The Uroepithelial-associated sensory web

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An important, but not well understood, function of epithelial cells is their ability to sense changes in their extracellular environment and then communicate these changes to the underlying nervous, connective, and muscular tissues. This communication is likely to be important for tube- and sac-shaped organs such as blood vessels, the lungs, the gut, and the bladder, whose normal function can be modulated by stimuli initiated within the epithelium. We propose that the uroepithelium, which lines the renal pelvis, ureters, and inner surface of the bladder, functions as an integral part of a 'sensory web.' Through uroepithelial-associated channels and receptors, the uroepithelium receives sensory 'inputs' such as changes in hydrostatic pressure and binding of mediators including adenosine triphosphate (ATP). These input signals stimulate membrane turnover in the outermost umbrella cell layer and release of sensory 'outputs' from the uroepithelium in the form of neurotransmitters and other mediators that communicate changes in the uroepithelial milieu to the underlying tissues, altering their function. The global consequence of this sensory web is the coordinated function of the bladder during the cycles of filling and voiding, and disruption of this web is likely to lead to bladder dysfunction.

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THE UROEPITHELIUM AND ITS INNERVATION

The uroepithelium is stratified and is comprised of three cell types, including basal cells, intermediate cells, and umbrella cells and like other epithelia it is in communication with the underlying tissue.[1–4] The outer umbrella cell layer interfaces with urine and forms the primary barrier that includes a mucin/glycosaminoglycan layer, which may prevent bacterial attachment and diffusion of urine components across the epithelium,[5] and an apical plasma membrane with low permeability to urea and water.[6] In addition, umbrella cell tight junctions form a tight seal between adjacent cells, and are comprised of multiple claudin species including claudin-1, -3, -4, -5, -7, -8, -12, and possibly claudin-13,[7,8] which regulate paracellular transport. The uroepithelium maintains the barrier even as the bladder undergoes cycles of filling and voiding. This accommodation likely reflects the ability of the highly wrinkled mucosal surface of the bladder to unfold, and the increases in mucosal surface area that result from fusion of a population of subapical discoidal/fusiform vesicles with the apical plasma membrane of the umbrella cell layer. Upon voiding, the mucosa refolds, and the membrane added to the apical surface of the umbrella cells is thought to be recovered by endocytosis.[4]

Recent studies indicate that the uroepithelium is intimately associated with the nervous system. Capsaicin-sensitive transient receptor potential channel, vanilloid subfamily member 1 (TRPV1)-positive primary afferent neurons are localized near to and within the uroepithelium, and are also in close proximity to blood vessels and smooth muscle cells. They exhibit a sensory and also an 'efferent' function that is mediated by release of peptides such as substance P and calcitonin gene-related peptide, which can affect bladder function and participate in sensory transmission within the spinal cord.[9,10] As described below, these inputs may also regulate the function of the uroepithelium. In addition, cholingeric (choline acetyltransferase positive) and adrenergic (tyrosine hydroxylase positive) nerve fibers are detected just below the uroepithelium, which may indicate the presence of efferent innervation as well. However, in guinea pig bladder some choline acetyltransferase-positive immunoreactivity near the uroepithelium may be present in sensory rather than efferent nerves.[11]

In addition to the nervous system, myofibroblasts are found in the suburothelial space of the bladder in both

humans and animals.[12] These cells, which arise from fibroblasts that undergo differentiation into smooth muscle -like cells, are extensively linked by gap junctions and have close contact with nerves. They can respond to neurotransmitters, such as ATP released from nerves or uroepithelial cells, indicating that they could act as intermediaries in urothelial–nerve interactions.[12]

THE UROEPITHELIAL-ASSOCIATED SENSORY WEB

In addition to acting as the primary barrier in the bladder, new data indicate that the uroepithelium may function as an integral part of a '**sensory web**.' This web includes the uroepithelium, closely apposed nerve fibers, interstitial cells, including myofibroblasts and mast cells, and the detrusor/sphincter muscles (Figure 1). The overall purpose of the sensory web is to coordinate the function of the bladder and its associated tissues. In the following sections, we describe how the uroepithelium receives extracellular signals, the impact these signals have on uroepithelial function, and how release of mediators by the uroepithelium can communicate changes in its environment to the underlying tissues and thus regulate bladder function.

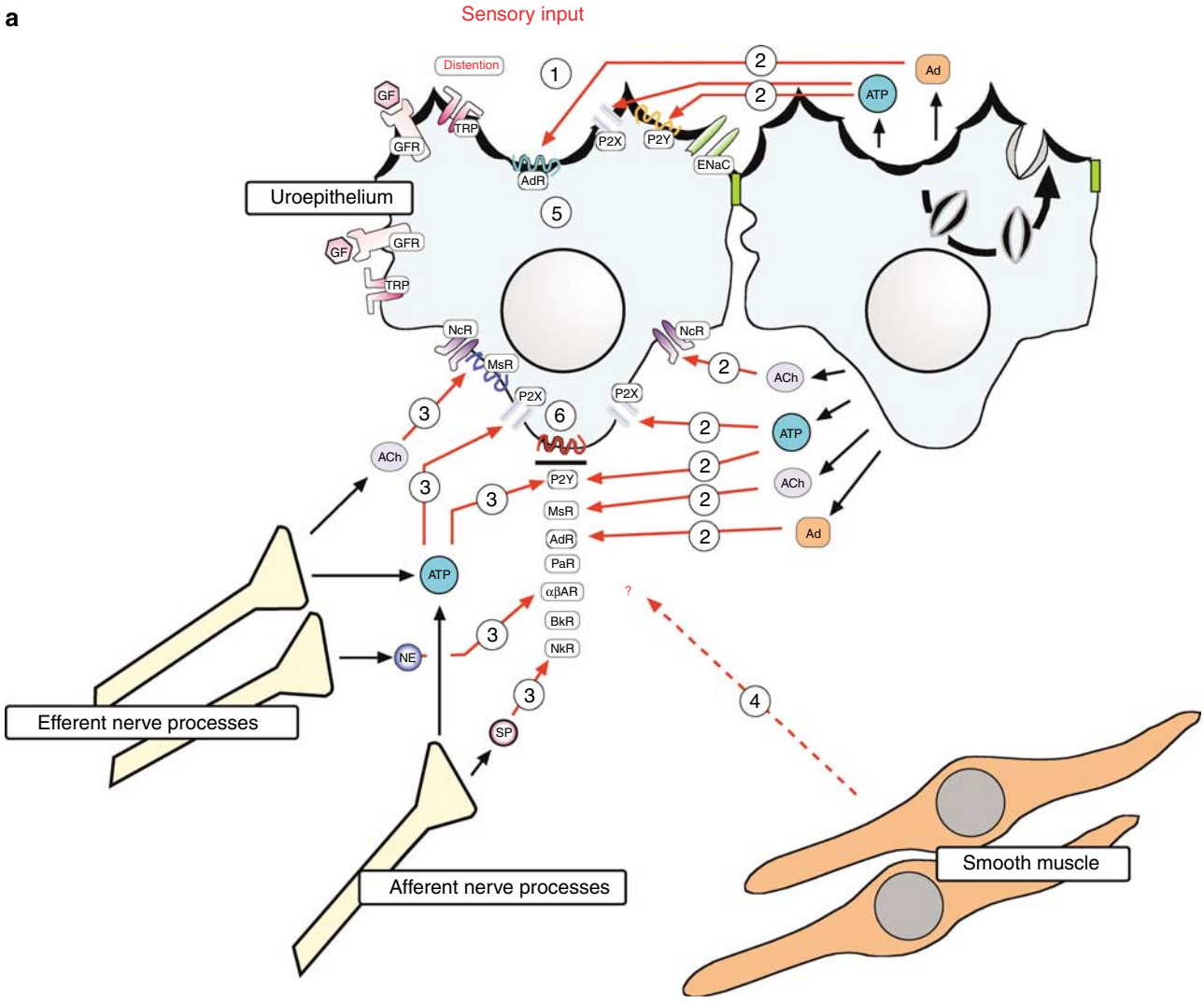

Figure 1 | The uroepithelial-associated sensory web. (**a**) Bladder distention during filling, or soluble mediators found in urine (1) or released from the uroepithelium (2), adjacent afferent/efferent nerve processes (3), or smooth muscle cells act as sensory inputs to stimulate cell surface receptors/channels present on the apical surface of the umbrella cells (5), the basolateral surfaces of the umbrella cells (6), and the plasma membranes of the underlying intermediate and basal cells. For clarity the intermediate/basal cell layers and interstitial cells are not shown, but are likely to play a significant role in the signaling web. (**b**) Receptor binding or channel activation results in changes in the uroepithelium including membrane turnover (i.e. exocytosis/endocytosis) at the apical plasma membrane of the umbrella cell (and possibly at the other plasma membrane domains of the uroepithelium) (7), and release of sensory outputs such as acetylcholine, adenosine, ATP, NO, and prostaglandins (8). (**c**) These sensory outputs can act in an autocrine manner to further alter uroepithelial function (2), or can bind to receptors on sensory afferent nerve processes (9) and/or bladder-associated smooth muscle (10) to regulate nervous and muscular function. Outputs may also affect efferent nerve processes (11). Hashed lines indicate presumptive pathways. Legend: α,β-AR, α,β -adrenergic receptor; Ach, acetylcholine; Ad, adenosine; AdR, adenosine receptor; ATP, adenosine triphosphate; BkR, brandykinin receptor; ENaC, epithelial sodium channel; GF, growth factor; GFR, growth factor receptor; MsR, muscarinic receptor; NcR, nicotinic receptor; NE, norepinephrine; NkR, neurokinin receptor; NO, nitric oxide; PaR, proteinase-activated receptor; PGs, prostaglandins; SP, substance P; Trp, trp channel family member.

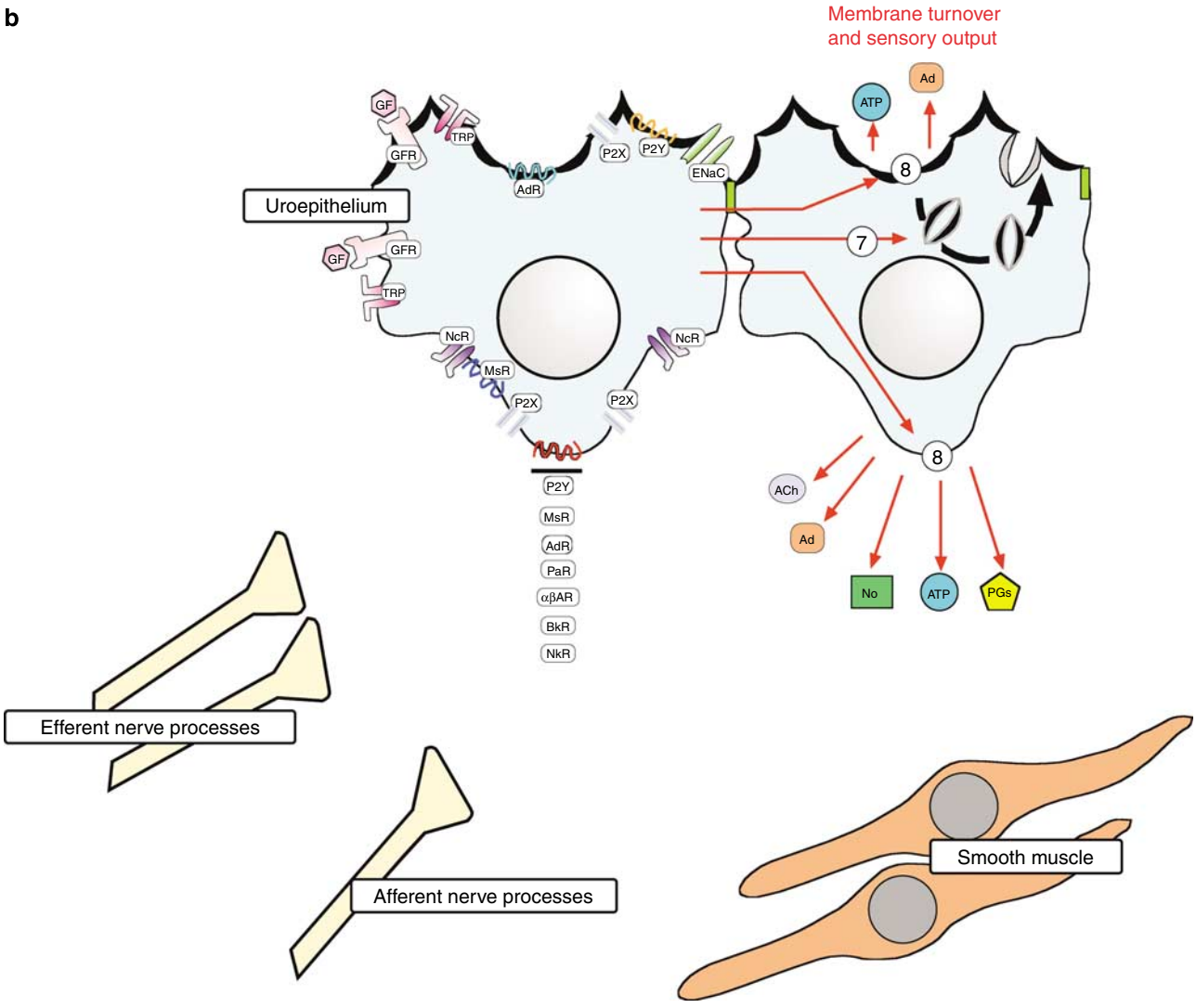

Figure 1 | Continued.

SENSORY INPUT PATHWAYS

The uroepithelium receives '**sensory inputs**' in a number of different forms and from a variety of sources (Figure 1a). These inputs include mechanical stimuli such as the increased stretch associated with bladder filling, soluble mediators such as growth factors found in the urine (e.g. epidermal growth factor (EGF)), or neurotransmitters such as ATP, adenosine, substance P, acetylcholine, or norepinephrine released from nerve processes or other cell/tissue types including the uroepithelium itself (Figure 1a).[13–16] The uroepithelium is primed to receive these input signals by expressing a growing list of receptors and ion channels, including the following: EGF family ErbB1–3 receptors,[17] A_1, A_{2a}, A_{2b}, and A_3 adenosine receptors,[18] α- and β-adrenergic receptors,[19,20] bradykinin receptors,[21] neurokinin receptor,[22] nicotinic and muscarinic receptors (M1–M5),[3,23,24] purinergic $P2X_{1–7}$ and $P2Y_{1,2,4}$ receptors,[14,25–30] protease-activated receptors,[31] the epithelial sodium channel,[32–34] and the TRP family channels TRPV1, TRPV2, TRPV4, and TRPM8.[3,35–37] Further work is sure to identify other mediators, signaling receptors, and channels that will further act as signal inputs for the uroepithelium.

IMPACT OF SENSORY INPUT PATHWAYS ON UROEPITHELIAL FUNCTION

Stimulation of uroepithelial-associated input pathways can lead to changes in the function of the uroepithelium, including increased membrane traffic and ion transport and alterations of barrier function (Figure 1b).[38–41] As described above, membrane trafficking pathways such as exocytosis/endocytosis in the umbrella cell layer plays an important role in maintaining the barrier function of the uroepithelium. However, exocytosis/endocytosis are also likely to play crucial roles in the sensory web, because these pathways regulate the composition of receptors and channels at the surface of the umbrella and other uroepithelial cells.

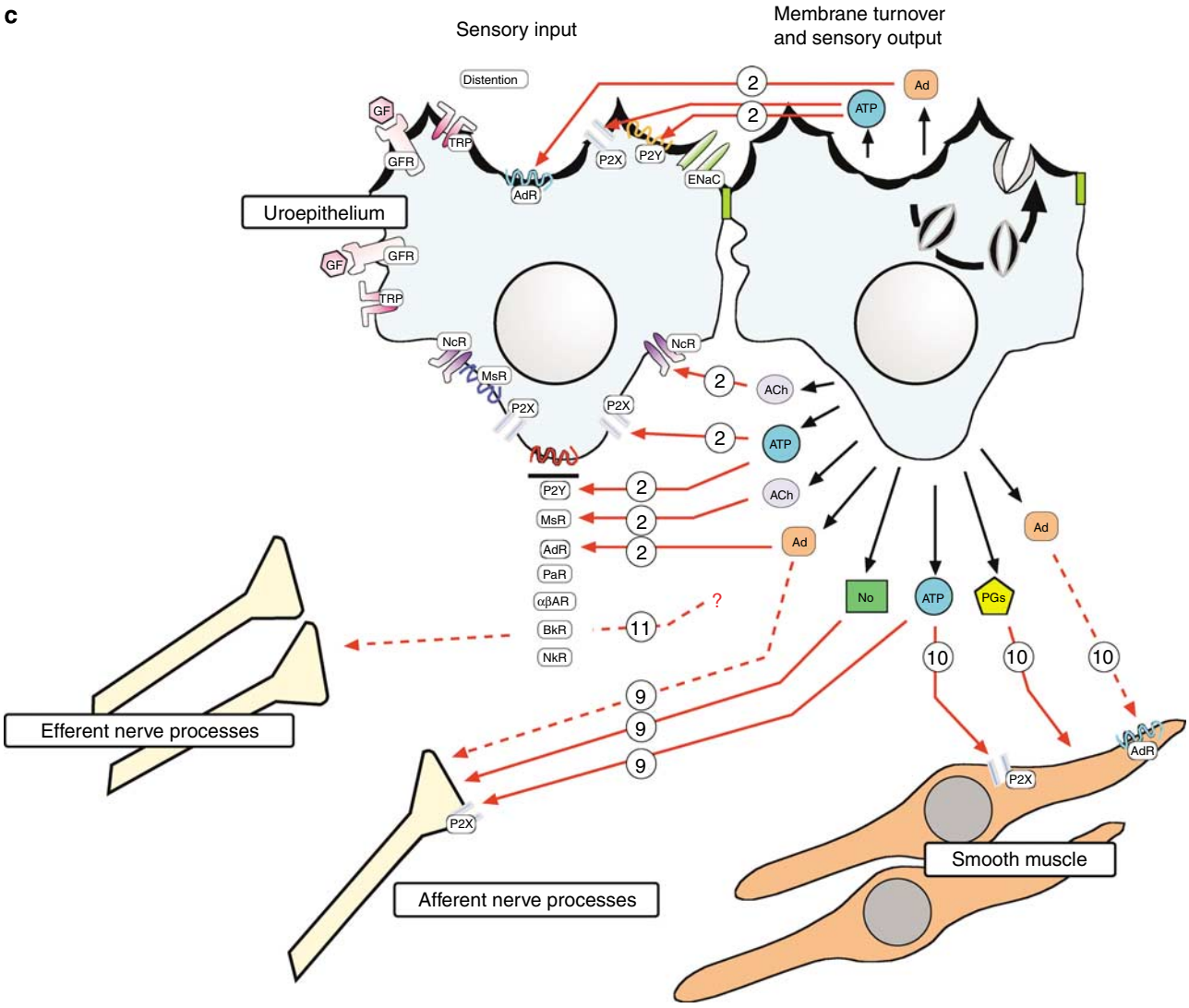

Figure 1 | Continued.

Furthermore, exocytosis is likely to play an important role in the release of mediators from the uroepithelium that allow communication with other cell types in the sensory web (see discussion below).

It is unknown whether all input pathways stimulate increased membrane turnover, however, we have observed that extracellular ATP (released from the uroepithelium) is an important autocrine mediator that acts as a proximal signal for both stretch-induced exocytosis and endocytosis.[14] In addition, we observe that adenosine, most likely acting through A_1 and A_{2a} receptors, stimulates membrane turnover in the umbrella cell layer and enhances stretch-induced changes in membrane traffic.[18] The signaling pathways that act downstream of ATP/adenosine to stimulate membrane traffic are not well understood, but are likely to involve changes in $[Ca^{2+}]_I$.[14,18] Other studies show that stretch-induced exocytosis is regulated by cAMP and its downstream effector PKA.[38]

Most recently, we reported that long-term changes in stretch-regulated exocytosis are dependent on EGF receptor autoactivation at the apical surface of the umbrella cell layer, downstream of heparin-binding EGF-like growth factor cleavage by a putative metalloproteinase.[42] Changes in apical exocytosis depend on protein synthesis, which occurs upon EGF receptor-dependent activation of downstream mitogen-activated protein kinase signaling pathways. In other cell types elevated $[Ca^{2+}]_I$ and activation of G-protein coupled receptors promote proteolytic processing and release of ErbB family ligands such as heparin-binding EGF-like growth factor, which rapidly bind to and activate the EGF receptor in a process known as transactivation.[43] While experimental evidence is forthcoming, transactivation of EGF receptors may be a common pathway for various input pathways to stimulate membrane turnover in the uroepithelium. Future studies are sure to significantly increase our understanding of the signaling pathways involved in transactivation and

whether there is specificity in terms of which input pathway is stimulated. By blocking transactivation pathways it may be possible to slow or prevent the release of mediators from the uroepithelium, which as described below may have therapeutic benefit.

Bladder filling not only stimulates exocytosis, but endocytosis as well, although the net effect of stretch is of an increase in surface area.[38] Beyond stretch and ATP, the stimuli and signaling cascades that regulate endocytosis are unknown. The endocytosed membrane components may be delivered to lysosomes where they are degraded.[38] It may seem counterintuitive that hydrostatic pressure would simultaneously induce exocytosis and endocytosis; however, hydrostatic pressure-induced endocytosis would modulate the increase in apical surface area brought about by exocytosis, ensure turnover of membrane components, and importantly for the uroepithelial-associated sensory web it would regulate the number and function of receptors and channels at the cell surface.

SENSORY OUTPUT FROM THE UROEPITHELIUM

In addition to stimulating membrane traffic in the uroepithelium, sensory inputs can stimulate the release of '**sensory outputs**' from the uroepithelium including growth factors, neurotransmitters, and other mediators (Figure 1b).[3] The nature of the signaling pathways that act downstream of the input pathways to stimulate the production of these mediators and the mechanism(s) of their release are currently unknown. However, once released, the sensory outputs are likely to act in an autocrine manner to further modify uroepithelial function (Figure 1c). In addition, they can act in a paracrine manner to modulate other cells and tissues associated with the broader sensory web (Figure 1c).[3] Potentially important sensory outputs produced by the uroepithelium include acetylcholine, adenosine, ATP, nitric oxide (NO), and prostaglandins.[3,13–15,18,19,44,45] Other studies have demonstrated that the uroepithelium can modulate the spontaneous activity of the smooth muscle[46] or muscle contraction, possibly via release of a soluble factor.[47,48]

NO is released from the uroepithelium in response to norepinephrine (an α/β-adrenergic receptor agonist), capsaicin (a neurotoxin that activates TRPV1 channels), and isoproterenol (a β-adrenoceptor agonist), and may have several functions including relaxation of smooth muscle, modulation of afferent and efferent nerve functions, and regulation of uroepithelial barrier function.[3,20] Prostaglandins are also released from the uroepithelium in response to stretch and may play roles in modulation of nerve and detrusor functions.[3] ATP is released from both surfaces of the uroepithelium in response to stretch[13–15] and can act via $P2X_2$- and $P2X_3$-containing receptors present on the uroepithelium to stimulate stretch-induced exocytosis in the uroepithelium.[14] The expression of P2X and P2Y purinergic receptor subtypes in nerve fibers and myofibroblasts located at or near the luminal surface of the bladder[10,12,49] and the sensitivity of these cells to ATP (indicated by an ATP-induced increase in $[Ca^{2+}]_i$)[2] suggests that basolateral ATP release from the uroepithelium may also influence the function of myofibroblasts and nerves. In addition, intercellular communication mediated by gap junctions in myofibroblasts could provide a mechanism for long-distance spread of signals from the uroepithelium to the detrusor muscle.[12] Adenosine is also produced by the uroepithelium and is released from both cell surfaces, particularly from the serosal surface of stretched epithelium.[18] It may play important roles in modulating sensory afferent function and the contraction of smooth muscle.

Recent studies have shown that urothelial cells express the plasma membrane choline transporter, the acetylcholine-synthesizing enzymes choline acetyltransferase and carnitine acetyltransferase, and the enzyme responsible for metabolism of acetylcholine (acetylcholinesterase).[50,51] Furthermore, the uroepithelium releases acetylcholine following both mechanical and chemical stimulation. Once released, there are a number of sites where urothelial-derived acetylcholine could exert its effects including smooth muscles, nerves, and uroepithelial associated-muscarinic and/or nicotinic receptors, thereby participating in feedback mechanisms to modify urothelial function (Figure 1c). Because stimulation of cholinergic receptors in urothelial cells elicits the release of NO and ATP, cholinergic mechanisms in the uroepithelium could alter bladder sensation indirectly by triggering purinergic stimulation of nearby afferent nerves.

TRANSMURAL SIGNALING PATHWAYS

Sensory input at the apical surface of the umbrella cell layer can regulate bladder function via a '**transmural signaling**' pathway (Figure 2). This is a subset of the signaling pathways shown in Figure 1a and allows input signals to be transmitted from the urinary space to the underlying tissues via the mucosal surface of the umbrella cell layer. The secondary messenger cascades that occur downstream of apical input, and the mediators released from the umbrella cells remain to be fully characterized. Although our understanding of these pathways is in its infancy, they may hold significant promise in increasing our comprehension of the sensory web and for the design of therapies that can modulate bladder function by targeting specific tissues and pathways in the sensory web.

The presence of transmural signaling pathways are suggested by studies in which agonists are instilled intravesically (into the bladder lumen), which results in changes in bladder capacity and activity. For example, short-term addition of ATP or ATP analogs such as α,β-methylene ATP into the bladder lumen stimulates detrusor overactivity,[52–55] and work in *ex vivo* bladder preparations or isolated ureters indicates that mucosal application of ATP or α,β-methylene ATP stimulates afferent nerve discharge.[52,54,55] The afferent stimulation observed in isolated bladder preparations is inhibited by extended treatment with α,β-methylene ATP (which downregulates homomeric $P2X_3$ and heteromeric $P2X_2/P2X_3$ receptors), pyridoxal-phosphate-6-azophenyl-2′, 4′-disulfonate (a broad-spectrum P2 receptor antagonist), or

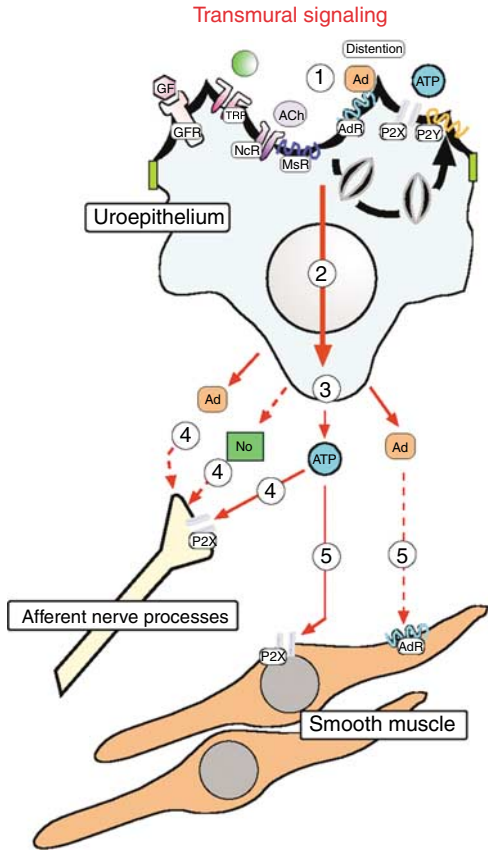

Figure 2 | Transmural signaling pathways. In transmural signaling, bladder distention or soluble mediators activate receptors/channels present at the apical surface of the umbrella cell layer (1). Activation of input pathways stimulate poorly understood signaling cascades that stimulate membrane turnover and release of sensory outputs (3), such as ATP. The uroepithelial-released mediators alter the function of underlying tissues including sensory nerves (4) and smooth muscle (5). See legend in Figure 1 for abbreviations. Cap, capsaicin.

capsaicin, thus implicating purinergic and TRPV1 receptors in this signaling cascade.[52,54,55] Additional studies show that intravesical administration of carbachol, nicotine, vanilloid compounds, and oxyhemoglobin (a scavenger of NO) also affect bladder function.[56–63] Thus, several mediators and their associated input pathways may be able to stimulate transmural signaling in the uroepithelium.

FUNCTION OF TRANSMURAL SIGNALING PATHWAYS

One proposed example of transmural signaling occurs as the bladder fills with urine.[2,14] Filling stretches the uroepithelium, activating mechanotransduction pathways (likely situated at the apical surface of umbrella cells) that result in the uroepithelial release of ATP from both surfaces of the epithelium. The function of the mucosally released ATP is unclear, but addition of exogenous ATP or its analogs to the mucosal surface of the epithelium can stimulate exocytosis in the umbrella cell layer,[14] and as described above may play a role in regulating bladder function via transmural signaling processes. The serosally released ATP has at least two functions. First, it can act via $P2X_2$ and $P2X_3$-containing receptors present on the uroepithelium to stimulate stretch-induced exocytosis in the uroepithelium,[14] and second, it is proposed to bind to receptors containing $P2X_3$ subunits present on the sensory afferent nerve processes, thus signaling bladder filling to the central nervous system.[2,55] Consistent with this hypothesis, knockout mice lacking $P2X_2$, $P2X_3$, or $P2X_2/P2X_3$ receptor subunits can still release ATP from their uroepithelium, but activation of bladder afferents is significantly decreased and knockout mice show reduced micturition frequencies and increased bladder capacities.[45,64]

CLINICAL SIGNIFICANCE OF THE SENSORY WEB

Defects in the uroepithelial-associated sensory web are likely to contribute to the pathogenesis of bladder diseases. For example, the uroepithelium of patients with the painful bladder disorder interstitial cystitis releases increased amounts of ATP and expresses higher levels of $P2X_2$ and $P2X_3$ receptor subunits,[27,29,30,65] both the likely consequence of increased exocytosis. A similar enhancement of ATP release has been detected in urothelial cells isolated from cats with a feline version of interstitial cystitis,[66] and increased ATP release is also observed in rats with chronic spinal cord injury or chemically irritated bladders.[67] ATP can also act in an autocrine manner to enhance its own release from uroepithelial cells,[68] which may potentiate ATP release from the uroepithelium of patients with chronic bladder disease. Once released, ATP can directly depolarize and initiate firing in sensory nerves by activating P2X channels,[64] or by activating P2Y receptors on afferent nerves to stimulate intracellular second messenger pathways that in turn modulate other ion channels. For example, ATP can enhance TRPV1 currents by lowering the threshold for protons, capsaicin, and heat in sensory neurons.[69] This action, which likely reflects activation of intracellular protein kinases and phosphorylation of the TRPV1 channel, represents a novel mechanism, by which large amounts of ATP released from damaged or sensitized cells in response to injury or inflammation may trigger the sensation of pain.

The transmural signaling pathways described above may offer important targets to treat various bladder disorders. In patients with neurogenic detrusor overactivity, there is an increased density of suburothelial $P2X_3$-immunoreactive sensory afferent nerves.[70] Intriguingly, a decrease in $P2X_3$ staining in these nerves is observed in patients who are responsive to intravesical administration of resiniferatoxin (which targets afferent nerves) or botulinum toxin A (which inhibits secretion).[70,71] While these treatments alter $P2X_3$ receptor expression in afferent nerves, it is equally possible that a mechanism of these treatments is to decrease release of ATP from the uroepithelium. Other data point to a possible role for intravesical infusion of antimuscarinics to treat bladder overactivity.[56–58] Furthermore, intravesical application of nicotine in the rat elicits two effects: a decrease in the frequency of reflex micturition in low concentrations and an increase in frequency in high concentrations.[56] The inhibitory effect at low concentrations is blocked by

methyllcaconitine, an antagonist of α7 nicotinic receptors, whereas the facilitatory effect at high concentrations is blocked by hexamethonium, an antagonist of α3-type nicotinic receptors. Methyllcaconitine alone does not alter reflex bladder activity; whereas hexamethonium alone decreases reflex bladder activity, indicating the existence of a tonically active nicotinic facilitatory mechanism. Other studies have shown that intravesical administration of vanilloid compounds produces beneficial effects in patients with bladder disorders such as neurogenic detrusor overactivity or interstitial cystitis,[60–63] and intravesical administration of oxyhemoglobin results in a bladder hyperactivity demonstrating an inhibitory role for NO in the control of bladder reflexes.[59]

SUMMARY

For decades the uroepithelium was viewed as a passive barrier that served to maintain the composition of urine before voiding. In contrast, more recent studies indicate that the uroepithelium is an active participant in the normal function of the bladder and exists as an integral part of a sensory web, in which it communicates the degree of bladder filling to the underlying nervous and muscular tissues and affects their functions. This communication is made possible by the input and output pathways of the uroepithelium, which allow it to respond to its chemical and physical environment and to engage in bidirectional communication with neighboring cells in the subjacent tissues. Defects in the uroepithelial expression of receptors or aberrant release of mediators such as ATP and acetylcholine may contribute to bladder diseases such as interstitial cystitis and detrusor overactivity. As such, uroepithelial-associated receptor and mediator release pathways may serve as important targets for the pharmacologic management of bladder disorders. Finally, study of the uroepithelial sensory web will further our comprehension of other organ systems where epithelial cells may modulate the function of the end organ.[1,2]

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