N. Afferent nerve regulation of bladder function in health and disease. *Handbook of Experimental Pharmacology* **194**, 91–138 (2009).
30. Vlaskovska, M. *et al.* P2X₃ Knock-Out Mice Reveal a Major Sensory Role for Urothelially Released ATP. *J. Neurosci.* **21**, 5670–5677 (2001).
31. Cockayne, D. A. *et al.* Urinary bladder hyporeflexia and reduced pain-related behaviour in P2X3-deficient mice. *Nature* **407**, 1011–1015 (2000).
32. Apodaca, G., Balestreire, E. & Birder, L. A. The Uroepithelial-associated sensory web. *Kidney International* **72**, 1057–1064 (2007).
33. Durnin, L. *et al.* Urothelial purine release during filling of murine and primate bladders. *Am. J. Physiol. Physiol.* **311**, F708–F716 (2016).
34. Heppner, T. J., Tykocki, N. R., Hill-Eubanks, D. & Nelson, M. T. Transient contractions of urinary bladder smooth muscle are drivers of afferent nerve activity during filling. *J. Gen. Physiol.* **147**, 323–335 (2016).
35. Yu, W. & Hill, W. G. Defining protein expression in the urothelium: a problem of more than transitional interest. *Am. J. Physiol. Physiol.* **301**, F932–F942 (2011).
36. Everaerts, W. *et al.* Where is TRPV1 expressed in the bladder, do we see the real channel? *Naunyn. Schmiedeberg's. Arch. Pharmacol.* **379**, 421–425 (2009).
37. Deruyver, Y., Voets, T., De Ridder, D. & Everaerts, W. Transient receptor potential channel modulators as pharmacological treatments for lower urinary tract symptoms (LUTS): myth or reality? *BJU Int.* **115**, 686–697 (2015).
38. Birder, L. A. *et al.* Vanilloid receptor expression suggests a sensory role for urinary bladder epithelial cells. *PNAS* **98**, 13396–13401 (2001).
39. Ost, D., Roskams, T., Van Der Aa, F. & De Ridder, D. Topography of the Vanilloid Receptor in the Human Bladder: More Than Just the Nerve Fibers. *J. Urol.* **168**, 293–297 (2002).
40. Lazzeri, M. *et al.* Immunohistochemical Evidence of Vanilloid Receptor 1 in Normal Human Urinary Bladder. *Eur. Urol.* **46**, 792–798 (2004).
41. Lazzeri, M. *et al.* Transient receptor potential vanilloid type 1 (TRPV1) expression

- changes from normal urothelium to transitional cell carcinoma of human bladder. *Eur. Urol.* **48**, 691–698 (2005).
42. Apostolidis, A. *et al.* Capsaicin receptor TRPV1 in urothelium of neurogenic human bladders and effect of intravesical resiniferatoxin. *Urology* **65**, 400–405 (2005).
  43. Liu, H.-T. & Kuo, H.-C. Increased expression of transient receptor potential vanilloid subfamily 1 in the bladder predicts the response to intravesical instillations of resiniferatoxin in patients with refractory idiopathic detrusor overactivity. *BJU Int.* **100**, 1086–1090 (2007).
  44. Heng, Y. J., Saunders, C. I. M., Kunde, D. A. & Geraghty, D. P. TRPV1, NK1 receptor and substance P immunoreactivity and gene expression in the rat lumbosacral spinal cord and urinary bladder after systemic, low dose vanilloid administration. *Regul. Pept.* **167**, 250–258 (2011).
  45. Yamada, T. *et al.* Differential Localizations of the Transient Receptor Potential Channels TRPV4 and TRPV1 in the Mouse Urinary Bladder. *J. Histochem. Cytochem.* **57**, 277–287 (2009).
  46. Mochizuki, T. *et al.* The TRPV4 Cation Channel Mediates Stretch-evoked Ca²⁺ Influx and ATP Release in Primary Urothelial Cell Cultures. *J. Biol. Chem.* **284**, 21257–21264 (2009).
  47. Xu, X. *et al.* Functional TRPV4 channels and an absence of capsaicin-evoked currents in freshly-isolated, guinea-pig urothelial cells. *Channels* **3**, 156–160 (2009).
  48. O'Mullane, L. M., Keast, J. R. & Osborne, P. B. Co-Cultures Provide a New Tool to Probe Communication Between Adult Sensory Neurons and Urothelium. *J. Urol.* **190**, 737–745 (2013).
  49. Shabir, S. *et al.* Functional expression of purinergic P2 receptors and transient receptor potential channels by the human urothelium. *Am. J. Physiol. Physiol.* **305**, F396–F406 (2013).
  50. Kullmann, F. A., Shah, M. A., Birder, L. A. & de Groat, W. C. Functional TRP and ASIC-like channels in cultured urothelial cells from the rat. *Am. J. Physiol. Renal Physiol.* **296**, F892–901 (2009).
  51. Cavanaugh, D. J. *et al.* Trpv1 reporter mice reveal highly restricted brain distribution and functional expression in arteriolar smooth muscle cells. *J. Neurosci.* **31**, 5067–77 (2011).

52. Menigoz, A. & Boudes, M. The expression pattern of TRPV1 in brain. *J. Neurosci.* **31**, 13025–13027 (2011).
53. Song, A. J. & Palmiter, R. D. Detecting and Avoiding Problems When Using the Cre/lox System. *Trends Genet* **34**, 333–340 (2018).
54. Tabula Muris. 2018 Available at: <https://tabula-muris.ds.czbiohub.org/>.
55. Schaum, N. *et al.* Single-cell transcriptomics of 20 mouse organs creates a Tabula Muris. *Nature* **562**, 367–372 (2018).
56. Lazzeri, M. *et al.* Immunohistochemical evidence of vanilloid receptor 1 in normal human urinary bladder. *Eur. Urol.* **46**, 792–798 (2004).
57. Streng, T. *et al.* Distribution and Function of the Hydrogen Sulfide–Sensitive TRPA1 Ion Channel in Rat Urinary Bladder. *Eur. Urol.* **53**, 391–400 (2008).
58. Du, S. *et al.* Differential Expression Profile of Cold (TRPA1) and Cool (TRPM8) Receptors in Human Urogenital Organs. *Urology* **72**, 450–455 (2008).
59. Gratzke, C. *et al.* Transient Receptor Potential A1 (TRPA1) Activity in the Human Urethra—Evidence for a Functional Role for TRPA1 in the Outflow Region. *Eur. Urol.* **55**, 696–704 (2009).
60. Stein, R. J. *et al.* Cool (TRPM8) and Hot (TRPV1) Receptors in the Bladder and Male Genital Tract. *J. Urol.* **172**, 1175–1178 (2004).
61. Zeisel, A. *et al.* Molecular Architecture of the Mouse Nervous System. *Cell* **174**, 999–1014.e22 (2018).
62. Gevaert, T. *et al.* Deletion of the transient receptor potential cation channel TRPV4 impairs murine bladder voiding. *J. Clin. Invest.* **117**, 3453–3462 (2007).
63. Everaerts, W. *et al.* Inhibition of the cation channel TRPV4 improves bladder function in mice and rats with cyclophosphamide-induced cystitis. *Proc. Natl. Acad. Sci.* **107**, 19084–19089 (2010).
64. Thorneloe, K. S. *et al.* N-((1S)-1-([4-((2S)-2-([(2,4-dichlorophenyl)sulfonyl]amino)-3-hydroxypropanoyl)-1-piperazinyl]carbonyl)-3-methylbutyl)-1-benzothiophene-2-carboxamide (GSK1016790A), a novel and potent transient receptor potential vanilloid 4 channel agonist induces urinary bladder contraction and hyperactivity: Part I. *J. Pharmacol. Exp. Ther.* **326**, 432–42 (2008).
65. Phan, T. X., Ton, H. T., Chen, Y., Basha, M. E. & Ahern, G. P. Sex-dependent expression of TRPV1 in bladder arterioles. *Am. J. Physiol. Physiol.* **311**, F1063–F1073 (2016).

66. Yu, W., Hill, W. G., Apodaca, G. & Zeidel, M. L. Expression and distribution of transient receptor potential (TRP) channels in bladder epithelium. *Am. J. Physiol. Physiol.* **300**, F49–F59 (2010).
67. Vandewauw, I., Owsianik, G. & Voets, T. Systematic and quantitative mRNA expression analysis of TRP channel genes at the single trigeminal and dorsal root ganglion level in mouse. *BMC Neurosci.* **14**, (2013).
68. Harding, S. D. *et al.* The GUDMAP database - an online resource for genitourinary research. *Development* **138**, 2845–2853 (2011).
69. GUDMAP. Available at: <https://www.gudmap.org/id/16-DW7R@2VC-DWXQ-2KDE>.
70. Usoskin, D. *et al.* Unbiased classification of sensory neuron types by large-scale single-cell RNA sequencing. *Nat. Neurosci.* **18**, 145–153 (2015).
71. Hockley, J. R. F. *et al.* Single-cell RNAseq reveals seven classes of colonic sensory neuron. *Gut* **68**, 633–644 (2019).
72. Watanabe, H. *et al.* Heat-evoked activation of TRPV4 channels in a HEK293 cell expression system and in native mouse aorta endothelial cells. *J. Biol. Chem.* **277**, 47044–51 (2002).
73. Güler, A. D. *et al.* Heat-Evoked Activation of the Ion Channel, TRPV4. *J. Neurosci.* **22**, 6408–6414 (2002).
74. Watanabe, H. *et al.* Anandamide and arachidonic acid use epoxyeicosatrienoic acids to activate TRPV4 channels. *Nature* **424**, 434–438 (2003).
75. Voets, T. Quantifying and modeling the temperature-dependent gating of TRP channels. in *Reviews of Physiology, Biochemistry and Pharmacology* **162**, 91–119 (Springer, Berlin, Heidelberg, 2012).
76. Vincent, F. *et al.* Identification and characterization of novel TRPV4 modulators. *Biochem. Biophys. Res. Commun.* **389**, 490–494 (2009).
77. Thorneloe, K. S. *et al.* An Orally Active TRPV4 Channel Blocker Prevents and Resolves Pulmonary Edema Induced by Heart Failure. *Sci. Transl. Med.* **4**, 159ra148 (2012).
78. Everaerts, W., Nilius, B. & Owsianik, G. The vanilloid transient receptor potential channel TRPV4: From structure to disease. *Prog. Biophys. Mol. Biol.* **103**, 2–17 (2010).
79. Tian, W. *et al.* Renal expression of osmotically responsive cation channel TRPV4 is restricted to water-impermeant nephron segments. *Am. J. Physiol. Physiol.* **287**, F17–F24 (2004).

80. Masuyama, R. *et al.* TRPV4-Mediated Calcium Influx Regulates Terminal Differentiation of Osteoclasts. *Cell Metab.* **8**, 257–265 (2008).
81. Casas, S., Novials, A., Reimann, F., Gomis, R. & Gribble, F. M. Calcium elevation in mouse pancreatic beta cells evoked by extracellular human islet amyloid polypeptide involves activation of the mechanosensitive ion channel TRPV4. *Diabetologia* **51**, 2252–2262 (2008).
82. Chung, M.-K., Lee, H. & Caterina, M. J. Warm temperatures activate TRPV4 in mouse 308 keratinocytes. *J. Biol. Chem.* **278**, 32037–46 (2003).
83. Fian, R. *et al.* The Contribution of TRPV4-Mediated Calcium Signaling to Calcium Homeostasis in Endothelial Cells. *J. Recept. Signal Transduct.* **27**, 113–124 (2007).
84. Lorenzo, I. M., Liedtke, W., Sanderson, M. J. & Valverde, M. A. TRPV4 channel participates in receptor-operated calcium entry and ciliary beat frequency regulation in mouse airway epithelial cells. *Proc. Natl. Acad. Sci.* **105**, 12611–12616 (2008).
85. Birder, L. A. *et al.* Activation of urothelial transient receptor potential vanilloid 4 by 4alpha-phorbol 12,13-didecanoate contributes to altered bladder reflexes in the rat. *J. Pharmacol. Exp. Ther.* **323**, 227–35 (2007).
86. Janssen, D. A. W. *et al.* The Mechanoreceptor TRPV4 is Localized in Adherence Junctions of the Human Bladder Urothelium: A Morphological Study. *J. Urol.* **186**, 1121–1127 (2011).
87. Janssen, D. A. W. *et al.* TRPV4 channels in the human urogenital tract play a role in cell junction formation and epithelial barrier. *Acta Physiol.* **218**, 38–48 (2016).
88. Roberts, M. W. G. *et al.* TRPV4 receptor as a functional sensory molecule in bladder urothelium: Stretch-independent, tissue-specific actions and pathological implications. *FASEB J.* **34**, 263–286 (2020).
89. Namasivayam, Eardley & Morrison. Purinergic sensory neurotransmission in the urinary bladder: an in vitro study in the rat. *BJU Int.* **84**, 854–860 (2001).
90. Rong, W., Spyer, K. M. & Burnstock, G. Activation and sensitisation of low and high threshold afferent fibres mediated by P2X receptors in the mouse urinary bladder. *J. Physiol.* **541**, 591–600 (2002).
91. Cockayne, D. A. *et al.* P2X2 knockout mice and P2X2/P2X3 double knockout mice reveal a role for the P2X2 receptor subunit in mediating multiple sensory effects of ATP. *J. Physiol.* **567**, 621–639 (2005).

92. Aizawa, N., Wyndaele, J.-J., Homma, Y. & Igawa, Y. Effects of TRPV4 cation channel activation on the primary bladder afferent activities of the rat. *Neurourol. Urodyn.* **31**, 148–155 (2012).
93. Mizuno, A., Matsumoto, N., Imai, M. & Suzuki, M. Impaired osmotic sensation in mice lacking TRPV4. *Am. J. Physiol. Physiol.* **285**, C96–C101 (2003).
94. Liedtke, W. & Friedman, J. M. Abnormal osmotic regulation in *trpv4* ^{-/-} mice. *PNAS* **100**, 13698–13703 (2003).
95. Yoshiyama, M. *et al.* Functional roles of TRPV1 and TRPV4 in control of lower urinary tract activity: dual analysis of behavior and reflex during the micturition cycle. *Am. J. Physiol. Physiol.* **308**, F1128–F1134 (2015).
96. Deruyver, Y. *et al.* Intravesical Activation of the Cation Channel TRPV4 Improves Bladder Function in a Rat Model for Detrusor Underactivity. *Eur. Urol.* **74**, 336–345 (2018).
97. Ihara, T. *et al.* The Circadian expression of *Piezo1* , *TRPV4* , *Connexin26* , and *VNUT* , associated with the expression levels of the clock genes in mouse primary cultured urothelial cells. *Neurourol. Urodyn.* **37**, 942–951 (2018).
98. Ihara, T. *et al.* The oscillation of intracellular Ca²⁺ influx associated with the circadian expression of *Piezo1* and *TRPV4* in the bladder urothelium. *Sci. Rep.* **8**, 5699 (2018).
99. Ihara, T. *et al.* The Clock mutant mouse is a novel experimental model for nocturia and nocturnal polyuria. *Neurourol. Urodyn.* **36**, 1034–1038 (2017).
100. Balsalobre, A., Damiola, F. & Schibler, U. A serum shock induces circadian gene expression in mammalian tissue culture cells. *Cell* **93**, 929–937 (1998).
101. Alpizar, Y. A. *et al.* TRPV4 Mediates Acute Bladder Responses to Bacterial Lipopolysaccharides. *Front. Immunol.* **11**, 799 (2020).
102. Caterina, M. J., Rosen, T. A., Tominaga, M., Brake, A. J. & Julius, D. A capsaicin-receptor homologue with a high threshold for noxious heat. *Nature* **398**, 436–441 (1999).
103. Kanzaki, M. *et al.* Translocation of a calcium-permeable cation channel induced by insulin-like growth factor-I. *Nat. Cell Biol.* **1**, 165–170 (1999).
104. Muraki, K. *et al.* TRPV2 Is a Component of Osmotically Sensitive Cation Channels in Murine Aortic Myocytes. *Circ. Res.* **93**, 829–838 (2003).
105. Bang, S., Kim, K. Y., Yoo, S., Lee, S.-H. & Hwang, S. W. Transient receptor potential V2

- expressed in sensory neurons is activated by probenecid. *Neurosci. Lett.* **425**, 120–125 (2007).
106. Qin, N. *et al.* TRPV2 Is Activated by Cannabidiol and Mediates CGRP Release in Cultured Rat Dorsal Root Ganglion Neurons. *J. Neurosci.* **28**, 6231–6238 (2008).
  107. Shibasaki, K., Murayama, N., Ono, K., Ishizaki, Y. & Tominaga, M. TRPV2 Enhances Axon Outgrowth through Its Activation by Membrane Stretch in Developing Sensory and Motor Neurons. *J. Neurosci.* **30**, 4601–4612 (2010).
  108. De Clercq, K. *et al.* The functional expression of transient receptor potential channels in the mouse endometrium. *Hum. Reprod.* **32**, 615–630 (2017).
  109. Katanosaka, Y. *et al.* TRPV2 is critical for the maintenance of cardiac structure and function in mice. *Nat. Commun.* **5**, (2014).
  110. Link, T. M. *et al.* TRPV2 has a pivotal role in macrophage particle binding and phagocytosis. *Nat. Immunol.* **11**, 232–239 (2010).
  111. Park, U. *et al.* TRP vanilloid 2 knock-out mice are susceptible to perinatal lethality but display normal thermal and mechanical nociception. *J. Neurosci.* **31**, 11425–11436 (2011).
  112. Caprodossi, S. *et al.* Transient Receptor Potential Vanilloid Type 2 (TRPV2) Expression in Normal Urothelium and in Urothelial Carcinoma of Human Bladder: Correlation with the Pathologic Stage. *Eur. Urol.* **54**, 612–620 (2008).
  113. Yamada, T. *et al.* TRPV2 Activation Induces Apoptotic Cell Death in Human T24 Bladder Cancer Cells: A Potential Therapeutic Target for Bladder Cancer. *Urology* **76**, 509.e1-509.e7 (2010).
  114. Ryazanova, L. V *et al.* TRPM7 is essential for Mg²⁺ homeostasis in mammals. *Nat. Commun.* **1**, (2010).
  115. Fonfria, E. *et al.* Tissue Distribution Profiles of the Human TRPM Cation Channel Family. *J. Recept. Signal Transduct.* **26**, 159–178 (2006).
  116. Jin, J. *et al.* Deletion of TRPM7 disrupts embryonic development and thymopoiesis without altering Mg²⁺ homeostasis. *Science*. **322**, 756–760 (2008).
  117. Watanabe, M. *et al.* Trpm7 Protein Contributes to Intercellular Junction Formation in Mouse Urothelium. *J. Biol. Chem.* **290**, 29882–92 (2015).
  118. Zygmunt, P. M. *et al.* Vanilloid receptors on sensory nerves mediate the vasodilator action of anandamide. *Nature* **400**, 452–457 (1999).

119. Nilius, B. & Szallasi, A. Transient receptor potential channels as drug targets: from the science of basic research to the art of medicine. *Pharmacol. Rev.* **66**, 676–814 (2014).
120. Acs, G., Palkovits, M. & Blumberg, P. M. [3H]Resiniferatoxin binding by the human vanilloid (capsaicin) receptor. *Mol. Brain Res.* **23**, 185–190 (1994).
121. Baumann, T. K., Burchiel, K. J., Ingram, S. L. & Martenson, M. E. Responses of adult human dorsal root ganglion neurons in culture to capsaicin and low pH. *Pain* **65**, 31–38 (1996).
122. Denda, M. *et al.* Immunoreactivity of VR1 on epidermal keratinocyte of human skin. *Biochem. Biophys. Res. Commun.* **285**, 1250–1252 (2001).
123. Birder, L. A. *et al.* Altered urinary bladder function in mice lacking the vanilloid receptor TRPV1. *Nat. Neurosci.* **5**, 856–860 (2002).
124. Sanchez, J. F., Krause, J. E. & Cortright, D. N. The distribution and regulation of vanilloid receptor VR1 and VR1 5' splice variant RNA expression in rat. *Neuroscience* **107**, 373–381 (2001).
125. Mouse Brain Atlas. Available at: www.mousebrain.org.
126. Maggi, C. A. The dual function of capsaicin-sensitive sensory nerves in the bladder and urethra. *Ciba Found. Symp.* **151**, 77–83 (1990).
127. Engel, M. A. *et al.* The proximodistal aggravation of colitis depends on substance P released from TRPV1-expressing sensory neurons. *J. Gastroenterol.* **47**, 256–265 (2012).
128. Meng, J. *et al.* Activation of TRPV1 mediates calcitonin gene-related peptide release, which excites trigeminal sensory neurons and is attenuated by a retargeted botulinum toxin with anti-nociceptive potential. *J. Neurosci.* **29**, 4981–92 (2009).
129. Gouin, O. *et al.* TRPV1 and TRPA1 in cutaneous neurogenic and chronic inflammation: pro-inflammatory response induced by their activation and their sensitization. *Protein Cell* **8**, 644–661 (2017).
130. Boillat, A., Alijevic, O. & Kellenberger, S. Calcium entry via TRPV1 but not ASICs induces neuropeptide release from sensory neurons. *Mol. Cell. Neurosci.* **61**, 13–22 (2014).
131. Yiangou, Y. *et al.* Capsaicin receptor VR1 and ATP-gated ion channel P2X3 in human urinary bladder. *BJU Int.* **87**, 774–779 (2002).
132. Birder, L. A. TRPs in bladder diseases. *Biochim. Biophys. Acta* **1772**, 879–84 (2007).

133. Wang, Z.-Y., Wang, P., Voznika Merriam, F. & Bjorling, D. E. Lack of TRPV1 inhibits cystitis-induced increased mechanical sensitivity in mice. *Pain* **139**, 158–167 (2008).
134. Daly, D., Rong, W., Chess-Williams, R., Chapple, C. & Grundy, D. Bladder afferent sensitivity in wild-type and TRPV1 knockout mice. *J. Physiol.* **583**, 663–674 (2007).
135. Grundy, L. *et al.* Histamine induces peripheral and central hypersensitivity to bladder distension via the histamine H 1 receptor and TRPV1. *Am J Physiol Ren. Physiol* **318**, 298–314 (2020).
136. Grundy, L., Daly, D. M., Chapple, C., Grundy, D. & Chess-Williams, R. TRPV1 enhances the afferent response to P2X receptor activation in the mouse urinary bladder. *Sci. Rep.* **8**, 197 (2018).
137. Jordt, S.-E. *et al.* Mustard oils and cannabinoids excite sensory nerve fibres through the TRP channel ANKTM1. *Nature* **427**, 260–265 (2004).
138. Bautista, D. M. *et al.* Pungent products from garlic activate the sensory ion channel TRPA1. *Proc. Natl. Acad. Sci. U. S. A.* **102**, 12248–52 (2005).
139. Meseguer, V. *et al.* TRPA1 channels mediate acute neurogenic inflammation and pain produced by bacterial endotoxins. *Nat. Commun.* **5**, 3125 (2014).
140. Bessac, B. F. *et al.* TRPA1 is a major oxidant sensor in murine airway sensory neurons. *J. Clin. Invest.* **118**, 1899–910 (2008).
141. Bautista, D. M., Pellegrino, M. & Tsunozaki, M. TRPA1: A Gatekeeper for Inflammation. *Annu. Rev. Physiol.* **75**, 181–200 (2013).
142. Andersson, D. A., Gentry, C., Moss, S. & Bevan, S. Transient receptor potential A1 is a sensory receptor for multiple products of oxidative stress. *J. Neurosci.* **28**, 2485–2494 (2008).
143. Moparthy, L. *et al.* Human TRPA1 is intrinsically cold- and chemosensitive with and without its N-terminal ankyrin repeat domain. *Proc. Natl. Acad. Sci. U. S. A.* **111**, 16901–16906 (2014).
144. Bandell, M. *et al.* Noxious cold ion channel TRPA1 is activated by pungent compounds and bradykinin. *Neuron* **41**, 849–857 (2004).
145. Vandewauw, I. *et al.* A TRP channel trio mediates acute noxious heat sensing. *Nature* **555**, 662–666 (2018).
146. Nagata, K., Duggan, A., Kumar, G. & García-Añoveros, J. Nociceptor and hair cell transducer properties of TRPA1, a channel for pain and hearing. *J. Neurosci.* **20**, 7131–

- 7142 (2005).
147. Kim, Y. S. *et al.* Expression of Transient Receptor Potential Ankyrin 1 in Human Dental Pulp. *J. Endod.* **38**, 1087–1092 (2012).
  148. El Karim, I. A. *et al.* Human Dental Pulp Fibroblasts Express the “Cold-sensing” Transient Receptor Potential Channels TRPA1 and TRPM8. *J. Endod.* **37**, 473–478 (2011).
  149. Story, G. M. *et al.* ANKTM1, a TRP-like Channel Expressed in Nociceptive Neurons, Is Activated by Cold Temperatures. *Cell* **112**, 819–829 (2003).
  150. Nassini, R., Materazzi, S., Benemei, S. & Geppetti, P. The TRPA1 Channel in Inflammatory and Neuropathic Pain and Migraine. in *Reviews of Physiology, Biochemistry and Pharmacology, Vol. 167* (eds. Nilius, B. *et al.*) 1–43 (Springer International Publishing, 2014). doi:10.1007/112_2014_18
  151. Moilanen, L. J., Hämäläinen, M., Lehtimäki, L., Nieminen, R. M. & Moilanen, E. Urate Crystal Induced Inflammation and Joint Pain Are Reduced in Transient Receptor Potential Ankyrin 1 Deficient Mice – Potential Role for Transient Receptor Potential Ankyrin 1 in Gout. *PLoS One* **10**, e0117770 (2015).
  152. Moilanen, L. J. *et al.* Monosodium iodoacetate-induced inflammation and joint pain are reduced in TRPA1 deficient mice – potential role of TRPA1 in osteoarthritis. *Osteoarthr. Cartil.* **23**, 2017–2026 (2015).
  153. Du, S., Araki, I., Yoshiyama, M., Nomura, T. & Takeda, M. Transient Receptor Potential Channel A1 Involved in Sensory Transduction of Rat Urinary Bladder Through C-Fiber Pathway. *Urology* **70**, 826–831 (2007).
  154. Everaerts, W. *et al.* The Capsaicin Receptor TRPV1 Is a Crucial Mediator of the Noxious Effects of Mustard Oil. *Curr. Biol.* **21**, 316–321 (2011).
  155. Minagawa, T., Aizawa, N., Igawa, Y. & Wyndaele, J.-J. The role of transient receptor potential ankyrin 1 (TRPA1) channel in activation of single unit mechanosensitive bladder afferent activities in the rat. *Neurourol. Urodyn.* **33**, 544–549 (2014).
  156. McNamara, C. R. *et al.* TRPA1 mediates formalin-induced pain. *Proc. Natl. Acad. Sci. U. S. A.* **104**, 13525–30 (2007).
  157. Eid, S. R. *et al.* HC-030031, a TRPA1 Selective Antagonist, Attenuates Inflammatory- and Neuropathy-Induced Mechanical Hypersensitivity. *Mol. Pain* **4**, 1744-8069-4–48 (2008).

158. Kamei, J. *et al.* Attenuated lipopolysaccharide-induced inflammatory bladder hypersensitivity in mice deficient of transient receptor potential ankinin1. *Sci. Rep.* **8**, 15622 (2018).
159. Uvin, P. *et al.* Essential Role of Transient Receptor Potential M8 (TRPM8) in a Model of Acute Cold-induced Urinary Urgency. *Eur. Urol.* **68**, 655–661 (2015).
160. McKemy, D. D., Neuhausser, W. M. & Julius, D. Identification of a cold receptor reveals a general role for TRP channels in thermosensation. *Nature* **416**, 52–58 (2002).
161. Kobayashi, K. *et al.* Distinct expression of TRPM8, TRPA1, and TRPV1 mRNAs in rat primary afferent neurons with $\alpha\delta$ /c-fibers and colocalization with trk receptors. *J. Comp. Neurol.* **493**, 596–606 (2005).
162. Dhaka, A., Earley, T. J., Watson, J. & Patapoutian, A. Visualizing Cold Spots: TRPM8-Expressing Sensory Neurons and Their Projections. *J. Neurosci.* **28**, 566–575 (2008).
163. Tsavaler, L., Shapero, M. H., Morkowski, S. & Laus, R. Trp-p8, a Novel Prostate-specific Gene, Is Up-Regulated in Prostate Cancer and Other Malignancies and Shares High Homology with Transient Receptor Potential Calcium Channel Proteins. *Cancer Res.* **61**, 3760–3769 (2001).
164. Ma, S. *et al.* Activation of the cold-sensing TRPM8 channel triggers UCP1-dependent thermogenesis and prevents obesity. *J. Mol. Cell Biol.* **4**, 88–96 (2012).
165. Bautista, D. M. *et al.* The menthol receptor TRPM8 is the principal detector of environmental cold. *Nature* **448**, 204–208 (2007).
166. Dhaka, A. *et al.* TRPM8 Is Required for Cold Sensation in Mice. *Neuron* **54**, 371–378 (2007).
167. Colburn, R. W. *et al.* Attenuated Cold Sensitivity in TRPM8 Null Mice. *Neuron* **54**, 379–386 (2007).
168. Rossato, M. *et al.* Human white adipocytes express the cold receptor TRPM8 which activation induces UCP1 expression, mitochondrial activation and heat production. *Mol. Cell. Endocrinol.* **383**, 137–146 (2014).
169. Gavva, N. R. *et al.* Transient receptor potential melastatin 8 (TRPM8) channels are involved in body temperature regulation. *Mol. Pain* **8**, (2012).
170. Lashinger, E. S. R. *et al.* AMTB, a TRPM8 channel blocker: evidence in rats for activity in overactive bladder and painful bladder syndrome. *Am. J. Physiol. Physiol.* **295**, F803–F810 (2008).

171. Andrews, M. D. *et al.* Discovery of a Selective TRPM8 Antagonist with Clinical Efficacy in Cold-Related Pain. *ACS Med. Chem. Lett.* **6**, 419–424 (2015).
172. Kobayashi, J. *et al.* Synthesis and optimization of novel α -phenylglycinamides as selective TRPM8 antagonists. *Bioorg. Med. Chem.* **25**, 727–742 (2017).
173. Aizawa, N. *et al.* RQ-00434739, a novel TRPM8 antagonist, inhibits prostaglandin E₂-induced hyperactivity of the primary bladder afferent nerves in rats. *Life Sci.* **218**, 89–95 (2019).
174. Gardiner, J. C. *et al.* The role of TRPM8 in the Guinea-pig bladder-cooling reflex investigated using a novel TRPM8 antagonist. *Eur. J. Pharmacol.* **740**, 398–409 (2014).
175. Hayashi, T. *et al.* Expression of the TRPM8-immunoreactivity in dorsal root ganglion neurons innervating the rat urinary bladder. *Neurosci. Res.* **65**, 245–251 (2009).
176. Ito, H. *et al.* Functional role of the transient receptor potential melastatin 8 (TRPM8) ion channel in the urinary bladder assessed by conscious cystometry and *ex vivo* measurements of single-unit mechanosensitive bladder afferent activities in the rat. *BJU Int.* **117**, 484–494 (2016).
177. Jun, J. H. *et al.* Function of the Cold Receptor (TRPM8) Associated with Voiding Dysfunction in Bladder Outlet Obstruction in Rats. *Int. Neurourol. J.* **16**, 69–76 (2012).
178. Dietrich, A., Fahlbusch, M. & Gudermann, T. Classical Transient Receptor Potential 1 (TRPC1): Channel or Channel Regulator? *Cells* **3**, 939–962 (2014).
179. Kim, J. *et al.* Isoform- and receptor-specific channel property of canonical transient receptor potential (TRPC)1/4 channels. *Pflugers Arch. Eur. J. Physiol.* **466**, 491–504 (2014).
180. Storch, U., Forst, A. L., Philipp, M., Gudermann, T. & Mederos Y Schnitzler, M. Transient receptor potential channel 1 (TRPC1) reduces calcium permeability in heteromeric channel complexes. *J. Biol. Chem.* **287**, 3530–3540 (2012).
181. Strübing, C., Krapivinsky, G., Krapivinsky, L. & Clapham, D. E. TRPC1 and TRPC5 form a novel cation channel in mammalian brain. *Neuron* **29**, 645–655 (2001).
182. Freichel, M. *et al.* Lack of an endothelial store-operated Ca²⁺ current impairs agonist-dependent vasorelaxation in TRP4^{-/-} mice. *Nat. Cell Biol.* **3**, 121–127 (2001).
183. Strübing, C., Krapivinsky, G., Krapivinsky, L. & Clapham, D. E. Formation of novel TRPC channels by complex subunit interactions in embryonic brain. *J. Biol. Chem.* **278**, 39014–9 (2003).

184. Tsvilovskyy, V. V *et al.* Deletion of TRPC4 and TRPC6 in mice impairs smooth muscle contraction and intestinal motility in vivo. *Gastroenterology* **137**, 1415–1424 (2009).
185. Phelan, K. D. *et al.* Heteromeric canonical transient receptor potential 1 and 4 channels play a critical role in epileptiform burst firing and seizure-induced neurodegeneration. *Mol. Pharmacol.* **81**, 384–92 (2012).
186. Stroh, O. *et al.* NMDA Receptor-Dependent Synaptic Activation of TRPC Channels in Olfactory Bulb Granule Cells. *J. Neurosci.* **32**, 5737–5746 (2012).
187. Boudes, M. *et al.* Crucial Role of TRPC1 and TRPC4 in Cystitis-Induced Neuronal Sprouting and Bladder Overactivity. *PLoS One* **8**, e69550 (2013).
188. Riccio, A. *et al.* Essential Role for TRPC5 in Amygdala Function and Fear-Related Behavior. *Cell* **137**, 761–772 (2009).
189. Nilius, B. *et al.* Voltage dependence of the Ca²⁺-activated cation channel TRPM4. *J. Biol. Chem.* **278**, 30813–20 (2003).
190. Launay, P. *et al.* TRPM4 regulates calcium oscillations after T cell activation. *Science*. **306**, 1374–1377 (2004).
191. Vennekens, R. *et al.* Increased IgE-dependent mast cell activation and anaphylactic responses in mice lacking the calcium-activated nonselective cation channel TRPM4. *Nat. Immunol.* **8**, 312–320 (2007).
192. Barbet, G. *et al.* The calcium-activated nonselective cation channel TRPM4 is essential for the migration but not the maturation of dendritic cells. *Nat. Immunol.* **9**, 1148–1156 (2008).
193. Gerzanich, V. *et al.* De novo expression of Trpm4 initiates secondary hemorrhage in spinal cord injury. *Nat. Med.* **15**, 185–191 (2009).
194. Schattling, B. *et al.* TRPM4 cation channel mediates axonal and neuronal degeneration in experimental autoimmune encephalomyelitis and multiple sclerosis. *Nat. Med.* **18**, 1805–1811 (2012).
195. Hof, T., Simard, C., Rouet, R., Sallé, L. & Guinamard, R. Implication of the TRPM4 nonselective cation channel in mammalian sinus rhythm. *Heart Rhythm.* **10**, 1683–1689 (2013).
196. Simard, C., Hof, T., Keddache, Z., Launay, P. & Guinamard, R. The TRPM4 non-selective cation channel contributes to the mammalian atrial action potential. *J. Mol. Cell. Cardiol.* **59**, 11–19 (2013).

197. Mathar, I. *et al.* Increased catecholamine secretion contributes to hypertension in TRPM4-deficient mice. *J. Clin. Invest.* **120**, 3267–3279 (2010).
198. Brink, P. A. *et al.* Gene for Progressive Familial Heart Block Type I Maps to Chromosome 19q13. *Circulation* **91**, 1633–1640 (1995).
199. Liu, H. *et al.* Gain-of-Function Mutations in *TRPM4* Cause Autosomal Dominant Isolated Cardiac Conduction Disease. *Circ. Cardiovasc. Genet.* **3**, 374–385 (2010).
200. Wang, H. *et al.* Gain-of-Function Mutations in TRPM4 Activation Gate Cause Progressive Symmetric Erythrokeratoderma. *J. Invest. Dermatol.* **139**, 1089–1097 (2019).
201. Smith, A. C. *et al.* Novel role for the transient potential receptor melastatin 4 channel in guinea pig detrusor smooth muscle physiology. *Am J Physiol Cell Physiol* **304**, 467–477 (2013).
202. Hristov, K. L. *et al.* Novel regulatory mechanism in human urinary bladder: central role of transient receptor potential melastatin 4 channels in detrusor smooth muscle function. *Am. J. Physiol. Physiol.* **310**, C600–C611 (2016).
203. Kullmann, F. A. *et al.* Involvement of TRPM4 in detrusor overactivity following spinal cord transection in mice. *Naunyn. Schmiedeberg's. Arch. Pharmacol.* **391**, 1191–1202 (2018).
204. Guinamard, R., Hof, T. & Del Negro, C. A. The TRPM4 channel inhibitor 9-phenanthrol. *Br. J. Pharmacol.* **171**, 1600–1613 (2014).
205. Grand, T. *et al.* 9-Phenanthrol inhibits human TRPM4 but not TRPM5 cationic channels. *Br. J. Pharmacol.* **153**, 1697–1705 (2008).
206. Burris, S. K., Wang, Q., Bulley, S., Neeb, Z. P. & Jaggar, J. H. 9-Phenanthrol inhibits recombinant and arterial myocyte TMEM16A channels. *Br. J. Pharmacol.* **172**, 2459–2468 (2015).
207. Garland, C. J. *et al.* TRPM4 inhibitor 9-phenanthrol activates endothelial cell intermediate conductance calcium-activated potassium channels in rat isolated mesenteric artery. *Br. J. Pharmacol.* **172**, 1114–1123 (2015).
208. Lee, H. *et al.* Premature contractions of the bladder are suppressed by interactions between TRPV4 and SK3 channels in murine detrusor PDGFR α + cells. *Sci. Rep.* **7**, 12245 (2017).
209. Koh, B. H. *et al.* Platelet-derived growth factor receptor- α cells in mouse urinary

- bladder: a new class of interstitial cells. *J Cell Mol Med* **16**, 691–700 (2012).
210. Griffin, C. S. *et al.* Muscarinic Receptor Induced Contractions of the Detrusor are Mediated by Activation of TRPC4 Channels. *J. Urol.* **196**, 1796–1808 (2016).
  211. Griffin, C. S., Thornbury, K. D., Hollywood, M. A. & Sergeant, G. P. Muscarinic receptor-induced contractions of the detrusor are impaired in TRPC4 deficient mice. *Sci. Rep.* **8**, 9264 (2018).
  212. Morelli, M. B. *et al.* Cross-talk between alpha 1D-adrenoceptors and transient receptor potential vanilloid type 1 triggers prostate cancer cell proliferation. *BMC Cancer* **14**, 921 (2014).
  213. Wissenbach, U. *et al.* Expression of CaT-like, a Novel Calcium-selective Channel, Correlates with the Malignancy of Prostate Cancer. *J. Biol. Chem.* **276**, 19461–19468 (2001).
  214. Sagredo, A. I. *et al.* TRPM4 channel is involved in regulating epithelial to mesenchymal transition, migration, and invasion of prostate cancer cell lines. *J. Cell. Physiol.* **234**, 2037–2050 (2019).
  215. Holzmann, C. *et al.* Transient receptor potential melastatin 4 channel contributes to migration of androgen-insensitive prostate cancer cells. *Oncotarget* **6**, 41783–41793 (2015).
  216. Chen, L. *et al.* Downregulation of TRPM7 suppressed migration and invasion by regulating epithelial–mesenchymal transition in prostate cancer cells. *Med. Oncol.* **34**, 1–11 (2017).
  217. Grolez, G. & Gkika, D. TRPM8 Puts the Chill on Prostate Cancer. *Pharmaceuticals* **9**, 44 (2016).
  218. Thebault, S. *et al.* Differential role of transient receptor potential channels in Ca²⁺ entry and proliferation of prostate cancer epithelial cells. *Cancer Res.* **66**, 2038–2047 (2006).
  219. Wang, H. P., Pu, X. Y. & Wang, X. H. Distribution profiles of transient receptor potential melastatin-related and vanilloid-related channels in prostatic tissue in rat. *Asian J. Androl.* **9**, 634–640 (2007).
  220. Uhlen, M. *et al.* Tissue-based map of the human proteome. *Science.* **347**, 1260419–1260419 (2015).
  221. Human Protein Atlas. Available at: <http://www.proteinatlas.org>.

222. Dinis, P. *et al.* The distribution of sensory fibers immunoreactive for the TRPV1 (capsaicin) receptor in the human prostate. *Eur. Urol.* **48**, 162–167 (2005).
223. Roman, K., Hall, C., Schaeffer, A. J. & Thumbikat, P. TRPV1 in experimental autoimmune prostatitis. *Prostate* **80**, 28–37 (2020).
224. Zhang, J. *et al.* Upregulation of TRPV1 in spinal dorsal root ganglion by activating NGF-TrkA pathway contributes to pelvic organ cross-sensitisation in rats with experimental autoimmune prostatitis. *Andrologia* **51**, (2019).
225. Funahashi, Y. *et al.* Bladder overactivity and afferent hyperexcitability induced by prostate-to-bladder cross-sensitization in rats with prostatic inflammation. *J. Physiol.* **597**, 2063–2078 (2019).
226. Eggermont, M., De Wachter, S., Eastham, J. & Gillespie, J. Innervation of the Epithelium and Lamina Propria of the Urethra of the Female Rat. *Anat. Rec.* **302**, 201–214 (2019).
227. Danziger, Z. C. & Grill, W. M. Sensory feedback from the urethra evokes state-dependent lower urinary tract reflexes in rat. *J. Physiol.* **595**, 5687–5698 (2017).
228. Birder, L. *et al.* Activation of Urothelial Transient Receptor Potential Vanilloid 4 by 4-Phorbol 12,13-Didecanoate Contributes to Altered Bladder Reflexes in the Rat. *J. Pharmacol. Exp. Ther.* **323**, 227–235 (2007).
229. Oliveira, M. G. *et al.* Autonomic dysregulation at multiple sites is implicated in age-associated underactive bladder in female mice. *Neurourol. Urodyn.* **38**, 1212–1221 (2019).
230. Berrout, J. *et al.* Function of transient receptor potential cation channel subfamily V member 4 (TRPV4) as a mechanical transducer in flow-sensitive segments of renal collecting duct system. *J. Biol. Chem.* **287**, 8782–8791 (2012).
231. Parlani, M., Conte, B., Goso, C., Szallasi, A. & Manzini, S. Capsaicin-induced relaxation in the rat isolated external urethral sphincter: characterization of the vanilloid receptor and mediation by CGRP. *Br. J. Pharmacol.* **110**, 989–994 (1993).
232. Weinhold, P. *et al.* TRPA1 Receptor Induced Relaxation of the Human Urethra Involves TRPV1 and Cannabinoid Receptor Mediated Signals, and Cyclooxygenase Activation. *J. Urol.* **183**, 2070–2076 (2010).
233. Deckmann, K. *et al.* Bitter triggers acetylcholine release from polymodal urethral chemosensory cells and bladder reflexes. *Proc. Natl. Acad. Sci. U. S. A.* **111**, 8287–8292

(2014).

234. Tizzano, M. *et al.* Nasal chemosensory cells use bitter taste signaling to detect irritants and bacterial signals. *Proc. Natl. Acad. Sci. U. S. A.* **107**, 3210–3215 (2010).
235. Krasteva, G. *et al.* Cholinergic chemosensory cells in the trachea regulate breathing. *Proc. Natl. Acad. Sci. U. S. A.* **108**, 9478–9483 (2011).
236. Liman, E. R. TRPM5. *Handb. Exp. Pharmacol.* **222**, 489–502 (2014).
237. Yu, Y. & de Groat, W. C. Sensitization of pelvic afferent nerves in the in vitro rat urinary bladder-pelvic nerve preparation by purinergic agonists and cyclophosphamide pretreatment. *Am. J. Physiol. Physiol.* **294**, F1146–F1156 (2008).
238. Park, B. S. & Lee, J.-O. Recognition of lipopolysaccharide pattern by TLR4 complexes. *Exp. Mol. Med.* **45**, e66–e66 (2013).
239. Alpizar, Y. A. *et al.* TRPV4 activation triggers protective responses to bacterial lipopolysaccharides in airway epithelial cells. *Nat. Commun.* **8**, 1059 (2017).
240. Charrua, A., Cruz, C. D., Cruz, F. & Avelino, A. Transient Receptor Potential Vanilloid Subfamily 1 is Essential for the Generation of Noxious Bladder Input and Bladder Overactivity in Cystitis. *J Urol* **177**, 1537–1541 (2007).
241. Vizzard, M. A. Alterations in spinal cord Fos protein expression induced by bladder stimulation following cystitis. *Am. J. Physiol. Integr. Comp. Physiol.* **278**, R1027–R1039 (2000).
242. Herrera, D. G. & Robertson, H. A. Activation of c-fos in the brain. *Progress in Neurobiology* **50**, 83–107 (1996).
243. Harris, J. A. Using c-fos as a neural marker of pain. *Brain Research Bulletin* **45**, 1–8 (1998).
244. Cox, P. J. Cyclophosphamide cystitis—Identification of acrolein as the causative agent. *Biochem. Pharmacol.* **28**, 2045–2049 (1979).
245. Aizawa, N. *et al.* KPR-2579, a novel TRPM8 antagonist, inhibits acetic acid-induced bladder afferent hyperactivity in rats. *Neurourol. Urodyn.* **37**, 1633–1640 (2018).
246. Franzén, K., Johansson, J.-E., Andersson, G., Pettersson, N. & Nilsson, K. Urinary incontinence in women is not exclusively a medical problem: A population-based study on urinary incontinence and general living conditions. *Scand. J. Urol. Nephrol.* **43**, 226–232 (2009).
247. Rothrock, N. E., Lutgendorf, S. K., Kreder, K. J., Ratliff, T. & Zimmerman, B. Stress and

- symptoms in patients with interstitial cystitis: a life stress model. *Urology* **57**, 422–427 (2001).
248. Valentino, R. J., Wood, S. K., Wein, A. J. & Zderic, S. A. The bladder–brain connection: putative role of corticotropin-releasing factor. *Nat. Rev. Urol.* **8**, 19–28 (2011).
  249. Wolfe-Christensen, C., Veenstra, A. L., Kovacevic, L., Elder, J. S. & Lakshmanan, Y. Psychosocial Difficulties in Children Referred to Pediatric Urology: A Closer Look. *Urology* **80**, 907–913 (2012).
  250. Mingin, G. C., Peterson, A., Erickson, C. S., Nelson, M. T. & Vizzard, M. A. Social stress induces changes in urinary bladder function, bladder NGF content, and generalized bladder inflammation in mice. *Am. J. Physiol. Integr. Comp. Physiol.* **307**, R893–R900 (2014).
  251. Mingin, G. C. *et al.* Social stress in mice induces urinary bladder overactivity and increases TRPV₁ channel-dependent afferent nerve activity. *Am. J. Physiol. Integr. Comp. Physiol.* **309**, R629–R638 (2015).
  252. Tykocki, N. R. *et al.* Development of stress-induced bladder insufficiency requires functional TRPV1 channels. *Am. J. Physiol. Physiol.* **315**, F1583–F1591 (2018).
  253. Wood, S. K., Baez, M. A., Bhatnagar, S. & Valentino, R. J. Social stress-induced bladder dysfunction: potential role of corticotropin-releasing factor. *Am. J. Physiol. Integr. Comp. Physiol.* **296**, R1671–R1678 (2009).
  254. Merrill, L. & Vizzard, M. A. Intravesical TRPV4 blockade reduces repeated variate stress-induced bladder dysfunction by increasing bladder capacity and decreasing voiding frequency in male rats. *Am J Physiol Regul Integr Comp Physiol* **307**, R471–R480 (2014).
  255. Maggi, C. A. *et al.* The contribution of capsaicin-sensitive innervation to activation of the spinal vesico-vesical reflex in rats: relationship between substance P levels in the urinary bladder and the sensory-efferent function of capsaicin-sensitive sensory neurons. *Brain Res.* **415**, 1–13 (1987).
  256. de Groat, W. C. *et al.* Mechanisms underlying the recovery of urinary bladder function following spinal cord injury. *J. Auton. Nerv. Syst.* **30**, S71–S77 (1990).
  257. Simone, D. A., Nolano, M., Johnson, T., Wendelschafer-Crabb, G. & Kennedy, W. R. Intradermal injection of capsaicin in humans produces degeneration and subsequent reinnervation of epidermal nerve fibers: Correlation with sensory function. *J.*

Neurosci. **18**, 8947–8959 (1998).

258. Phé, V. *et al.* Intravesical vanilloids for treating neurogenic lower urinary tract dysfunction in patients with multiple sclerosis: A systematic review and meta-analysis. A report from the Neuro-Urology Promotion Committee of the International Continence Society (ICS). *Neurourol. Urodyn.* **37**, 67–82 (2018).
259. Craft, R. M., Cohen, S. M. & Porreca, F. Long-lasting desensitization of bladder afferents following intravesical resiniferatoxin and capsaicin in the rat. *Pain* **61**, 317–323 (1995).
260. de Sèze, M. *et al.* Intravesical instillation of capsaicin in urology: A review of the literature. *Eur. Urol.* **36**, 267–77 (1999).
261. Andrade, E. L. *et al.* TRPA1 receptor modulation attenuates bladder overactivity induced by spinal cord injury. *Am. J. Physiol. Physiol.* **300**, F1223–F1234 (2011).
262. Osman, N. I. & Chapple, C. R. Contemporary concepts in the aetiopathogenesis of detrusor underactivity. *Nat. Rev. Urol.* **11**, 639–648 (2014).
263. Abrams, P. *et al.* The standardisation of terminology in lower urinary tract function: report from the standardisation sub-committee of the International Continence Society. *Urology* **61**, 37–49 (2003).
264. Chapple, C. R. *et al.* The Underactive Bladder: A New Clinical Concept? *Eur. Urol.* **68**, 351–353 (2015).
265. Hoag, N. & Gani, J. Underactive Bladder: Clinical Features, Urodynamic Parameters, and Treatment. *Int. Neurourol. J.* **19**, 185–189 (2015).
266. Takaoka, E. *et al.* Effect of TRPV4 activation in a rat model of detrusor underactivity induced by bilateral pelvic nerve crush injury. *Neurourol. Urodyn.* **37**, 2527–2534 (2018).
267. Mukerji, G. *et al.* Pain during ice water test distinguishes clinical bladder hypersensitivity from overactivity disorders. *BMC Urol.* **6**, 31 (2006).
268. Mukerji, G. *et al.* Cool and menthol receptor TRPM8 in human urinary bladder disorders and clinical correlations. *BMC Urol.* **6**, (2006).
269. Liu, B. *et al.* Increased severity of inflammation correlates with elevated expression of TRPV1 nerve fibers and nerve growth factor on interstitial cystitis/bladder pain syndrome. *Urol. Int.* **92**, 202–8 (2014).
270. Dornelles, F. N., Andrade, E. L., Campos, M. M. & Calixto, J. B. Role of CXCR2 and

- TRPV1 in functional, inflammatory and behavioural changes in the rat model of cyclophosphamide-induced haemorrhagic cystitis. *Br. J. Pharmacol.* **171**, 452–467 (2014).
271. Dinis, P., Charrua, A., Avelino, A. & Cruz, F. Intravesical resiniferatoxin decreases spinal c-fos expression and increases bladder volume to reflex micturition in rats with chronic inflamed urinary bladders. *BJU Int.* **94**, 153–157 (2004).
  272. de Sèze, M. *et al.* Capsaicin and neurogenic detrusor hyperreflexia: A double-blind placebo-controlled study in 20 patients with spinal cord lesions. *Neurourol. Urodyn.* **17**, 513–523 (1998).
  273. Lazzeri, M. *et al.* Intravesical Resiniferatoxin for the Treatment of Hypersensitive Disorder: a Randomized Placebo Controlled Study. *J. Urol.* **164**, 676–679 (2000).
  274. Ham, B. K., Kim, J. H., Oh, M. M., Lee, J. G. & Bae, J. H. Effects of Combination Treatment of Intravesical Resiniferatoxin Instillation and Hydrodistention in Patients with Refractory Painful Bladder Syndrome/Interstitial Cystitis: A Pilot Study. *Int. Neurourol. J.* **16**, 41–46 (2012).
  275. Payne, C. K. *et al.* Intravesical Resiniferatoxin for the Treatment of Interstitial Cystitis: a Randomized, Double-blind, Placebo Controlled Trial. *J. Urol.* **173**, 1590–1594 (2005).
  276. Meotti, F. C., Forner, S., Lima-Garcia, J. F., Viana, A. F. & Calixto, J. B. Antagonism of the transient receptor potential ankyrin 1 (TRPA1) attenuates hyperalgesia and urinary bladder overactivity in cyclophosphamide-induced haemorrhagic cystitis. *Chem. Biol. Interact.* **203**, 440–447 (2013).
  277. Deberry, J. J., Schwartz, E. S. & Davis, B. M. TRPA1 mediates bladder hyperalgesia in a mouse model of cystitis. *Pain* **155**, 1280–1287 (2014).
  278. Alagiri, M., Chottiner, S., Ratner, V., Slade, D. & Hanno, P. M. Interstitial cystitis: Unexplained associations with other chronic disease and pain syndromes. *Urology* **49**, 52–57 (1997).
  279. Grundy, L. & Brierley, S. M. Cross-organ sensitization between the colon and bladder: to pee or not to pee? *Am. J. Physiol. Liver Physiol.* **314**, G301–G308 (2018).
  280. Malykhina, A. P. Neural mechanisms of pelvic organ cross-sensitization. *Neuroscience* **149**, 660–672 (2007).
  281. Furuta, A. *et al.* Cross-sensitization mechanisms between colon and bladder via transient receptor potential A1 stimulation in rats. *Int. Urogynecol. J.* **25**, 1575–1581

(2014).

282. Geirsson, G. *et al.* Positive Bladder Cooling Test in Neurologically Normal Young Children. *J. Urol.* **151**, 446–448 (1994).
283. Bors, E. H. & Blinn, K. A. Spinal Reflex Activity from the Vesical Mucosa in Paraplegic Patients. *Arch. Neurol. Psychiatry* **78**, 339–354 (1957).
284. Ronzoni, G., Menchinelli, P., Manca, A. & De Giovanni, L. The ice-water test in the diagnosis and treatment of the neurogenic bladder. *Br. J. Urol.* **79**, 698–701 (1997).
285. Geirsson, G., Lindström, S. & Fall, M. The Bladder Cooling Reflex and the Use of Cooling as Stimulus to the Lower Urinary Tract. *J. Urol.* **162**, 1890–1896 (1999).
286. Winchester, W. J. *et al.* Inhibition of TRPM8 channels reduces pain in the cold pressor test in humans. *J. Pharmacol. Exp. Ther.* **351**, 259–69 (2014).
287. Everaerts, W. & De Ridder, D. TRPM8 antagonists to treat lower urinary tract symptoms: don't lose your cool just yet. *BJU Int.* **117**, 384–385 (2016).
288. Ghei, M. & Malone-Lee, J. Using the circumstances of symptom experience to assess the severity of urgency in the overactive bladder. *J. Urol.* **174**, 972–6 (2005).
289. Imamura, T. *et al.* Cold environmental stress induces detrusor overactivity via resiniferatoxin-sensitive nerves in conscious rats. *Neurourol. Urodyn.* **27**, 348–352 (2008).
290. Kondo, A. *et al.* Prevalence of hand-washing urinary incontinence in healthy subjects in relation to stress and urge incontinence. *Neurourol. Urodyn.* **11**, 519–523 (1992).
291. Imamura, T., Ishizuka, O. & Nishizawa, O. Cold stress induces lower urinary tract symptoms. *Int. J. Urol.* **20**, 661–669 (2013).
292. Victor, E., O'Connell, K. A. & Blaivas, J. G. Environmental cues to urgency and leakage episodes in patients with overactive bladder syndrome: a pilot study. *J Wound Ostomy Cont. Nurs* **39**, 181–6 (2012).
293. Zinner, N. R. OAB. Are we barking up the wrong tree? A lesson from my dog. *Neurourol. Urodyn.* **30**, 1410–1411 (2011).
294. Boyden, E. S., Zhang, F., Bamberg, E., Nagel, G. & Deisseroth, K. Millisecond-timescale, genetically targeted optical control of neural activity. *Nat. Neurosci* **8**, 1263–1268 (2005).
295. Chow, B. Y. *et al.* High-performance genetically targetable optical neural silencing by light-driven proton pumps. *Nature* **463**, 98–102 (2010).

296. Samineni, V. K. *et al.* Optogenetic silencing of nociceptive primary afferents reduces evoked and ongoing bladder pain. *Sci. Rep.* **7**, 15865 (2017).
297. Park, J. H. *et al.* Optogenetic Modulation of Urinary Bladder Contraction for Lower Urinary Tract Dysfunction. *Sci. Rep.* **7**, 40872 (2016).
298. Yao, J. *et al.* A corticopontine circuit for initiation of urination. *Nat. Neurosci.* **21**, 1541–1550 (2018).
299. Mickle, A. D. *et al.* A wireless closed-loop system for optogenetic peripheral neuromodulation. *Nature* **565**, 361–365 (2019).
300. DeBerry, J. J. *et al.* Differential Regulation of Bladder Pain and Voiding Function by Sensory Afferent Populations Revealed by Selective Optogenetic Activation. *Front. Integr. Neurosci.* **12**, 5 (2018).
301. Smith-Anttila, J. A. *et al.* Sensory and Motor Systems Identification of a Sacral, Visceral Sensory Transcriptome in Embryonic and Adult Mice. *eNeuro* **7**, (2020).
302. Girard, B. M., Merrill, L., Malley, S. & Vizzard, M. A. Increased TRPV4 Expression in Urinary Bladder and Lumbosacral Dorsal Root Ganglia in Mice with Chronic Overexpression of NGF in Urothelium. *J. Mol. Neurosci.* **51**, 602–614 (2013).
303. Sharopov, B. R., Gulak, K. L., Philypov, I. B., Sotkis, A. V. & Shuba, Y. M. TRPV1 alterations in urinary bladder dysfunction in a rat model of STZ-induced diabetes. *Life Sci.* **193**, 207–213 (2018).
304. Shimizu, N. *et al.* Effects of nerve growth factor neutralization on TRP channel expression in laser-captured bladder afferent neurons in mice with spinal cord injury. *Neurosci. Lett.* **683**, 100–103 (2018).
305. Owsianik, G., D’hoedt, D., Voets, T. & Nilius, B. Structure–function relationship of the TRP channel superfamily. in *Reviews of Physiology, Biochemistry and Pharmacology* 61–90 (Springer Berlin Heidelberg, 2006). doi:10.1007/s10254-005-0006-0

Acknowledgements

This work was supported by grants from the Research Foundation—Flanders (FWO; GOA6113N), and the Research Council of KU Leuven (C1-TRPL; Thomas Voets, and Wouter Everaerts). Wouter Everaerts is a senior clinical researcher of the Research Foundation-Flanders (FWO Vlaanderen).

Author contributions

W.E., M.V. and A.S. researched data for article. W.E., M.V. and T.V. wrote the article and reviewed/edited the manuscript before submission. All authors substantially contributed to discussion of content.

Competing interests

The authors declare no competing interests.

Peer review information

Nature Reviews Urology thanks Scott Earley, Luke Grundy and Janet Keast for their contribution to the peer review of this work.

Related links

Tabula Muris database: <https://tabula-muris.ds.czbiohub.org/>

Key points

- Transient receptor potential (TRP) channels are expressed throughout all layers of the bladder wall and their function is closely related to the location of expression.
- High urothelial expression of TRPM4, TRPM7 and TRPV4 has been observed, of which the latter is considered a stretch sensor in urothelial cells.
- TRPV1, TRPA1 and TRPM8 are expressed on afferent nerves in the bladder and are involved in changes in bladder function during inflammation (TRPV1 and TRPA1) or cold stimulation (TRPM8).
- Despite involvement of TRP channels in animal models for overactive bladder, underactive bladder and cold-induced LUTS, translation to clinical applications remains limited.

Table

Table 1. TRP channel expression in different structures in the bladder

	Urothelium	Smooth muscle	Nerve endings in bladder wall	DRG L6-S1 ^a	Retrogradely labeled bladder neurons ^b
TRPV4	++ 24,46,54,62,64	++/- 62,64,88,96	++/- 302	+/- 45,67,302	++/- 48
TRPV2	++ 24,38,54	++ 38	?	++ 67	+/- 48
TRPM7	++ 24,54,117	++ 117	?	++/- 67	?
TRPM4	++ 35,54	++ 23,202	?	++/- 67	?
TRPV1	--/+ 22,24,35,38,45,46,51,54	-- 22	++ 22,57,269	++ 67,303	++ 48,135,303,304
TRPA1	--/+ 46,54,153	-- 153	++ 57	++ 67,153	++ 48,153
TRPM8	--/+ 46,48,54,174	-- 174	++ 268	++ 174	++ 48,175
TRPC4	--/+ 35,54	++ 210	++ 66	++ 187	++ 187

++, present; ++/-, most likely present but conflicting or limited available evidence; +/-, unclear; --, absent or very low expression; --/+, most likely absent but conflicting or limited available evidence; ?, no data available; DRG, dorsal root ganglia. ^aData from non-labelled DRG neurons at the spinal cord level of bladder innervation. ^bData from DRG neurons labelled by injection of tracer or dye in the bladder.

Table 2. TRP channels as targets in the LUT

Pathology	TRP channel	Preclinical evidence	Clinical evidence	Refs
Detrusor overactivity				
Cystitis-induced	TRPV1	Reduced voiding frequency and pain in <i>Trpv1</i> -KO mice	–	240
Cystitis-induced	TRPA1	Reduced voiding frequency and pain in <i>Trpa1</i> -KO mice	–	158
Cystitis-induced	TRPV4	Reduced voiding frequency and <i>c-Fos</i> expression in <i>Trpv4</i> -KO mice; TRPV4 antagonist reduces voiding frequency during cystitis	–	63
Cystitis-induced	TRPM8	TRPM8 antagonist reduces C-fibre afferent nerve firing and detrusor overactivity during cystitis	–	173, 245
Stress-induced	TRPV1	TRPV1 antagonist reverses stress-induced afferent nerve activity and bladder overactivity	–	251
Stress-induced	TRPV4	Intravesical TRPV4 antagonist reverses stress-induced bladder	–	254

		overactivity		
Neurogenic	TRPV1	TRPV1 desensitization using capsaicin treatment suppresses neurogenic DOA	Intravesical capsaicin instillation improves bladder capacity, frequency and urge incontinence	256, 258
Neurogenic	TRPA1	<i>Trpa1</i> upregulated in DRG neurons after spinal cord injury; TRPA1 antagonist decreases non-voiding contractions during neurogenic DOA	–	261
Detrusor underactivity				
neurogenic	TRPV4	Intravesical TRPV4 agonist instillation increases voiding frequency and reduces postvoid residual volume	–	96, 266
stress-induced	TRPV1	<i>Trpv1</i> -KO mice do not develop bladder underactivity in response to stress	–	252
Bladder pain				
bladder pain syndrome	TRPM8	–	Increase in TRPM8-immunoreactive suburothelial nerve fibres in BPS patients; density of TRPM8-expressing fibres correlates with frequency and pain scores	268
bladder pain syndrome (humans) ; cystitis-induced (rodents)	TRPV1	TRPV1 antagonist reduces nociceptive behavior and mechanical hypersensitivity in cyclophosphamide-induced cystitis model of BPS; <i>Trpv1</i> -KO mice do not develop urinary frequency and cutaneous hypersensitivity of the hind paws	Increase in TRPV1-expressing suburothelial nerve fibres in BPS patients; intravesical RTX reduces pain scores and possibly frequency scores	133, 271, 269
cystitis-induced	TRPA1	TRPA1 antagonist reduces cutaneous hypersensitivity of the hind paws, number of non-voiding contractions and visceromotor reaction to bladder distension in cyclophosphamide-induced cystitis model of BPS	–	276, 277
Cold-induced LUTS				
bladder-cooling reflex	TRPM8	Intravesical TRPM8 agonist reduces volume threshold for micturition; TRPM8 antagonist reduces duration of bladder contraction evoked by cold temperature	Intravesical TRPM8 agonist increases threshold temperature needed to induce reflex contraction	174, 285, 286
cold-induced	TRPM8	TRPM8 antagonist inhibits the	–	159

urgency		induction of detrusor contraction by cold stimuli; <i>Trpm8</i> -KO mice do not develop detrusor contraction in response to cold stimulation		

KO, knockout.

Figure legends

Figure 1. Schematic representation of the shared structure of transient receptor potential (TRP) channels. A functional TRP channel comprises four subunits organized around a central pore through which ions flow in or out of the cell. Each subunit consists of 6 transmembrane spanning helices, named S1–S6. The pore-forming region is located between S5 and S6, and the entire transmembrane section is highly conserved among different TRP channel subfamilies. The intracellular amino (N) and carboxy (C) terminal sections are highly variable in length and composition, and contain sequences that are important for regulation, organization and trafficking of the channel. These intracellular sections contain, for example, ankyrin repeats, calmodulin binding sites or binding sites for protein kinases³⁰⁵. **a)** TRP channel composed of four subunits **b)** Top view of four subunits around the central pore opening **c)** Schematic representation of a single TRP subunit, showing six transmembrane domains (S1–S6) and the intracellular N-terminal and C-terminal domains.

Figure 2 | Schematic representation of transient receptor potential (TRP) channels in excitable (sensory nerve endings and smooth muscle cells) and non-excitable (urothelial cells) cells in the bladder wall. Activation of a TRP channel by chemical stimuli (such as capsaicin, protons, bacterial lipopolysaccharides) or physical stimuli (such as heat, cold or membrane stretch) opens the central pore, allowing cations to flow into the cell. In urothelial cells, the Ca^{2+} influx through TRP channels causes the release of messengers such as ATP and NO. In afferent nerve fibres, TRP activation causes cation influx and direct depolarization of the cell. When this depolarization is sufficient to activate voltage-gated channels, an action potential is generated. Similarly, binding of urothelial-released messengers, such as ATP, to ligand-gated ion channels can cause depolarization of the afferent nerve. Upon stimulation, afferent nerves can also release inflammatory mediators such as substance P and CGRP that cause neurogenic inflammation. In smooth muscle cells, the Ca^{2+} influx through an activated TRP channel causes Ca^{2+} -induced contraction. Smooth muscle cells and suburothelial interstitial cells also express P2Y purinergic receptors, but the effects of their activation are unknown in these cell types (as indicated by the ?).

Figure 3. Single cell transient receptor potential channel transcriptomics in mouse mucosa. Based on their expression profile, urothelial cells (expressing *Epcam* and *uroplakins*) can be separated from mesenchymal cells (expressing *Dcn* and *Vim*). **a |** Bladder cells, collected by fluorescence-activated cell sorting, visualized with t-distributed stochastic neighbour embedding (t-SNE) plot and coloured by cell type. Bladder epithelial cells (characterized by high *Epcam* expression) are classified into luminal epithelial cells based on higher expression

levels of *Grhl3* and uroplakin transcripts such as *Upk3a* and *Upk1b*, or basal epithelial cells based on *Krt5* and *Krt14* expression. Mesenchymal cells (characterized by high *Dcn* expression) are divided into two clusters expressing *Car3* and one cluster expressing *Scara5*. **b–d** | t-NSE plots of bladder cells expressing *Dcn* (part **b**), *Epcam* (part **c**) and *Upk3a* (part **d**). **e** | Dot plot showing level of expression (colour scale) and number of expressing cells (point diameter) for *Trp* genes within each cluster of bladder cells. Data from the Tabula Muris database⁵⁴.

Figure 4: Schematic representation of the bladder wall with the presumed cellular localization of TRP channels. For each TRP channel, examples of known physical and chemical agonists (if any) are listed, together with the proposed function in the bladder and possible clinical applications. Urothelial expression is well established for TRPM4, TRPV2, TRPV4 and TRPM7, but controversial for TRPA1, TRPV1 and TRPM8. Expression of TRPA1, TRPV1, TRPM8 and TRPC4 is well established on afferent nerves. ROS, reactive oxygen species.

Glossary terms

afferent nerve: a nerve that conveys information from the organs to the central nervous system (as opposed to efferent nerves that send information from the central nervous system to the organs)

agonist: a chemical compound that activates a receptor or ion channel

antagonist: a chemical compound that inhibits a receptor or ion channel

neurogenic inflammation: inflammation caused by the release of inflammatory mediators from nerve endings

detrusor–sphincter dyssynergia: detrusor contraction accompanied by involuntary contraction (instead of relaxation) of the urethral sphincter

Toc blurb

Transient receptor potential (TRP) channels have an important role in sensory mechanisms of the lower urinary tract. Vanneste et al. discuss the involvement of TRP channels in normal and abnormal bladder sensations and their potential as therapeutic targets.