More than just a barrier: urothelium as a drug target for urinary bladder pain

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Birder, Lori A. More than just a barrier: urothelium as a drug target for urinary bladder pain. Am J Physiol Renal Physiol 289: F489–F495, 2005; doi:10.1152/ajprenal.00467.2004.—Although the urinary bladder urothelium has classically been thought of as a passive barrier to ions/solutes, a number of novel properties have been recently attributed to these cells. Studies have revealed that the urothelium is involved in sensory mechanisms (i.e., ability to express a number of sensor molecules or respond to thermal, mechanical, and chemical stimuli) and can release chemical mediators. Localization of afferent nerves next to the urothelium suggests these cells may be targets for transmitters released from bladder nerves or that chemicals released by urothelial cells may alter afferent excitability. Taken together, these and other findings highlighted in this review suggest a sensory function for the urothelium. Elucidation of mechanisms impacting on urothelial function may provide insights into the pathology of bladder dysfunction.

THE BLADDER UROTHELIUM: AN EFFECTIVE BARRIER

The urinary bladder urothelium is a specialized lining of the urinary tract, extending from the renal pelvis to the urethra. The urothelium is composed of at least three layers: a basal cell layer attached to a basement membrane, an intermediate layer, and a superficial apical layer with large hexagonal cells (diameters of 25–250 μm), which are also termed “umbrella cells” (5, 50) (Fig. 1A). The umbrella cells and perhaps intermediate cells may have projections to the basement membrane (5, 39, 54). Basal cells, which are thought to be precursors for other cell types, normally exhibit a low (3–6 mo) turnover rate; however, accelerated proliferation can occur in pathology. For example, using a model (protamine sulfate) that selectively damages the umbrella cell layer, it has been shown that the urothelium rapidly undergoes both functional and structural changes to restore the barrier in response to injury (48).

Apical urothelial cells function as a barrier against most substances found in urine, thus protecting the underlying tissues (5, 50, 55, 78). When this function is compromised during injury or inflammation, it can result in the passage of toxic substances into the underlying tissue (neural/muscle layers), resulting in urgency, frequency, and pain during voiding. The superficial or umbrella cells play a prominent role in maintaining this barrier and exhibit a number of properties, including specialized membrane lipids, asymmetric unit membrane particles, and a plasmalemma with stiff plaques (5, 42, 50). These plaques are thought to cover nearly 90% of the urothelial cell surface, and each plaque is composed of nearly 1,000 subunits. The proteins [uroplakins (UPs)] that make up these subunits consist of two tetraspan proteins, UPIa and UPIb, and two type 1 proteins, UPII and UPIII, which are organized into two heterodimer pairs (UPla/II and UPIb/III) (50, 77). These cells are also interconnected with extensive junctional complexes, which include cytoskeletal elements and various cytoplasmic as well as transmembrane proteins, which play a role in cell-cell adhesion (1, 5, 36, 50). The “watertight” function of the apical membrane is due, in part, to these specialized lipid molecules and uroplakin proteins that reduce the permeability of the urothelium to small molecules (water, urea, protons), whereas the tight junction complexes reduce the movement of ions and solutes between cells (5, 50, 68, 73) (Fig. 1B).

“SENSOR” FUNCTION OF THE UROTHELIUM

While the urothelium has been historically viewed as primarily a “barrier,” it is becoming increasingly appreciated as a responsive structure capable of detecting physiological and chemical stimuli and releasing a number of signaling molecules. Data accumulated over the last several years now indicate that urothelial cells display a number of properties similar to sensory neurons (nociceptors/mechanoceptors) and that both types of cells use diverse signal-transduction mechanisms to detect physiological stimuli. Examples of “sensor molecules” (i.e., receptors/ion channels) associated with neurons that have been identified in the urothelium are depicted in Table 1. These include receptors for bradykinin (27, 32); neurotrophins (trkA and p75) (60, 75); purines (P2X and P2Y) (15, 20, 49, 64, 69); norepinephrine (α and β) (10, 14); acetylcholine (nicotinic and muscarinic) (8, 9, 25, 37); protease-activated receptors (PARs) (31, 58); amiloride/mechano-sensitive Na+ channels (21, 51, 52, 57, 62, 73); and a number of transient receptor potential (TRP) channels (TRPV1, TRPV2, TRPV4, TRPM8) (7, 12, 13, 47, 63).

TRP CHANNELS: MEDIATORS OF SENSORY TRANSDUCTION IN UROTHELIAL CELLS

The TRP superfamily is a diverse family of proteins that are expressed in many cell types, including neurons, smooth muscle, and nonexcitable cells. These channels, which share sequence homology and structural similarities including six predicted transmembrane segments, can be divided into three...
main classes (TRPC, TRPV, and TRPM). Because of the prominent role in bladder and nociceptive function, this review will focus mainly on the sensory role of various TRP channels found in bladder urothelium.

One example of a urothelial sensor molecule is the TRP channel TRPV1, known to play a prominent role in nociception and in urinary bladder function (66). It is well established that painful sensations induced by capsaicin, the pungent substance in hot peppers, are caused by stimulation of TRPV1, an ion channel protein (22, 23), which is activated by capsaicin as well as by moderate heat, protons, and lipid metabolites such as anandamide (endogenous ligand of both cannabinoid and vanillloid receptors). TRPV1 is expressed throughout the afferent limb of the micturition reflex pathway (Fig. 2), including urinary bladder unmyelinated (C fiber) nerves that detect bladder distension or the presence of irritant chemicals (24). In the urinary bladder, one of the more remarkable findings in our own studies is that TRPV1 is not only expressed by afferent nerves that form close contact with urothelial cells but also by the urothelial cells themselves (12) (Fig. 2A). Furthermore, urothelial TRPV1 receptor expression correlates with the sensitivity to vanillloid compounds, as exogenous application of capsaicin or resiniferatoxin increases intracellular calcium and evokes transmitter [nitric oxide (NO)] release (12, 13) in

Table 1. Polymodal properties of urinary bladder urothelial cells

<table>
<thead>
<tr>
<th>Sensor Function/ Stimulus</th>
<th>Urothelial Sensor Molecules</th>
<th>Neuronal Sensor Molecules</th>
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<tbody>
<tr>
<td>ATP</td>
<td>P2X/P2Y</td>
<td>P2X/P2Y</td>
</tr>
<tr>
<td>Capsaicin/resiniferatoxin</td>
<td>TRPV1</td>
<td>TRPV1</td>
</tr>
<tr>
<td>Heat</td>
<td>TRPV1; TRPV2; TRPV4</td>
<td>TRPV1; TRPV2; TRPV3; TRPV4</td>
</tr>
<tr>
<td>Cold</td>
<td>TRPM8; TRPA1</td>
<td>TRPM8; TRPA1</td>
</tr>
<tr>
<td>H^+</td>
<td>TRPV1</td>
<td>TRPV1; ASIC; DRASIC</td>
</tr>
<tr>
<td>Osmolarity</td>
<td>In part TRPV4</td>
<td>In part TRPV4</td>
</tr>
<tr>
<td>Bradykinin</td>
<td>B1; B2</td>
<td>B1; B2</td>
</tr>
<tr>
<td>Acetylcholine</td>
<td>Nicotinic/muscarinic</td>
<td>Nicotinic/muscarinic</td>
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<tr>
<td>Norepinephrine</td>
<td>α,β-Subtypes</td>
<td>α,β-Subtypes</td>
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<tr>
<td>Nerve growth factor</td>
<td>p75/trkA</td>
<td>p75; trkA</td>
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<tr>
<td>Mechanosensitivity</td>
<td>Amiloride-sensitive Na^+ channels</td>
<td>Amiloride-sensitive Na^+ channels</td>
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TRP, transient receptor potential; ASIC, acid-sensing ion channel; DRASIC, dorsal root acid-sensing ion channel.
Cultured cells. These responses were eliminated in TRPV1 null mice. In neurons, TRPV1 is thought to integrate/amplify the response to various stimuli and thus plays an essential role in the development of inflammation-induced hyperalgesia (34, 40). Thus it seems likely that urothelial TRPV1 might participate in a similar manner in the detection of irritant stimuli following bladder inflammation or infection.

While anatomically normal, TRPV1 null mice exhibited a number of alterations in bladder function including a reduction of in vitro, stretch-evoked ATP release and membrane capacitance as well as a decrease in hypotonically evoked ATP release from cultured TRPV1 null urothelial cells (13). These findings demonstrate that the functional significance of TRPV1 in the bladder extends beyond pain sensation to include participation in normal bladder function and is essential for normal mechanically evoked purinergic signaling by the urothelium.

In contrast to TRPV1, TRPV2 and TRPV4, which are detectors of warm temperatures (TRPV4 can also be gated by hypotonic stimuli) (2, 29, 53), TRPM8 has been shown to be activated by cold (25–28°C) temperatures as well as by cooling agents (menthol, icilin) and is expressed in a subset of sensory neurons as well as in nonneural cells. TRPV1, TRPV4, and TRPM8 are localized throughout the urothelium, in contrast to TRPV2, which seems to be expressed primarily in apical cells. This expression suggests that these cells express a range of thermoreceptors underlying both “cold” and “heat” stimuli (63). While the functional role of these thermosensitive channels in the urothelium remains to be clarified, it seems likely that a primary role for these proteins may be to recognize noxious stimuli in the bladder. However, the diversity of stimuli that can activate these proteins suggests a much broader sensory and/or cellular role. For example, TRPM8 expression has been shown to be increased in some epithelia in malignant disorders (prostate tumors), suggesting a role in proliferating cells (72). Thus further studies are needed to fully elucidate the role of TRP channels in the urothelium and influence on bladder function.

**“TRANSDUCER” FUNCTION OF THE UROTHELIUM AND CELL-CELL SIGNALING**

Release of chemical mediators (NO, ATP, acetylcholine, substance P, PGs) (10, 11, 19, 26, 28, 33) from urothelial cells suggests that these cells exhibit specialized sensory and signaling properties that could allow reciprocal communication with neighboring urothelial cells as well as nerves or other cells (i.e., immune, myofibroblasts, inflammatory) in the bladder.
Recent studies have shown that both afferent as well as autonomic axons are located in close proximity to the urothelium (8, 12). For example, peptide- and TRPV1-immunoreactive nerve fibers have been found localized throughout the urinary bladder musculature and in a plexi beneath and extending into the urothelium (Fig. 2B). Confocal microscopy revealed that TRPV1-immunoreactive nerve fibers are in close association with basal urothelial cells such that their fluorescent signals overlapped within 0.5-μm optical sections. This type of communication suggests that these cells may be targets for transmitters released from bladder nerves or other cells or that chemicals released by urothelial cells may also alter the excitability of bladder nerves. In support of this idea is evidence that ATP (released from urothelial cells during stretch) can activate a population of suburothelial bladder afferents expressing P2X3 receptors, signaling changes in bladder fullness and pain (19, 33). Accordingly, P2X3 null mice exhibit a urinary bladder hyporeflexia, suggesting that this receptor and neural-epithelial interactions are essential for normal bladder function (30). This type of regulation may be similar to epithelial-dependent secretion of mediators in airway epithelial cells which are thought to modulate submucosal nerves and bronchial smooth muscle tone and may play an important role in inflammation (41, 43). Thus it is possible that activation of bladder nerves and urothelial cells can modulate bladder function directly or indirectly via the release of chemical factors in the urothelial layer.

ATP released from the urothelium or surrounding tissues may also play a role in the regulation of membrane trafficking. This is supported by recent studies in the urinary bladder where urothelial-derived ATP release purportedly acts as a trigger for exocytosis in part via autocrine activation of urothelial purinergic (P2X; P2Y) receptors (Wang E, Birder L, and Apodaca G, unpublished observations). These findings suggest a mechanism whereby urothelial cells sense or respond to extracellular ATP concentration and thereby translate extracellular stimuli into functional processes.

PATHOLOGY-INDUCED UROTHELIAL PLASTICITY AND EFFECT ON BARRIER FUNCTION

Recent evidence has demonstrated that inflammation or injury can alter the expression and/or sensitivity of a number of urothelial sensor molecules (75, 76). Sensitization can be triggered by various mediators [ATP, NO, nerve growth factor (NGF), PGE2] which may be released by both neuronal and nonneuronal cells (urothelial cells, fibroblasts, mast cells) located near the bladder luminal surface. For example, an important component of the inflammatory response is ATP release from various cell types including urothelial, which can initiate painful sensations by exciting purinergic (P2X) receptors on sensory fibers (19, 30). Recently, it has been shown in sensory neurons that ATP can potentiate the response of vanilloids by lowering the threshold for protons, capsaicin, and heat (70). This 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. These findings have clinical significance and suggest that alterations in afferents or epithelial cells in pelvic viscera may contribute to the sensory abnormalities in a number of pelvic disorders, such as interstitial cystitis (IC), a chronic clinical disease characterized by urgency, frequency, and bladder pain.

Fig. 3. Schematic depicting possible involvement of urothelial “sensor molecules” and/or release mechanisms in bladder function. During bladder storage, increased pressure can lead to release of acetylcholine (ACh) from both neural and nonneural (urothelial) sources. Released ACh can stimulate muscarinic receptors on smooth muscle cells. Muscarinic receptors present on bladder nerves as well as on urothelium may also be activated by released ACh. Targeting muscarinic receptors activated by urothelially derived release of ACh (1) and/or other urothelial-release mechanisms (2) may be important in chronic bladder conditions and in the aging bladder. Urothelial cells also express TRPV1 receptors, which can be activated during bladder distension as well as by vanilloid compounds. Stimulation of urothelial TRPV1 can lead to release of transmitters such as ATP, which can activate purinergic (P2X3) receptors on nearby bladder afferents. These afferents also contain TRPV1, which are activated by vanilloid compounds. Other purinergic receptors present on smooth muscle cells as well as on urothelium may also be activated by urothelial-derived ATP. Intravesical vanilloid treatment could lead to activation/desensitization of urothelial TRPV1 or depletion of urothelial-derived mediators (3).
upon filling (an innocuous stimulus) (35, 56, 59). A comparable disease in cats is termed feline IC (16–18, 74), where we reported alterations in stretch-activated release of urothelially derived ATP (11) consistent with augmented release of ATP in urothelial cells from some patients with IC (65).

Although the urothelium maintains a tight barrier to ion and solute flux, a number of factors such as tissue pH, mechanical or chemical trauma, or bacterial infection can modulate this barrier function of the urothelium (3, 39). For example, inflammation, injury (spinal cord transection), or IC, all of which increase endogenously generated levels of NO, increase permeability to water/urea in addition to producing ultrastructural changes in the apical layer (6, 71). Although the mechanism is unknown, these findings may be similar to those in other epithelia where excess production of NO has been linked to changes in epithelial integrity (38). Disruption of epithelial integrity may also be due to substances such as antiproliferative factor, which has been shown to be secreted by bladder epithelial cells from IC patients and can inhibit epithelial proliferation, thereby adversely affecting barrier function (44, 45). Uropathogenic *Escherichia coli* can also bind to uroplakin proteins present on the apical surface of superficial umbrella cells (3, 61). This could be an initial step leading to a cascade of events thought to be part of symptoms associated with urinary tract infections. In addition, acute injury (spinal cord transection) results in altered urothelial barrier function ( ultrastructural changes accompanied by increased permeability). The disruptions and accompanying changes in permeability can be blocked by pretreatment with a ganglionic blocker (6), suggesting an involvement of the autonomic nervous system in the acute effects of spinal cord injury on bladder urothelium. Taken together, modification of the urothelium and/or loss of epithelial integrity in a number of bladder pathologies could result in passage of toxic/irritating urinary constituents through the epithelium, leading to changes in the properties of sensory pathways.

**UROTHELIAL RECEPTORS/RELEASE MECHANISMS AS TARGETS FOR DRUG TREATMENT**

It is conceivable that the effectiveness of some agents currently used in the treatment of bladder disorders may involve urothelial receptors and/or release mechanisms (Fig. 3). For example, antimuscarinic drugs, currently the standard treatment for detrusor overactivity (4), target muscarinic receptors on bladder smooth muscle. These agents prevent receptor stimulation by acetylcholine released from bladder effector nerves and result in increased bladder capacity. Because these drugs are thought to be effective during the storage phase of micturition, when parasympathetic nerves are silent, it is postulated that the release of acetylcholine from the urothelium may contribute to detrusor overactivity. Accordingly, targeting muscarinic receptors activated by acetylcholine released from the urothelium and/or other urothelial-release mechanisms may prove to be an effective therapy. Intravesical instillation of vanilloids (capsaicin or resiniferatoxin) improves urodynamics parameters in patients with neurogenic detrusor overactivity and reduces bladder pain in patients with hypersensitivity disorders, presumably by desensitizing bladder nerves (46, 67). This treatment could also target TRPV1 on urothelial cells, whereby a persistent activation might lead to receptor desensitization or depletion of urothelial transmitters.

**SUMMARY**

These findings suggest that urothelial cells exhibit specialized sensory and signaling properties that could allow them to respond to their chemical and physical environments and to engage in reciprocal communication with neighboring urothelial cells as well as nerves within the bladder wall. Taken together, pharmacological interventions aimed at targeting urothelial receptor/ion channel expression or release mechanisms may provide a new strategy for the clinical management of bladder disorders.

**ACKNOWLEDGMENTS**

The author is grateful to Drs. Gerald Apodaca, William C. de Groat, and Thomas Kleyman for helpful suggestions and critical reading of this manuscript and to Tim Voit for assistance with graphics.

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