

Receptors, channels, and signalling in the urothelial sensory system in the bladder

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Abstract | The storage and periodic elimination of urine, termed micturition, requires a complex neural control system to coordinate the activities of the urinary bladder, urethra, and urethral sphincters. At the level of the lumbosacral spinal cord, lower urinary tract reflex mechanisms are modulated by supraspinal controls with mechanosensory input from the urothelium, resulting in regulation of bladder contractile activity. The specific identity of the mechanical sensor is not yet known, but considerable interest exists in the contribution of transient receptor potential (TRP) channels to the mechanosensory functions of the urothelium. The sensory, transduction, and signalling properties of the urothelium can influence adjacent urinary bladder tissues including the suburothelial nerve plexus, interstitial cells of Cajal, and detrusor smooth muscle cells. Diverse stimuli, including those that activate TRP channels expressed by the urothelium, can influence urothelial release of chemical mediators (such as ATP). Changes to the urothelium are associated with a number of bladder pathologies that underlie urinary bladder dysfunction. Urothelial receptor and/or ion channel expression and the release of signalling molecules (such as ATP and nitric oxide) can be altered with bladder disease, neural injury, target organ inflammation, or psychogenic stress. Urothelial receptors and channels represent novel targets for potential therapies that are intended to modulate micturition function or bladder sensation.

Transient receptor potential (TRP) channels and purinergic receptors are expressed in neural and non-neural elements of the lower urinary tract (LUT) and are thought to contribute to physiological and pathological function. Targeting TRP channels and purinergic receptors in the LUT could affect mechanosensation, chemosensation, pain perception, and excitability of bladder sensory nerves to modulate the afferent limb of the micturition reflex and affect the overall function of the urinary bladder.

Anatomy of the lower urinary tract

The LUT is under both voluntary (somatic) and involuntary (autonomic) control and is comprised of multiple tissues and cell types that include the urinary bladder and the urethra. The urinary bladder is a hollow, smooth-muscle organ and its primary function is to store and release soluble waste from the kidney. To sustain continuous storage and elimination phases, the urinary bladder wall is organized into mucosal, muscular, and serosal and adventitial layers¹.

The mucosal layer consists of transitional epithelial cells that line the lumen of the bladder and a lamina propria beneath the basement membrane of the epithelial cells. The transitional epithelial cells, termed the urothelium, function not only as an impermeable, nonadherent barrier, but also as a sensory component that is capable of responding to multiple stimuli². The lamina propria of the urinary bladder contains interstitial cells of Cajal (ICC), vasculature, and nerve terminals that have all been proposed to integrate epithelial and smooth muscle input to maintain normal bladder function³. Cells similar to ICC, as observed in the gastrointestinal tract, have been found in the urinary bladder of various species (including mouse, guinea pig, rabbit, and human); however, whether species differences exist is not known, their specific functions have not yet been determined, and much controversy currently exists regarding the exact function of these cells⁴.

The muscular layer is organized into three smooth-muscle compartments that are collectively termed the detrusor. The detrusor smooth muscle is structurally

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Key points

- Complex neural pathways coordinate the activities of the urinary bladder. The bladder reflex exists in two modes of operation, storage and elimination. The elimination phase is triggered by urothelial mechanosensors
- Urothelial cells exhibit specialized sensory and signalling properties enabling responses to stimuli and release of chemical mediators, and express diverse receptors and ion channels linked to mechanoceptive and nociceptive sensations
- The urothelium secretes many signalling molecules (including neurotrophins, neuropeptides, acetylcholine, prostaglandins, nitric oxide, and cytokines); but ATP seems to be the main messenger in voiding reflexes and pain
- Transient receptor potential (TRP) channels from different subfamilies are expressed in the bladder, exhibit specific distributions in the lower urinary tract, and are implicated in its normal and pathological physiology
- The urothelium expresses purinergic receptors and releases neuroactive chemicals, including ATP, from its apical and basolateral surfaces in response to stimuli
- Current research is focusing on the identification of novel targets in the sensory limb of the micturition reflex (such as TRP channels and purinergic neurotransmission) to treat sensory voiding disorders

different from that of the urethral smooth muscle in that it consists of inner and outer longitudinal layers and a middle circular layer⁵. The serosa surrounds the superior and lateral external surfaces of the bladder wall, whereas the retroperitoneal bladder wall is surrounded by loose connective tissue termed adventitia¹.

The urethra functions as the outlet from the urinary bladder to the external world and its tissue wall is organized into mucosal, muscular, and adventitial layers (FIG. 1). The cells lining most of the urethral mucosa are nonkeratinized, stratified squamous epithelial cells as the urethra does not have to accommodate the mechanical forces experienced by the urinary bladder⁶. In men, the smooth muscle layer of the urethra is arranged into inner longitudinal and outer circular layers and is termed the internal urethral sphincter⁷. However, in women, the smooth muscle layers do not seem to establish an internal sphincter⁷. The striated muscle layer surrounding the proximal urethra is termed the external urethral sphincter and provides voluntary control over bladder filling.

Neural control of micturition

The storage and elimination of urine are important and necessary in daily life and involve intricate neural signalling pathways requiring coordination of the urinary bladder and urethra^{5,8} (FIG. 1). The micturition reflex is often referred to as a spinobulbospinal system as information travels from the spinal cord to the brainstem and back to the spinal cord to relay information regarding urinary bladder filling⁹. During the emptying phase of the micturition cycle, parasympathetic nerve cotransmission involving ATP and acetylcholine acting on smooth muscle P2X purinoceptor 1 (P2X₁) and muscarinic receptors (M₂ and M₃) mediates urinary bladder contraction. During the storage phase of the micturition cycle, the sympathetic nervous system inhibits detrusor smooth muscle contraction, enabling the bladder to relax and increase in size. In addition, the urethral sphincters contract in response to background stimulation from the sympathetic and somatic nervous systems⁹. The switch to the emptying phase is triggered by tension in the bladder

that stimulates stretch receptors (which are slowly adapting mechanoreceptors) within the bladder⁹. These receptors activate A δ and C fibres (sensory afferent nerves) that convey information about bladder filling and noxious stimuli, respectively, from the bladder neck and urethra to sacral spinal cord levels S2–S4 via the pelvic, pudendal, and hypogastric nerves¹⁰. Low-level afferent nerve discharge is present throughout bladder filling. During bladder filling, as hydrostatic pressure rises, thinly myelinated A δ afferent fibres of the hypogastric and pelvic nerves increase their activity¹⁰ (FIG. 2). Bladder afferent nerves that terminate peripherally in the urinary bladder might also signal through unmyelinated C fibres that respond to nociceptive stimuli (such as chemicals, inflammation, and elevated intravesical pressures). C fibres are quiescent (silent C fibres) during normal bladder filling, but their activation might contribute to the development of LUT symptoms and pathological disorders of the urinary bladder¹⁰. The sensation of bladder fullness is experienced in humans with intravesical pressures of 5–15 mmHg¹¹. On passing this tension threshold, bladder afferent discharge increases to stimulate micturition reflexes (FIG. 2). Urinary urgency occurs in humans when intravesical pressures reach 20–25 mmHg and, if not relieved, pain and/or discomfort occurs when pressures exceed 30 mmHg¹¹.

A δ and C fibres from the urinary bladder enter the spinal cord through the Lissauer tract and synapse in laminae (I, V, VII, and X), which contain parasympathetic preganglionic neurons and neurons that project to the periaqueductal grey (PAG)⁹. Signals then travel from the PAG to the pontine micturition centre (PMC) and back to the lumbosacral spinal cord to synapse on preganglionic sympathetic and parasympathetic neurons⁹. Multiple higher brain centres project to the PAG including the hypothalamus, preoptic region, central nucleus of the amygdala, bed nucleus of the stria terminalis, and prefrontal cortex. During the filling phase, signals from these higher brain centres inhibit the PAG reducing excitation of the PMC, which prevents inappropriate voiding or incontinence¹⁰. However, during the voiding phase, activation of the PMC suppresses preganglionic sympathetic control (which releases the contraction of the sphincter and removes the inhibition of the detrusor) and enables the parasympathetic system to stimulate detrusor smooth muscle contraction and the expulsion of urine.

The sensory component of the urothelium

The urothelium has previously been considered a passive barrier; however, a large and growing body of literature now demonstrates that it has additional and important physiological roles^{12,13}. Current evidence indicates that the urothelium has specialized sensory and transduction or signalling properties that enable it to respond to chemical or mechanical stimuli (such as changes in bladder pressure or tension, and tonicity of urine)^{12,13}, as well as acting as a functional barrier against urine solutes. The distribution of anatomical components (FIG. 1) suggests that reciprocal communication is possible between urothelial cells that are

located close to bladder nerves and other cell types such as smooth muscle cells and ICC^{12,14}. For example, following activation of surface proteins, evidence exists that ATP is released from the urothelium and can bind to purinergic receptors on nerve cells within the bladder wall, thereby stimulating afferent nerve activity, resulting in bladder sensation^{15–17}. The sensory inputs and outputs of the urothelium that result in receptor-mediated and channel-mediated events at

the urothelium and subsequent release or secretion of chemical mediators have been described using the term ‘urothelial-associated sensory web’ (REF. 12).

Anatomical proximity of nerve fibres to the urothelium

Findings of a 2002 study established that both sensory afferent and autonomic efferent nerves are located in a suburothelial plexus in close proximity to, and extending into the urothelium¹⁸ (FIG. 3). Adrenergic (tyrosine hydroxylase positive) and cholinergic (choline acetyltransferase positive) nerves have also been observed in close proximity to the urothelium¹⁹. Additionally, the suburothelial plexus exhibits immunoreactivity for neuropeptides (such as calcitonin gene-related peptide, substance P, corticotropin-releasing factor (CRF)), and receptors such as P2X₃ and P2X_{2/3} receptors and transient receptor potential vanilloid receptor 1 (TRPV1)^{2,20,21} (FIG. 3). The sensory function of these nerve fibres was indicated by intravesical administration of the ultra-potent C fibre neurotoxin resiniferatoxin, which reduced the density of TRPV1 and P2X₃ immunoreactive suburothelial nerves in humans with neurogenic detrusor overactivity^{16,22,23}.

Urothelial expression of receptors and ion channels

Many different receptors and ion channels have been identified in the urothelium and many are implicated in mechanoceptive or nociceptive sensations^{2,12,13,24–26}. These receptors and ion channels include purinergic (such as P2X_{1–7} and P2Y₁, P2Y₂, and P2Y₄)^{2,27} (TABLE 1), adrenergic (α and β)², cholinergic (muscarinic; M₁–M₅ and nicotinic α_1 – α_9 , β_2 and β_4)², protease-activated receptors²⁸, acid sensing ion channels (ASIC) (such as ASIC2a

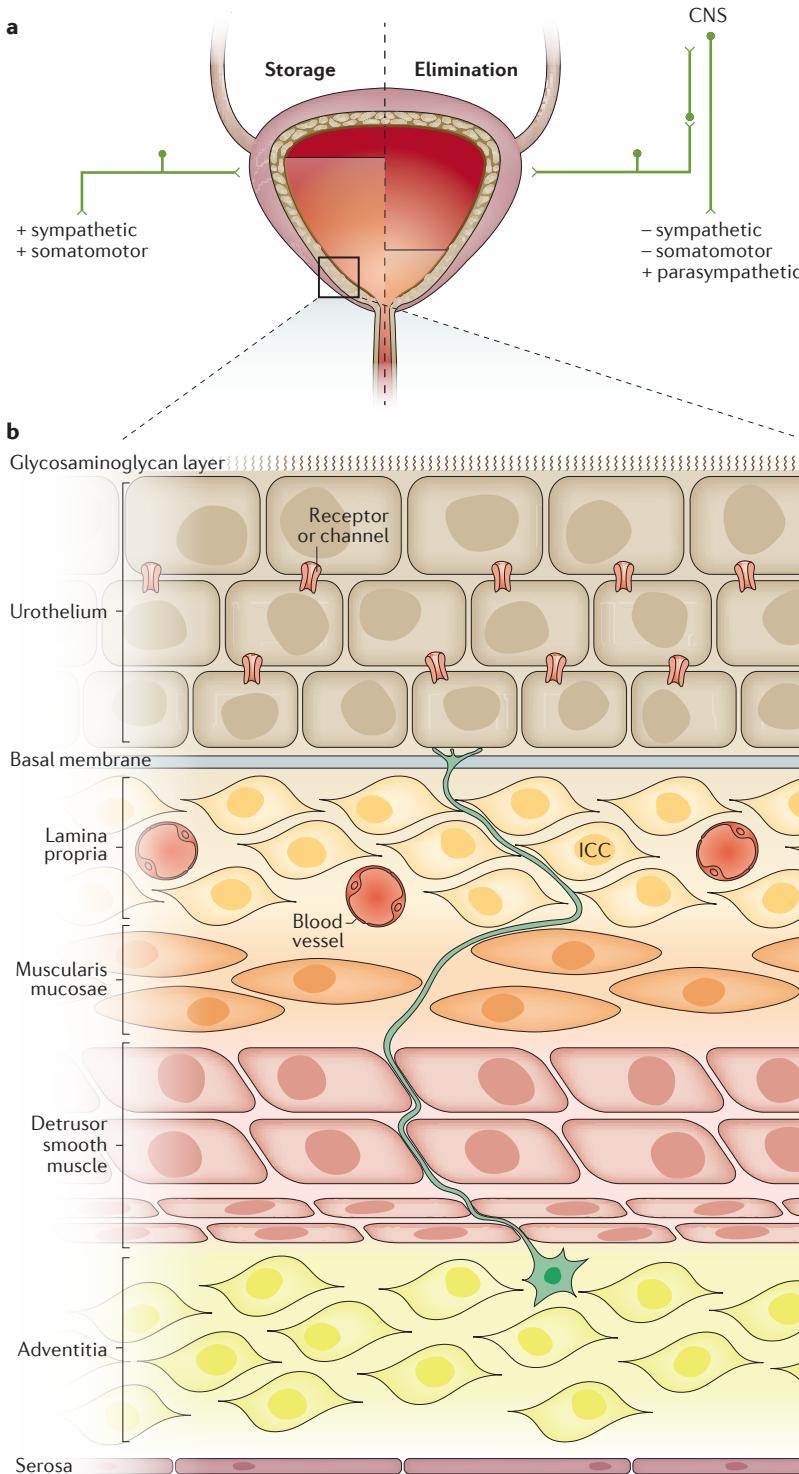


Figure 1 | An overview of micturition reflex control and cell layers of the wall of the urinary bladder. **a** | Storage and elimination (voiding) of urine. The neural pathways that control lower urinary tract function maintain a reciprocal relationship between the urinary bladder and the urethral outlet. Storage reflexes are activated during bladder filling and are organized primarily in the spinal cord, whereas voiding is mediated by reflex mechanisms that are organized in the brain. During bladder filling and storage, the parasympathetic innervation of the detrusor is inhibited and the smooth and striated parts of the urethral sphincter are activated, preventing involuntary bladder emptying. During bladder filling the parasympathetic efferent pathway to the bladder, including a population of CNS (for example pontine micturition centre) neurons, is turned off. As bladder filling continues and a critical level of bladder distension is achieved, the afferent activity from mechanoreceptors in the bladder switches the pathway to the elimination mode. During elimination (voiding), parasympathetic activity is activated resulting in urinary bladder contraction, whereas sympathetic activity and somatomotor activity is withdrawn. **b** | Anatomical components of the urinary bladder wall. Numerous receptors (including purinergic, adrenergic, cholinergic, neurotrophin, and neuropeptide) and ion channels (transient receptor potential channels) are expressed by the anatomical components of the urinary bladder wall including the urothelium, bladder sensory nerves, interstitial cells of Cajal (ICC), and detrusor smooth muscle.

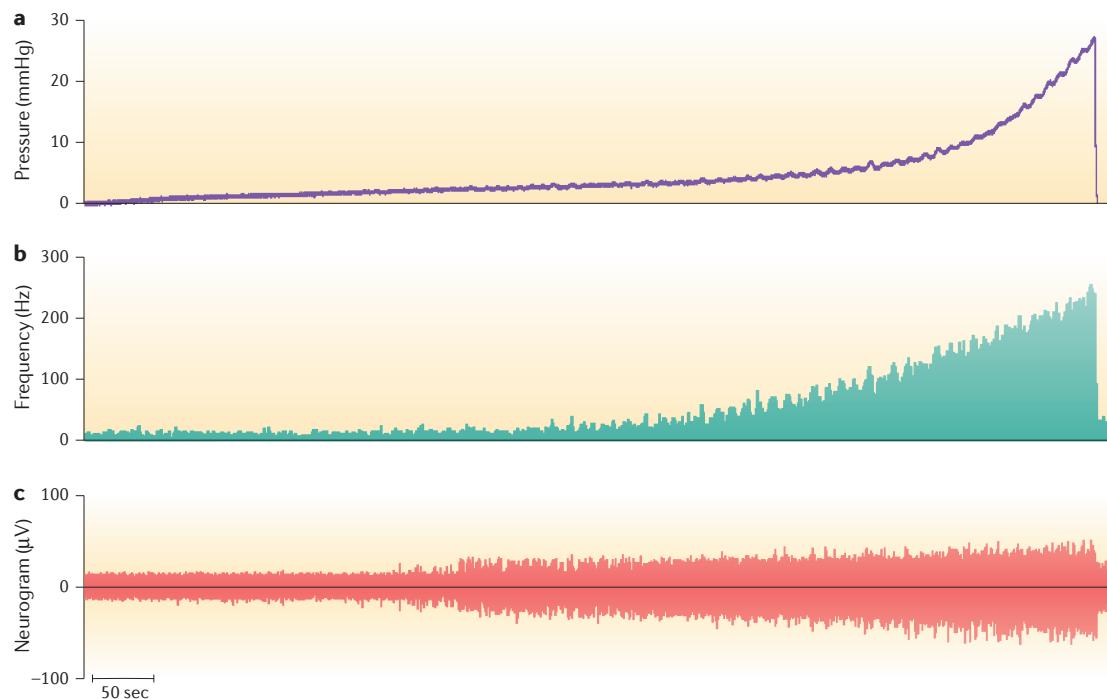


Figure 2 | Illustration of low-level afferent nerve discharge that is present throughout bladder filling. In humans, the sensation of bladder fullness is experienced at intravesical pressures of 5–15 mmHg. On passing this tension threshold, bladder afferent discharge increases to stimulate micturition reflexes. Urinary urgency occurs in humans when intravesical pressures reach 20–25 mmHg and if not relieved, pain and/or discomfort occur when pressures exceed 30 mmHg¹¹.

and ASIC3)²⁹, neurotrophin receptors ($p75^{\text{NTR}}$ and tropomyosin receptor kinases A and B)^{30–33}, CRF receptors 1 and 2 (REF. 21), transient receptor potential (TRP) (including TRPV1, TRPV2, TRPV4, TRPM7, TRPM8, TRPA1) channels^{2,24,34–36} (TABLE 1), neuropeptide receptors (such as pituitary adenyl cyclase-activating polypeptide (PACAP) type I receptor and vasoactive intestinal polypeptide (VIP) receptor 2)^{24,25,37}, and chemokine receptors (such as CXCR4, CX3CR1)³⁸. The expression of these receptors and ion channels means that the urothelium can respond to diverse stimuli from many sources including: stretching and distension during bladder filling^{2,24,34–36}, soluble factors such as nerve growth factor (NGF)²⁴, neuroactive compounds such as PACAP²⁵, VIP²⁵, CRF²¹, acetylcholine², ATP or noradrenaline² (which are released from nerves and inflammatory cells), chemo-kines (including CXCL1, CXCL12, CX3CL1, CCL2), which are released from inflammatory cells^{38–40}, and changes in pH resulting from inflammation^{2,41}.

Urothelial secretion of transmitters and mediators

A variety of signalling molecules (such as neurotrophins, neuropeptides, ATP, acetylcholine, prostaglandins, prostacyclin, nitric oxide and cytokines) are secreted by the urothelium^{2,24,36,42} and it is able to communicate, possibly in a reciprocal manner, with other cell types including bladder nerves, smooth muscle cells, interstitial cells and inflammatory cells^{2,27}. ATP seems to be the main messenger released from urothelial cells during purinergic mechanosensory transduction that acts on P2X₃ receptors on sensory nerves to generate signals that indicate bladder fullness, and pain^{17,43} (FIG. 3).

In this Review, we discuss examples of receptors that are expressed in the urothelium (FIGS 1,3; TABLE 1), their responses on activation and their regulation in experimental models and human studies of bladder dysfunction, where appropriate. This Review will highlight several principal components (including TRP channels and purinergic receptors and receptor signalling) that might underlie the afferent limb of the micturition reflex and to describe how their modulation might influence the sensory processing of bladder filling (FIGS 1,3; TABLE 1).

Transient receptor potential channels

TRP channels are a superfamily of nonspecific cation channels that are generally, but variably, permeable to Ca²⁺ (REFS 44,45) and might act as sensors of stretch and/or chemical irritation in the LUT^{35,46–48}. More than 50 TRP channels have been described in species from yeast to human and 28 have been discovered so far in mammals⁴⁹. The TRP channel superfamily consists of seven subfamilies: TRPC (canonical), TRPM (melastatin), TRPV (vanilloid), TRPA (ankyrin), TRPP (polycystin), TRPML (mucolipin), and TRPN (no mechanopotential).

TRP channels in the lower urinary tract

Multiple TRP channels from different subfamilies, including TRPV1, TRPV2, TRPV4, TRPM7, TRPM8, and TRPA1 are expressed in the urinary bladder, have specific tissue distributions in the LUT, are activated by numerous exogenous and endogenous mediators^{34,35,50} (FIG. 3; TABLE 1), and might have functional roles in the

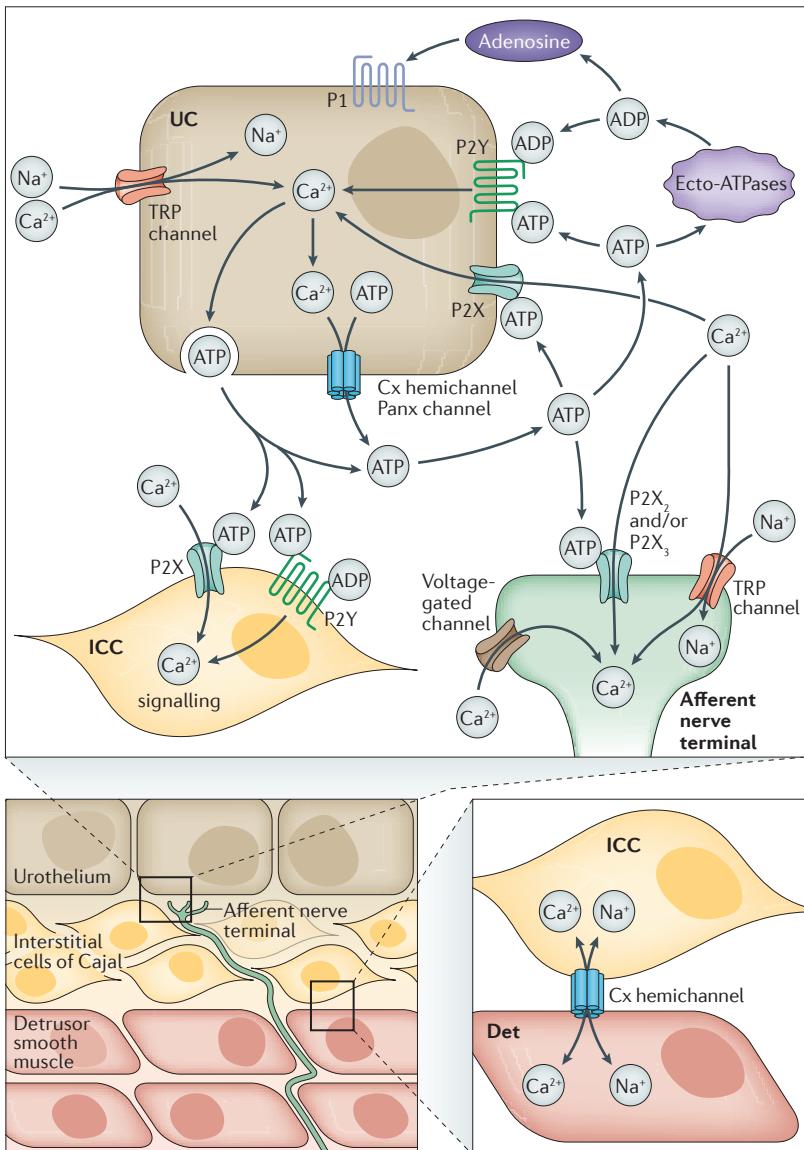


Figure 3 | Hypothetical model of purinergic mechanosensory transduction and the involvement of TRP channels in the LUT focusing on potential interactions among bladder sensory nerves, urothelial cells, detrusor smooth muscle cells and ICC. Distension activates transient receptor potential (TRP) channels expressed by the urothelium leading to release of ATP, which then acts on purinergic receptors (P2X₁ and/or P2X₃) on suburothelial sensory nerves to convey sensory and/or nociceptive signals to the central nervous system (CNS). Several TRP channels are expressed by the urothelium and bladder sensory nerve fibres and are activated by diverse stimuli. Roles in lower urinary tract (LUT) physiology have been proposed for several TRP channels; however, the exact localization and function of TRP channels in the LUT in health and disease are still being determined. Release of ATP can be mediated by exocytosis (such as vesicular release), pannexin (Panx) channels and connexin (Cx) hemichannels. Expression of both P2X and P2Y receptors in interstitial cells of Cajal (ICC) also suggests that ATP release from the urothelium can influence the function of ICC as well as bladder sensory nerves. The signal resulting from ATP release depends on many factors including the purinergic receptors subtypes expressed, activation of G-protein-mediated and Ca²⁺-mediated signalling and by the expression of ATPases and ectonucleotidases that degrade ATP. The ATP signal might influence sites beyond the urothelium and suburothelium. For example, gap junction proteins (such as connexins) are expressed by ICC and detrusor smooth muscle cells (Det) suggesting that gap junction-mediated intercellular communication could be an underlying mechanism for long-distance spread of signals from the urothelial cells to Det. Stimulation of urothelial receptors and channels can release mediators that target bladder sensory nerves and other cell types; urothelial cells can also be targets for neurotransmitters released from nerves or other cell types and can be activated by either autocrine (autoregulation) or paracrine (release from nearby nerve fibres or other cells) mechanisms. P1, purinergic 1 receptor; TRPA1, transient receptor potential channel ankyrin 1; TRPV, transient receptor potential channel vanilloid family; TRPM8, transient receptor potential channel melastatin 8; UC, urothelial cell.

micturition reflex^{46,51}. Many of these channels are also implicated in bladder disorders including overactive bladder (OAB) and interstitial cystitis/bladder pain syndrome (IC/BPS)^{49,52}. TRPV1 is the most widely studied TRP channel with regards to its role in urinary bladder function, but other studies also suggest the involvement of TRPV4 in both normal urinary bladder function and dysfunction⁴⁶. Other TRP channels have also been observed in the LUT (TABLE 1).

Members of the ankyrin, vanilloid, and canonical families of TRP channels have distributions and functions specific to the LUT (TABLE 1). For example, TRPA1 is expressed in unmyelinated C fibres throughout the urinary bladder⁵³ and colocalizes with TRPV1-containing peptidergic nerve fibres⁵⁴. TRPA1 transcripts, detected in the urothelium, are upregulated in the bladder mucosa of humans with bladder outlet obstruction⁵⁵; however, the results of functional experiments in mice suggest that TRPA1 does not contribute to mechanosensation in the urothelium⁵⁶. TRPA1 does seem to have a role in

the afferent limb of the micturition reflex as intravesical TRPA1 agonists (such as allyl isothiocyanate and cinnamaldehyde) have been demonstrated to decrease micturition-threshold pressure, intercontraction intervals, and voided volume and increase micturition frequency in rats^{53,57}. These agonists might act at the level of bladder afferent nerves to contract the detrusor smooth muscle and alter bladder function⁵⁸.

TRPV2 is expressed in superficial cells of the urothelium^{56,59}, detrusor smooth muscle cells⁵⁹, and nerve fibres within the suburothelium⁵⁹. The TRPV2 agonist tetrahydrocannabinol (THC) has been shown to reliably induce Ca²⁺ influx into ATP-primed urothelial cells, suggesting that ATP might facilitate TRPV2 trafficking to the plasma membrane⁵⁹. Patch clamp studies also demonstrated THC-induced currents in urothelial cells, supporting the functional expression of TRPV2 in the urinary bladder⁵⁶. TRPV2 knockout mice exhibit decreased embryonic weight and perinatal viability with surviving adult mice still demonstrating reduced body

weight⁶⁰. Behavioural assays and neurophysiological studies failed to demonstrate the necessity for TRPV2 in the detection of noxious heat or mechanical stimulation under normal or pathological conditions⁶⁰. Additional work is needed to determine the role of TRPV2 in bladder sensation, as this receptor does not seem to be essential for heat or mechanical nociception or hypersensitivity but is important for perinatal survival⁶⁰.

TRPCs are calcium-permeable, nonselective cationic channels implicated in neuronal cell growth, remodelling, axon guidance, and growth-cone signalling. Cyclophosphamide (CYP)-induced cystitis has been shown to increase TRPC1 and TRPC4 expression in bladder sensory neurons and also increase sprouting of bladder sensory neurons in bladder mucosa. CYP-treated *Trpc1/c4*^{-/-} mice failed to exhibit increased bladder sensory neuron sprouting and failed to demonstrate increased voiding frequency with CYP treatment⁶¹.

Members of the TRPM channel family also have diverse functions in the LUT (TABLE 1). TRPM8 is expressed in the urothelium⁶² and in A δ fibres and C fibres throughout the suburothelium of the urinary

bladder^{54,55}. Similar to other TRP channels, the functional expression of TRPM8 in the urothelium is unclear as pharmacological manipulation experiments suggest little to no functional response to agonists in urothelial cells⁵⁶. However, TRPM8 is functionally involved in the micturition reflex through bladder afferent nerve activation⁶³, a cold-induced urgency reflex⁶⁴ and a bladder-cooling reflex that is observed in patients with supraspinal neuronal lesions⁶⁵. TRPM8-containing C fibres might underlie these reflexes in humans owing to the absence of urothelial cell responses to TRPM8 agonists during Ca²⁺ imaging or patch-clamp experiments⁵⁶ and because C fibre density is increased in pathological bladder conditions (such as spinal cord injury)⁵⁵. Additionally, C-fibre activation via TRPM8 seems to contribute to the cooling reflex in guinea pigs⁶⁶, whereas, activation of A δ fibres might underlie this reflex in rats⁶⁷. TRPM2 is a nonselective calcium-permeable cation channel, activated by free intracellular adenosine diphosphate ribose in response to oxidative stress and cell-death signals. TRPM2 mRNA expression is increased in human bladder samples from patients with non-ulcerative interstitial

Table 1 | Properties of major TRP channels and purinergic receptors and channels in the LUT^{2,10,14,22,23,32,33,44}

Channel or receptor	Major activators	Distribution	Function
TRPV			
TRPV1	Heat (>43 °C), pH ≤ 5.9, fatty acids (such as anandamide), vanilloids (such as Cap and RTX), and endocannabinoids	UC*, Det*, C fibres	Bladder distension, bladder hyper-reflexia, pain, and thermosensation
TRPV2	Noxious heat (>52 °C), mechanical (such as stretch or swelling), THC, and 2-APB	UC, Det, BSN	UC mechanosensation, chemosensation, and thermosensation
TRPV4	Moderate heat (>24 °C), hypotonic cell swelling, shear stress, phorbol esters (such as 4 α -PDD), BAA, EETs, and synthetic compounds (such as GSK1016790A)	UC, Det, BSN	Det contractility, bladder distension, voiding dysfunction, detrusor sphincter dyssynergia, and pain
TRPM			
TRPM7	Fluid flow, pH <6.0, and the δ opioid antagonist naltriben	UC	UC mechanosensation, and sheer stress
TRPM8	Cold (8–25 °C), cooling compounds (such as geraniol, lemonol, and eucalyptol), menthol, icilin, lipidergic mediators, and PIP2	UC, A δ fibres, and C fibres	Bladder cooling reflex, voiding dysfunction, and pain
TRPA			
TRPA1	Noxious cold (<17 °C), mechanical (such as stretch, or swelling), allyl isothiocyanate, and aldehydes (such as acrolein and cinnamaldehyde)	UC*, A δ fibres, and C fibres	Det contractility, urethral mechanosensation, and bladder hyper-reflexia
Purinergic receptor or channel			
P2X (1–7)	ATP	UC, Det, ICC, and BSN [†]	Visceral mechanosensation, and BSN sensitivity
P2Y (1, 2, 4, and 6)	ATP and nucleosides (ADP, UTP, and UDP)	UC, Det, ICC, and BSN [†]	Visceral mechanosensation, and BSN sensitivity
P1	Adenosine	UC, Det, and SubU [‡]	Voiding threshold

2-APB, 2-aminoethoxydiphenyl borate; 4 α -PDD, 4 α -phorbol 12,13-didecanoate; BAA, bisandrographolide; BSN, bladder sensory nerves; Cap, capsaicin; Det, detrusor smooth muscle cells; EETs, epoxyeicosatrienoic acids; ICC, interstitial cells of Cajal; LUT, lower urinary tract; P, purinergic; PIP2, phosphatidylinositol 4,5-bisphosphate; RTX, resiniferatoxin; SubU, suburothelium; THC, tetrahydrocannabinol; TRP, transient receptor potential; TRPA, transient receptor potential ankyrin-type channel; TRPM, transient receptor potential melastatin-type channel; TRPV, transient receptor potential vanilloid-type channel; UC, urothelial cells; UDP, uridine diphosphate; UTP, uridine triphosphate. *Species differences. [†]Lack of consensus in the literature.

cystitis⁶⁸. TRPM2 overexpression resulted in apoptosis of T24 bladder cancer cells, suggesting that it is a potential therapeutic target for bladder carcinogenesis⁶⁹. TRPM7 is expressed in the cytoplasm of urothelial cells⁵¹ and its expression was proven to be higher in a cancer cell line generated from bladder than in normal urothelial cells. TRPM7 expression in bladder cancer cells might act as a negative regulator of cell proliferation and prevent excess mechanical stress enabling cells to be maintained in a healthier state⁷⁰.

TRPV1 and TRPV4 expression in the lower urinary tract
 TRPV1 has been detected in all human genitourinary tract tissues and most LUT structures in mice^{46,52}, but its expression pattern has recently been questioned, particularly in the urothelium. TRPV1 expression has been observed in neuronal and non-neuronal human and rat LUT tissues including the urothelium, suburothelial nerve plexus, detrusor smooth muscle, ICC, and sensory afferent neurons^{46,52} (FIG. 3; TABLE 1). The expression and function of TRPV1 channels in the bladder, specifically in the urothelium, is controversial^{34,46}. Birder *et al.*¹⁸ described ATP release from cultured rat urothelial cells on application of the TRPV1 agonist, capsaicin, which was blocked by co-administration of the TRPV1 antagonist, capsazepine. Furthermore, capsaicin activation of TRPV1 induced increased intracellular calcium and inward cation currents in cultured urothelial cells from rats and humans^{71,72}. The expression of TRPV1 in the urothelium has been reported using several methods including quantitative PCR, immunohistochemistry, and western blotting in different species^{71,73,74}. However, the specificity of TRPV1 antibodies has been questioned and absence of appropriate controls (such as use of knockout animals) has been criticized⁷⁵. Considerable support exists for TRPV1 expression in small diameter bladder afferent fibres in close proximity to the urothelium and in bladder sensory neurons in the dorsal root ganglia (DRG) but not by the urothelial cells^{51,76,77}. Moreover, two independent groups did not record TrpV1-induced calcium currents in cultured urothelial cells from guinea pigs and mice^{56,78}. In 2011, Cavanaugh *et al.*⁷⁹ designed a *Trpv1*-reporter mouse that enables the expression of a nuclear β-galactosidase (*lacZ*) and the placental alkaline phosphatase with the putative TrpV1 expression pattern without disturbing TrpV1 function. These investigators reported that TrpV1 is primarily restricted to nociceptors in the DRG, with minimal expression in a few brain regions, and no TRPV1 expression in urinary bladder.

The intravesical administration of the TRPV1 activators, capsaicin or resiniferatoxin (TABLE 1), which desensitize afferent neurons, has been introduced into clinical practice for treatment of neurogenic detrusor overactivity (NDO) resulting in symptom improvement and major adverse effects^{34,46,80}, emphasizing the need for the identification of additional targets (such as TRPV, TRPM, and TRPA channels and purinergic receptors and/or channels) (TABLE 1) that could result in improved urinary bladder function⁸¹. Intravesical instillation of capsaicin in patients with NDO reduced urinary frequency and urge incontinence and increased

bladder capacity; however, treatment resulted in severe side effects (including bladder pain, suprapubic burning, bladder overactivity, and hot flashes)^{34,46,80} making treatment poorly tolerated. Findings from two studies^{82,83} have demonstrated that interaction between TRPV1 and anoctamin-1 (ANO1), a calcium-activated chloride channel, is a pain-enhancing mechanism in sensory neurons, suggesting that blockade of TRPV1–ANO1 interactions might be a novel analgesic target. TRPV1 is the most widely studied TRP channel regarding its role in urinary bladder function, but other studies also suggest the involvement of TRPV4 in both normal bladder function and dysfunction⁴⁶.

The expression of TRPV4 has also been established in different tissues and systems throughout the body including the central nervous system (CNS) and peripheral nervous system (PNS). Within the urinary bladder, TRPV4 was first demonstrated to be expressed in basal and intermediate urothelial cells⁸⁴ and expression in the urothelium has been subsequently confirmed by other studies^{51,72,76,85,86}. The functional expression of TRPV4 in urothelial cells has been established following measurements of ionic currents and Ca²⁺ signalling events induced by agonists (such as 4α-phorbol 12,13-didecanoate (4α-PDD) or GSK1016790A) or stretch^{72,78,85}. TRPV4 is also expressed in the detrusor smooth muscle; however, transcript levels were found to be approximately 20-fold to 36-fold higher in the urothelium than the detrusor smooth muscle^{78,87} perhaps suggesting a more prominent role in urothelial function. TRPV4 expression has also been observed in the DRG neurons innervating viscera^{76,88–90} but functional evidence is lacking⁸⁸.

TRPV and bladder function

Pharmacological approaches and the use of *Trpv1*-knockout mice have started to demonstrate the roles of TrpV1 in the micturition reflex⁵². Investigation of bladder function of *Trpv1*-knockout mice has revealed a higher frequency of nonvoiding bladder contractions than in wild-type mice, as well as a reduction in reflex voiding during bladder filling¹⁸. Additionally, the *Trpv1*-knockout mouse model does not develop bladder overactivity during acute bladder inflammation, suggesting that TrpV1 is involved in bladder hyperreflexia in response to inflammation⁵².

CYP-induced cystitis results in significantly increased TrpV1 and TrpV4 expression 4 h and 48 h after injection (acute cystitis), and in models of chronic cystitis ($P=0.01$); however, *Trpv1* transcript expression was significantly reduced in urothelial and suburothelial tissues from rats that underwent 4 h and 48 h acute cystitis but significantly increased in detrusor tissue from rats with chronic CYP-induced cystitis ($P<0.01$)³⁶. The lack of correlation between *Trpv1* transcript and protein expression might reflect changes in post-transcriptional and post-translational mechanisms including increased protein stability³⁶. The increased *Trpv1* expression demonstrated with CYP-induced cystitis is consistent with the hypothesis that TRPV1 contributes to bladder hyperreflexia in response to inflammation⁵².

The deletion of *Trpv4* in mice has also helped to elucidate its physiological role in diverse functions, including the micturition reflex. Authors of studies have reported mild phenotypic effects in *Trpv4*-knockout mice such as abnormal responses to both osmotic and somatosensory mechanical stimuli where hyperalgesia is absent, deficits in osmotic homeostasis leading to cell-size changes and functional abnormalities, increased blood osmolarity causing dehydration, alkalosis, or acidosis, increased bone mass, ageing-related hearing impairment, and reduced water consumption, all potentially being fatal^{45,52}. Urodynamic measurements showed that *Trpv4*-knockout mice have abnormal urine voiding patterns characterized by a decreased frequency of voiding contractions and increased frequency of nonvoiding contractions, longer intermicturition intervals and increased total urine volume per void^{45,84,85}. Pharmacological manipulation has also been an effective tool to help define the role or roles of TRPV4 in bladder function. Administration of the TRPV4 agonist 4α-PDD to conscious rats resulted in an increase in the amplitude of reflex bladder contractions during cystometry⁸⁵. GSK1016790A, a highly selective TRPV4 agonist that is ~300-fold more potent than 4α-PDD, similarly induced bladder hyperactivity *in vivo* in mice⁸⁷ and rats⁹¹. Systemic administration of the selective and potent TRPV4 antagonist HC-067047 decreased voiding frequency and increased bladder capacity in mice and rats following CYP-induced cystitis⁴⁵.

TRPV1, TRPV4 and purinergic signalling

Studies suggest that TRPV1 and TRPV4 regulate ATP release from the urothelium that occurs in response to bladder distension^{18,84}. Sensory neurons terminating in the bladder wall were originally thought to initiate the micturition reflex; however, the urothelium has now been proven to participate in the sensory response to bladder filling^{76,92}. Mechanical stretch evokes the release of ATP from urothelial cells and mechanosensitive contributions of TRPV4 and possibly TRPV1 channels present on the urothelial cell surface might contribute to this process (FIG. 1). Functional evidence in support of TRPV4 involvement in the release of ATP was demonstrated with the administration of 4α-PDD to cultured urothelial cells, which subsequently released ATP, an effect that was blocked by ruthenium red, a pan inhibitor of TRP channels⁸⁵. Similarly, capsaicin evoked ATP release from cultured urothelial cells¹⁸ and bladder mucosal strips⁹³ that was abolished with the coadministration of the TRPV1 antagonist, capsazepine, suggesting a role of TRPV1 in ATP release. The influence of TRPV1 and TRPV4 on ATP release has also been confirmed in knockout mice. ATP release is suppressed in response to a hypotonic stimulus¹⁸ or mechanical stretch⁸⁶ of cultured urothelial cells from *Trpv1*-knockout and *Trpv4*-knockout mice. The importance of transmembrane Ca²⁺ flux through TRPV channels is still disputed^{86,94}, studies provide support for TRPV-mediated sensory transduction at the level of the urothelium that results in ATP release^{86,94}. Subsequent P2X receptor signalling on bladder sensory neurons (among other cell types) might contribute to the afferent limb of the micturition reflex.

ATP and purinergic receptors

The urothelium releases neuroactive transmitters, in particular ATP, from its apical and basolateral surfaces in response to physical and chemical stimuli^{2,95}. ATP and other nucleosides (including ADP, UTP, and UDP) that are derived from the urothelium can be released through several mechanisms that include transporters, ion channels, or vesicular exocytosis^{96,97} (FIG. 3). Extracellular ATP that is not degraded by ectonucleotidases or exonucleotidases is then able to stimulate autocrine or paracrine pathways that might aid in sensory transduction to the CNS⁹⁸. The transduction pathways within the urinary bladder are affected by receptor subtype expression and their proximity to the urothelium. The tissues and cell types that might contribute to purinergic or pyrimidinergic signalling include the urothelium^{98,99}, ICC²⁷, smooth muscle cells^{100,101} and suburothelial nerve fibres²⁴ (FIG. 3).

Nucleosides and their metabolites seem to have an intricate role in ATP release and purinergic receptor signalling in the urothelium. More specifically, ADP has been demonstrated to evoke ATP release from the urothelium, whereas adenosine inhibits ATP release via A1 receptors^{98,102}. The urothelium also expresses diverse purine and pyrimidine receptor subtypes enabling distinct signal transduction^{103–106}. The activation of P2Y, but not P2X, receptors on the urothelium causes ATP release, suggesting urothelial P2Y receptors might contribute to purinergic neurotransmission^{103,107}. However, P2X₂ and P2X₃ receptor expression is altered in urothelium from patients with interstitial cystitis indicating that P2X receptors are involved in urinary bladder sensory transduction in bladder injury or disease¹⁰⁵.

ICC in the lamina propria (ICC-LP) of the urinary bladder also express P2X₁, P2Y₂, P2Y₄ and P2Y₆ receptors and are proposed to form a functional syncytium with smooth muscle cells^{108,109}. In response to ATP, ICC-LP generate P2Y-dependent intracellular calcium transients followed by inward currents¹⁰⁹. These ATP-generated transients could then propagate to smooth muscle cells via gap junctions to alter contractility¹¹⁰. The mechanism coupling ICC-LP to sensory activity is not yet known, but the localization of ICC-LP and their responsiveness to ATP suggest they have a regulatory role in the afferent limb of the micturition reflex¹¹⁰. In addition to the functional syncytium with ICC-LP, urinary bladder smooth muscle cells express P2X₁ to P2X₆ receptors and P2Y receptors¹¹¹. P2X₁ receptors are the predominant receptors in purinergic cotransmission mediating bladder contractility¹¹². P2Y₆ receptors have also been demonstrated to augment P2X-mediated bladder contraction, suggesting that extracellular UDP and P2Y₆-receptor activation might have a role in pathological bladder conditions involving smooth muscle tone¹¹³.

Bladder afferent nerves that terminate throughout the layers of the urinary bladder have been shown to express P2X_{1–7}, P2Y₁, P2Y₂, and P2Y₄ receptors^{114–116}. The proximity of afferent nerve terminals to the basal layer of the urothelium indicates that sensory nerves have a role in purinergic sensory transduction, but the data are not yet conclusive. Studies have established

the functional contributions of P2X₂ and P2X₃ receptors to the afferent limb of the micturition reflex¹¹⁷. P2X₃-receptor-mediated mechanisms contribute to mechanosensory transduction in the urinary bladder because intravesical- α,β -methylene-ATP, a P2X₃ agonist, potentiates both low-threshold, non-nociceptive fibres and high-threshold, nociceptive fibres¹¹⁸. Furthermore, mice lacking P2X₂ or P2X_{2/3} receptor subunits exhibit decreased urinary bladder reflexes and decreased pelvic afferent nerve activity in response to bladder distension¹¹⁹. The functional contributions of P2Y₂ receptors to bladder sensory neuron sensitization have also been demonstrated¹²⁰. P2Y₂ receptor activation is able to facilitate P2X₂-evoked currents through G-protein-coupled mechanisms, whereas the facilitation of P2X₃-evoked currents was independent of G-protein-coupled mechanisms¹²⁰. These results suggest that UTP might prime P2X receptor signalling to contribute to neuron hyperexcitability in pathological bladder conditions¹²⁰. In summary, purinergic signalling through P2X receptors contributes to mechanosensory transduction within bladder afferent nerves and contractility within detrusor smooth muscle cells, whereas, P2Y receptors contribute to altered ATP release from the urothelium, calcium transients in ICC-LP, and the sensitization of P2X receptors in detrusor smooth muscle cells and afferent nerve terminals. The variety of cell types involved in purinergic and pyrimidinergic neurotransmission in the urinary bladder suggests a fundamental role in the sensory processing of micturition function making purinergic and pyrimidinergic receptors potential targets to improve voiding dysfunction.

Roles in urinary bladder dysfunction

TRPV1 and TRPV4 channels

Studies of TRP channels (such as TRPA1, TRPV1, and TRPV4) in the LUT have suggested functional roles in symptoms of OAB⁴⁶, and IC/BPS^{49,52}. In particular, TRPV1 and TRPV4 exhibit plasticity in LUT expression in patients with NDO, OAB, and IC/BPS. Patients with NDO were found to have increased TRPV1 immunoreactivity in suburothelial nerve fibres and basal cell layers of urothelium compared with controls¹²¹. Localized treatment with intravesical instillation of capsaicin improved symptoms in 84% of these patients, but side effects, including suprapubic burning, urgency, and hot flashes, lasted for 2 weeks after treatment⁸⁰. An increase in TRPV1 transcript levels in trigonal mucosa is also observed in patients with OAB¹²². In addition, patients with IC/BPS have an increase in the numbers of suburothelial TRPV1-immunoreactive nerve fibres⁴⁹. Intravesical capsaicin treatment significantly ($P<0.01$) improved frequency and nocturia symptoms in these patients, but not urgency or pain¹²³.

Some studies also suggest that alterations to TRPV4 expression after stress might contribute to urinary bladder dysfunction. Individuals with IC/BPS or OAB exhibit symptom exacerbation (such as urinary frequency) during times of stress^{124–127}. Repeated variate stress (RVS)^{128,129} in rats produces urinary bladder dysfunction characterized by decreased bladder capacity, decreased

void volume and increased voiding frequency¹³⁰. In addition to urodynamic changes following RVS exposure, TrpV4 expression in the urothelium also increased. The intravesical administration of the TRPV4 antagonist HC067047 improved bladder capacity and voiding frequency, suggesting TRPV4 might contribute to bladder dysfunction following RVS¹³⁰. Systemic administration of HC067047 also increased bladder capacity and decreased voiding frequency in mice with CYP-induced cystitis, suggesting that TrpV4 might have a fundamental role in bladder function following injury⁴⁵.

Stress is also known to potentiate the immune response, and RVS increased inflammatory mediators in the urinary bladder including NGF¹³⁰. The role of NGF in the inflammatory milieu of the urinary bladder is well established, and NGF has also been suggested as a potential biomarker for IC/BPS^{131,132}. We have shown previously that urinary-bladder-derived NGF modulates several TRP channels, including TRPV4 (REFS 36,89); therefore, NGF in the urinary bladder after RVS might contribute to increased TRPV4 expression, but future studies are necessary to address this possibility.

Purinergic receptor expression and signalling

Increased levels of urinary ATP have been demonstrated in patients with interstitial cystitis¹³³, OAB¹³⁴, and other functional disorders of the urinary bladder⁹⁵. Primary bladder urothelial cells taken from these patients also exhibit increased ATP release in response to mechanical stretch¹³³, hypotonic stress¹³⁵, or electrical field stimulation¹³⁶. In addition to increased urothelial-derived ATP release, changes in P2X receptor subtype expression within various bladder tissues have also been demonstrated in patients with interstitial cystitis (P2X₂ and P2X₃)¹⁰⁶, detrusor instability (P2X₂)¹³⁷ or bladder outlet obstruction (P2X₁)¹³⁸. The established role, or roles of purinergic signalling in bladder sensation suggest that these neurochemical alterations might contribute to the development of LUT symptoms in these patients. Thus, an alternative therapeutic approach to improve bladder function might be to target purinergic neurotransmission within sensory components of the micturition reflex¹³⁹.

Targeting receptors and/or channels

Determining the distribution and function of purinergic receptors or channels and TRP channels in the LUT (TABLE 1) in preclinical studies has the potential to produce safe and effective treatments for sensory voiding disorders. Based on the distribution and function of purinergic receptors and TRP channels in urothelial cells and bladder sensory nerves (TABLE 1), targeting these receptors and channels could alter mechanosensation (such as urgency), chemosensation and pain perception (such as pain that is attributed to the urinary bladder), and/or the excitability of bladder sensory nerves, therefore, modulating the afferent limb of the micturition reflex to the CNS and the overall function of the urinary bladder. Clinical studies are determining the risks and benefits of purinergic receptor and TRP channel antagonists in patients with IC/BPS. For example, results from a phase II clinical trial (NCT01569438)¹⁴⁰

with the selective, non-narcotic, and orally-administered P2X₃ receptor antagonist AF-219 showed a significant ($P=0.034$) reduction in urinary urgency and a positive trend in the improvement of pain in patients with IC/BPS¹⁴¹. The distribution of the P2X₃ receptor is restricted to sensory nerves, including bladder sensory nerves (TABLE 1), making it an ideal target for affecting bladder sensory nerve excitability.

TRPV1 has been considered a promising target in IC/BPS based on the function of TRPV1 in the LUT and the benefits of intravesical vanilloids in NDO⁸¹. However, intravesical capsaicin and its ultrapotent analogue RTX failed to improve pain scores or symptoms in patients with severe pelvic pain or interstitial cystitis^{124,142}. By contrast, hydrodistension combined with intravesical RTX significantly reduced pain at 1 month ($P=0.022$) and 3 months ($P=0.041$) follow-up duration in patients with IC/BPS¹⁴³. Disappointing results with TRPV1 antagonists have resulted in the pursuit of TRPV1 antagonists for clinical development. Unfortunately, use of TRPV1 antagonists (such as AMG517 and XEND0501) produced marked and persistent hyperthermia resulting in early termination of clinical trials^{44,144}. Intravesical administration could circumvent systemic adverse effects but its effects on pain and bladder dysfunction in IC/BPS patients need to be determined. The function of another member of the TRPV family, TRPV4, in the LUT (TABLE 1) in preclinical studies also suggests that TRPV4 antagonists could be an effective treatment for LUT storage disorders; however, no clinical studies have been reported. Preclinical studies with HC067047, a selective antagonist of TRPV4, have demonstrated significant ($P\leq 0.01$) improvement in urinary bladder function (such as reduced urinary frequency) in rodents treated with CYP-induced or RVS-induced bladder dysfunction^{45,145}. Similarly, TRPA1 antagonists need clinical validation in LUT dysfunction but pre-clinical studies are suggestive of a clinical benefit. For example, in CYP-induced cystitis in mice, the TRPA1

antagonist HC030031 reduced urinary bladder hyperalgesia as demonstrated by visceromotor reflexes quantified by abdominal muscle electromyography¹⁴⁶. TRPM8 might also be a target, as preclinical studies suggest that TRPM8 antagonists reduce urinary frequency^{147,148}, but they have not advanced to clinical trials. Additional clinical evaluation of purinergic-receptor, purinergic-channel, and TRP-channel antagonists is necessary to determine whether these targets can be effective and safe treatments for sensory voiding disorders.

Conclusions

Urinary bladder function is often taken for granted and given little thought, but attention is quickly redirected when neural injury, disease, or psychogenic stress disrupts this function. Normal urinary bladder reflex function as well as how the micturition reflex is affected by pathology is not yet completely understood, and much is still to be learned. For example, the identity of the sensory transducer at the level of the urothelium that responds to urinary bladder distension is a critical first step in the micturition reflex and is not known. That activation of the urothelium can result in the release of chemical mediators including ATP is better understood, although studies focusing on ATP release mechanisms from the urothelium are less well understood. The micturition reflex is altered with bladder pathology and numerous changes to the urothelium have been documented. Changes in the urothelial cell expression of channels and receptors including TRP and purinergic channels and receptors are observed in IC/BPS, OAB, NDO, and stress. Intensive research efforts are underway focused on identifying urothelial receptors and/or channels that can be targeted by pharmacological tools to improve urinary bladder function. Such a strategy requires a clearer understanding of the identity and function of urothelial TRP channels and ATP release mechanisms in normal and pathological LUT physiology.

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Competing interests statement

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