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Urothelial Signaling

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Abstract

Beyond serving as a simple barrier, there is growing evidence that the urinary bladder urothelium exhibits specialized sensory properties and play a key role in the detection and transmission of both physiological and nociceptive stimuli. These urothelial cells exhibit the ability to sense changes in their extracellular environment including the ability to respond to chemical, mechanical and thermal stimuli that may communicate the state of the urothelial environment to the underlying nervous and muscular systems. Here, we review the specialized anatomy of the urothelium and speculate on possible communication mechanisms from urothelial cells to various cell types within the bladder wall.

Keywords

urothelium; sensory web; TRP channels; barrier function

Anatomy and Barrier Function of the Urothelium

The uroepithelium forms the interface between the urinary space and the underlying vasculature, connective, nervous, and muscular tissues. Urothelium is composed of at least three layers: a basal cell layer attached to a basement membrane, an intermediate layer, and a superficial or apical layer composed of large hexagonal cells (diameters of 25-250 μm) known as 'umbrella cells' (Apodaca, 2004; Lewis, 2000). The umbrella cells are interconnected by tight junctions (which are composed of multiple proteins such as the claudins) and are covered on their apical surface (nearly 70-80%) by crystalline proteins called uroplakins that assemble into hexagonal plaques (Hicks, 1975; Liang, 2001; Sun, 2006). Uroplakins and other urothelial cellular differentiation markers, such as cytokeratin 20, are not expressed in the stratified epithelium of the urethra. In some species, the umbrella cells and perhaps also the intermediate cells have projections to the basement membrane (Apodaca, 2004).

The ability of the bladder to maintain a barrier, despite large alterations in urine volume and increases in pressure during bladder filling and emptying, is dependent on several features of the umbrella cell layer. These features include tight-junction complexes that reduce the movement of ions and solutes between cells and specialized lipid molecules and uroplakin proteins in the apical membrane, which reduce the permeability of the cells to small molecules (water, urea, protons) (Acharya et al., 2004; Apodaca, 2004). Mice lacking genes for uroplakins

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IIIa and II (UPIIIa and UPII) exhibit a number of abnormalities that lead to a compromised urothelial barrier. The apical surface of the urothelium is also covered with a sulfated polysaccharide glycosaminoglycan (GAG) or mucin layer that is thought to act as a nonspecific anti-adherence factor and as a defense mechanism against infection (Parson, 1990; Parsons, 1977; Parsons et al., 1991). In addition, during bladder filling the umbrella cells become flat and squamous and this shape change is accompanied by vesicular traffic (i.e. exocytosis/endocytosis), adding membrane to the apical surface thereby increasing overall urinary bladder surface area (Balestreire, 2007; Hicks, 1975; Wang et al., 2005). There is evidence that this stretch-induced exocytosis is dependent on activation of epidermal growth factor receptor (EGFR) (Balestreire, 2007; Cheng, 2002). These processes allow the bladder to accommodate increasing volumes of urine during filling without compromising barrier function. Exocytosis/endocytosis (vesicular recycling) may also play an important role in modulating the release of a number of neurotransmitters/mediators as well as regulating the function of many receptors and ion channels in urothelial cells (Apodaca, 2007; Birder et al., 2007).

Urothelial Heterogeneity

Studies of different species have shown that the major part of the urinary tract is lined with a fully differentiated urothelium (Sun, 2006). Findings in cultured cells reveal a distinct difference in morphology of ureteral and bladder urothelial cells, supporting a difference in cell lineage. There seems to be no apparent difference between the urothelium of the trigone compared to the detrusor. This is in contrast to the proximal urethra, a region in which a complete 'barrier' is unnecessary, which transitions from urothelium to a stratified or columnar epithelium accompanied by a lack of urothelial-specific differentiation markers (Romih et al., 2005; Sun, 2006; Thomas, 2005). There are also reports of microvilli on the apical surface of the urethral epithelium. The presence of microvilli may play a role in increasing the surface area of the cell, participation in both sensory and transducer functions as well as in fluid transport. Taken together, present evidence suggests at least 3 urothelial lineages: 1) those of the ureter/renal pelvis, 2) detrusor/trigone and 3) bladder neck/proximal urethra (Liang, 2005). The functional significance of these findings has yet to be determined.

Evidence Suggesting a Role for Urothelial Cells in Visceral Sensation

While urothelial cells are often viewed as bystanders in the process of visceral sensation, recent evidence has supported the view that these cells function as primary transducers of some physical and chemical stimuli and are able to communicate with underlying cells including bladder nerves, smooth muscle and even inflammatory cells. (Figure 1A)

There are at least 3 lines of evidence that suggest that urothelial cells participate in the detection of both physical and chemical stimuli. First, bladder nerves (afferent and efferent) are localized in close proximity, and some within, the urothelium (Birder et al., 2007; Birder et al., 2002; Jen, 1995; Kunze, 2006). Ultrastructural studies of nerves in the human bladder have found only unmyelinated nerves in the urothelial and immediate suburothelial layer (Wiseman, 2002). Peptidergic, P2X- and TRPV1-immunoreactive nerve fibers presumed to arise from afferent neurons in the lumbosacral dorsal root ganglia are distributed throughout the urinary bladder musculature as well as in a plexus beneath and extending into the urothelium (Birder, 2007; Birder et al., 2002). (Figure 1B) These axon collaterals are able to release neurotransmitters onto the various tissues in the bladder wall. In humans with neurogenic detrusor overactivity intravesical administration of resiniferatoxin, a C-fiber afferent neurotoxin, reduces the density of TRPV1 and P2X3 immunoreactive suburothelial nerves, indicating that these are sensory nerves (Apostolidis, 2005; Brady, 2004). In addition, immunohistochemical studies have also revealed both adrenergic (tyrosine hydroxylase-positive) as well as cholinergic (choline acetyltransferase, ChAT-positive) nerves in close

proximity to the urothelium (Jen, 1995). While this would commonly indicate the presence of efferent autonomic nerves it has been reported in the guinea pig bladder that some ChAT immunoreactivity near the urothelium may be present in sensory rather than efferent nerves (Gillespie, 2006). A network of cells with morphologic characteristics similar to those of myofibroblasts or interstitial cells is also detected in the suburothelial space of the bladder in both humans and animals (Brading, 2005; Ost, 2002; Sui, 2004). These cells, which are extensively linked by gap junctions and have close contacts with nerves, can respond to neurotransmitters such as ATP released from nerves or urothelial cells, suggesting that they can act as intermediaries in urothelial-nerve interactions (Brading, 2005; Ikeda et al., 2007; Sui, 2004). Thus, the anatomic substrates for bidirectional urothelial-neural communication exist within the urinary bladder.

Involvement of the Urothelium in ‘Sensing’ Chemical and Mechanical Stimuli

A second line of evidence suggesting that urothelial cells play a role in sensory function is the expression of numerous receptors/ion channels similar to that found in both nociceptors and mechanoreceptors. Examples of neuronal “sensor molecules” (receptors / ion channels) that have been identified in urothelium include receptors for purines (P2X₁₋₇ and P2Y_{1,2,4}), adenosine (A₁, A_{2a}, A_{2b} and A₃), norepinephrine (α and β), acetylcholine (muscarinic and nicotinic), protease-activated receptors (PARs), amiloride- and mechanosensitive epithelial sodium channels (ENaC), bradykinin (B1 and B2), neurotrophins (p75, trkA, EGF family ErbB1-3), vascular endothelial growth factor (VEGF) and neuropilins (NRP); pituitary adenylate cyclase-activating peptide (PACAP); corticotrophin releasing factor (CRF1 and CRF2), estrogens (ER α and ER β), endothelins, ASIC-like and various TRP channels (TRPV1, TRPV2, TRPV4, TRPM8 and TRPA1) (Beckel et al., 2006; Birder et al., 2003; Birder et al., 2002; Birder et al., 2007; Burnstock, 2001; Carattino et al., 2008; Chess-Williams, 2002; Chopra, 2005; Chopra et al., 2008; Ferguson, 1997; Girard, 2008; Kullmann, 2008; Kullmann, 2009; LaBerge, 2006; Ossovskaya, 2004; Saban, 2008; Taylor, 2000; Yu, 2006). The expression of these various receptors enable the urothelium to respond to a number of “sensory inputs” from a variety of sources. These inputs include increased stretch during bladder filling, soluble factors (many found in the urine) such as epidermal growth factor (EGF), or chemical mediators, peptides and/or transmitters such as substance P, calcitonin gene-related peptide (CGRP), corticotrophin releasing factor (CRF), acetylcholine, adenosine or norepinephrine released from nerves, inflammatory cells and even nearby blood vessels (Apodaca, 2004; Apodaca, 2007a; Birder et al., 2007; Hanna-Mitchell et al., 2007; LeBerge et al., 2006).

The third line of evidence for a sensor role for urothelial cells is the secretion of transmitters or mediators such as neurotrophins, peptides, ATP, acetylcholine, prostaglandins, prostacyclin, nitric oxide (NO) and cytokines that are capable of modulating, activating or inhibiting sensory nerves, smooth muscle and/or cells of the immune and inflammatory systems (Apodaca, 2007b; Birder et al., 2007). For example, the secretion of a diffusible factor from the urothelium (sonic hedgehog, Shh) has been proposed to induce smooth muscle differentiation (Shiroyanagi, 2007). In addition, the release of urothelial-derived factors in response to mechanical as well as chemical stimulation has been shown to either facilitate or inhibit the activity of bladder afferent nerves (Andersson, 1995; Birder et al., 2007). There is evidence that intravesical administration of oxyhemoglobin results in a bladder hyperactivity demonstrating an inhibitory role of NO in the control of bladder reflexes (Pandita, 2000). It has also been shown that removal of the urothelium using *in vitro* detrusor preparations can significantly increase the detrusor muscle contractile response evoked by acetylcholine. These findings suggest that the urothelium can release a factor that exerts an inhibitory effect on bladder smooth muscle contractility (Hawthorn, 2000). Though this “urothelial-derived inhibitory factor or UDIF” has not yet been identified, it is unlikely to be NO, adenosine, GABA, a cyclo-oxygenase product or mediated by the small conductance Ca²⁺-sensitive K⁺-

channel (Chaiyaprasithi et al., 2003; Fovaeus et al., 1999; Hawthorn et al., 2000; Murakami et al., 2007).

In some types of cells, intercellular calcium ‘waves’ may be a common way of translating extracellular stimuli into functional processes that can spread to nearby cells, ultimately leading to release of neuroactive mediators. Studies using optical mapping techniques have revealed that both chemical and physical stimulation of cross-sections of the bladder can generate a series of calcium ‘waves’ which propagate across the urothelial-suburothelial layers before invading the underlying smooth muscle (Ikeda et al., 2007). There is also evidence that a suburothelial population of interstitial cells may be an intermediate stage in the sensory response to bladder wall stimuli (Brading, 2005; Grol, 2008; Ikeda et al., 2007; Sui, 2004). These cells may act as a variable amplifier of the sensory response mediating signals between the urothelium and sensory afferents or the detrusor smooth muscle layer, either directly or via the activation of afferent nerve fibers (Fry et al., 2007).

The mechanism underlying release of chemical mediators from the urothelium, including whether all sensory “inputs” stimulate membrane turnover (i.e. vesicular exocytosis) is not well understood. It is also not known whether different layers of the urothelium may secrete different mediators with differing effects. What little is known about the roles and dynamics of membrane-bound cytoplasmic vesicles in urothelial cell physiology is derived from measurements of membrane capacitance and microscopy of fixed tissues and cells. For example, there is evidence that once released, ATP can act as an important autocrine mediator, which can induce membrane turnover as well as enhance both stretch induced exocytosis and endocytosis (Wang et al., 2005). Alterations in membrane turnover can not only increase apical surface area (as described above) but also regulate the number and function of receptors and channels at the cell surface.

Urothelial Sensor Targets and Mediators: Role in Pathology

ATP and Purinergic Receptors

Since the first report of distension-evoked ATP release from the urothelium, there has been abundant evidence supporting a role for urothelially-derived release of ATP in autocrine and paracrine signaling within the lower urinary tract. ATP is released from both the apical and basolateral urothelial surfaces in response to bladder stretch and can act on P2X2 and P2X3 urothelial receptors to stimulate stretch-induced exocytosis (Wang et al., 2005). (Figure 1C) It has been suggested that the amiloride-sensitive apical sodium channel or ENaC, expressed within the urothelium may be involved in mechanotransduction by controlling basolateral release of ATP (Du, 2007a). Additionally, the expression of both P2X and P2Y receptors in nerve fibers and suburothelial interstitial cells in close proximity to the bladder lumen and the sensitivity of these cells to ATP raises the possibility that basolateral ATP release from urothelial cells could influence not only adjacent urothelial cells, but also interstitial cells and nerves (Fry, 2007; Sui, 2006). The intercellular communication mediated by gap junctions in interstitial cells may provide a mechanism for long-distance spread of signals from the urothelium to the detrusor muscle (Ikeda et al., 2007).

Pathology results in augmented release of ATP from the bladder urothelium, which can cause painful sensations via excitation of purinergic receptors on nearby sensory fibers (Birder et al., 2003; Sun, 2001). This type of noncholinergic mechanism is likely to play an important role in a number of bladder pathologies such as bladder pain syndrome/interstitial cystitis (BPS/IC), a chronic clinical disease characterized by urgency, frequency and bladder pain upon filling (Gillenwater et al., 1998; Nickel, 2003; Parsons, 2007; Parsons et al., 1998). Studies of a comparable disease in cats (i.e. feline interstitial cystitis, FIC) revealed an augmented stretch-evoked release of urothelial-derived ATP and changes in purinergic receptor profiles in

urothelial cells (Birder et al., 2003). This observation is consistent with similar findings in patients with BPS/IC and suggests that urothelial sensor molecules exhibit plasticity in pathologic conditions (Sun, 2001). ATP can also act in an autocrine manner to enhance its own release from urothelial cells. This autofeedback mechanism may be more important in pathological conditions. Enhanced release of ATP into the bladder lumen in response to bladder distension has also been observed in chronic spinal cord injured rats and in rats with chemically irritated bladders (Girard, 2008; Salas, 2007). ATP released from the urothelium can directly depolarize and initiate firing in sensory nerves by activating ionotropic P2X channels or by activating metabotropic P2Y receptors on afferent nerves (as well as the urothelium) to stimulate intracellular second messenger pathways that in turn modulate other ion channels. For example, it has been shown in sensory neurons that ATP can enhance TRPV1 currents by lowering the threshold for protons, capsaicin and heat (Tominaga, 2001). This action which is presumably mediated by activating intracellular protein kinases and phosphorylation of the TRPV1 channel, represents a 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.

TRPV1 Channels

The ability of capsaicin to evoke NO release from rat urothelium, reported in 1998, provided the first, albeit indirect, demonstration that TRPV1 channels are expressed in urothelial cells and that urothelial cells and afferent nerves, which also express these channels, share a number of common properties (Birder, 1998). This ion-channel protein is activated by capsaicin, as well as moderate heat, protons and lipid metabolites such as anandamide (an endogenous ligand of both cannabinoid and vanilloids receptors) (Caterina et al., 1997; Hicks, 1975).

TRPV1 is not only expressed by afferent nerves that form close contact with the bladder epithelium but also in non-neuronal cells such as urothelial cells (Figure 1D) and interstitial cells (Birder, 2001; Ost, 2002). Activation of urothelial cells with capsaicin or resiniferatoxin can increase intracellular calcium and evoke transmitter (NO or ATP) release. Similar to that in sensory neurons, urothelial-response to vanilloids are enhanced by low pH, blocked by TRPV1 antagonists and eliminated in TRPV1 null mice (Birder, 2001; Birder et al., 2002). In afferent neurons, TRPV1 is thought to integrate/amplify the response to various stimuli and to play an essential role in the development of inflammation-induced hyperalgesia. It seems likely that urothelial-TRPV1 might participate in a similar manner, in the detection of irritant stimuli following bladder inflammation or infection.

Though TRPV1-null mice are anatomically normal, they exhibit a number of alterations in bladder function including a higher frequency of low-amplitude, non-voiding bladder contractions suggesting the possibility of a small but ongoing role for TRPV1 in normal urine storage function. In addition, TRPV1 null mice exhibit a reduction in stretch- and hypotonic-evoked ATP release from the urothelium and also an attenuated response of bladder afferents to distension (Birder et al., 2002; Daly, 2007). These findings demonstrate that the functional significance of TRPV1 in the bladder extends beyond pain sensation to include participation in normal voiding function, and is essential for mechanically evoked signaling by bladder afferents as well as the urothelium.

TRPV1 has attracted considerable attention as a potential contributor to bladder diseases. TRPV1 knockout mice also display differences in the response to chemically induced irritation or inflammation of the bladder as compared to their wild-type counterparts (Charrua, 2007; Szallasi, 2006; Wang, 2008). Furthermore patients suffering from neurogenic detrusor overactivity exhibit significant increases in the number of TRPV1-expressing nerves as well as increased TRPV1 expression within the urothelium (Apostolidis, 2005; Brady, 2004). Intravesical instillation of vanilloids (capsaicin or resiniferatoxin) improves urodynamic

parameters in patients with neurogenic detrusor overactivity and reduces pain in patients with hypersensitivity disorders, presumably by desensitizing bladder nerves (Apostolidis, 2006; Chancellor, 1999; Fowler, 1992; Kalsi, 2005). However, this treatment which had some clinical success, could also target TRPV1 on urothelial cells, whereby a persistent activation might lead to receptor desensitization or depletion of urothelial transmitters.

Additional TRP channels

Much less is known about the involvement of other TRPs in bladder function or disease. TRPV4 which is a nonselective cation channel activated by a number of stimuli including heat, shear stress, changes in osmolarity and lipid ligands is expressed mainly within the epithelium of the urinary bladder (Birder, 2007; Gevaert, 2007; Liedtke, 2005). While a definitive role for TRPV4 in bladder function has not been established, there is evidence that null mice exhibit impaired voiding responses and, intravesical instillation of a TRPV4 agonist in the rat triggers a novel voiding reflex which could regulate the late phase of micturition (Birder, 2007; Gevaert, 2007). In addition, in the awake ewe, TRPV4 may also be involved in a urethra to bladder reflex, proposed to facilitate bladder emptying (Combrisson, 2007).

While little is known about the involvement of the cold-sensing TRP channels (TRPM8 and TRPA1) in bladder function, the effect of cold temperatures on lower urinary tract function has long been of interest (Cheng, 1997). The instillation of cold solutions (known as the ice water test) elicits involuntary detrusor contracts in patients with either chronic spinal cord lesions or following bladder outlet obstruction (Chai, 1998; Gotoh, 1999). This reflex may be mediated by activation of C-type bladder afferent nerves sensitive to cold temperatures. The finding that intravesical instillation of menthol facilitates the bladder cooling reflex in both the cat and human suggests that TRPM8, a cold- and menthol-sensing channel expressed in both nerves and the urothelium, may be involved in triggering the reflex (Jiang, 2002; Peier, 2002). Another member of the TRP family, TRPA1 (characterized as a thermoreceptor activated by noxious cold), is expressed in C-fiber afferents as well as urothelium and agonists to this channel induce bladder hyperreflexia (Du, 2007b; Streng, 2008). In addition, hydrogen sulfide, which may be formed during infection/inflammation, is an activator of TRPA1 (Streng, 2008).

While the functional role of 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. In lung epithelium, activation of a variant of TRPM8 results in secretion of mediators that are thought to play a role in airway inflammation (Sabnis, 2008). However, the diversity of stimuli that can activate these proteins suggests a much broader sensory and/or cellular role. For example, TRPM8 expression is increased in some epithelia in prostate tumors, suggesting a role in proliferating cells (Bidaux, 2005). Thus, further studies are needed to fully elucidate the role of TRP channels in urothelium and their influence on bladder function.

Acetylcholine and the Urothelium

The presence of muscarinic and nicotinic receptors in the urothelium has attracted interest in the role of acetylcholine as a chemical mediator of neural-urothelial interactions (Beckel et al., 2006; Hanna-Mitchell et al., 2007; Hawthorn, 2000; Templeman et al., 2002). These receptors could be stimulated by acetylcholine released from urothelial cells as well as by cholinergic nerves that have been detected in close proximity to the urothelial cells in the rat bladder (Hanna-Mitchell et al., 2007; Kullmann, 2008). Exogenous muscarinic and nicotinic cholinergic agonists applied to cultured urothelial cells can elicit an increase in intracellular calcium concentration and evoke the release of NO and ATP (Beckel et al., 2006; Kullmann, 2008). In bladder strips or whole bladder preparations muscarinic agonists also stimulate the release of a smooth muscle inhibitory factor from the urothelium (Hawthorn, 2000; Templeman

et al., 2002). Electrical stimulation of the pelvic nerve or reflex activation of the autonomic nervous system by spinal cord injury (Apodaca et al., 2003) can elicit changes in urothelial permeability as well as changes in the morphology of the urothelium in the rat raising the possibility that autonomic or sensory nerves make 'synaptic connections' with urothelial cells.

There is evidence that the urothelium expresses the full complement of muscarinic receptors as well as enzymes necessary for the synthesis and release (except vesicular choline transporter) of acetylcholine (Hanna-Mitchell et al., 2007; Kullmann, 2008; Lips, 2007; Zarghooni, 2007). Further, the urothelium is able to release acetylcholine following both chemical and mechanical stimulation. Once released, urothelial-derived acetylcholine is likely to exert effects via a number of sites including smooth muscle, nerves as well as urothelial associated-muscarinic or nicotinic receptors, the latter that could contribute to feedback mechanisms modifying urothelial function. Stimulation of urothelial-cholinergic receptors elicits release of mediators such as nitric oxide and ATP, which could alter bladder sensation by stimulating nearby sensory afferent nerves.

It has been speculated that the mucosa could be affected by antimuscarinics via the bloodstream as well as affecting luminal muscarinic receptors (since some of these agents and/or metabolites are likely to be excreted in urine) (Andersson, 2008). Thus, targeting muscarinic receptors and/or urothelial release mechanisms may play an important role in the treatment for a number of bladder disorders. However, the mechanism of release is uncertain. Recently, botulinum toxin type A has been used to evaluate the release mechanism. Botulinum toxin type A is used clinically to treat a number of bladder disorders including neurogenic detrusor overactivity, detrusor-sphincter dyssnergia as well as painful bladder syndrome/interstitial cystitis (Chancellor, 2005; Tiwari, 2006). Studies have shown that this treatment may inhibit detrusor muscle contractions by suppressing the release of transmitters such as ATP and acetylcholine from bladder nerves by blocking exocytosis. Recent evidence suggests that botulinum toxins prevent the release of transmitters such as ATP from the urothelium, which may suggest urothelial-released mediators contribute to sensory urgency (Khera et al., 2004; Smith, 2005).

Pathology-Induced Urothelial Plasticity and Effect on Barrier Function

Basal cells, which are thought to be precursors for other cell types, normally exhibit a low (3-6 month) turnover rate, in fact the slowest turnover of any mammalian epithelial cells (Hicks, 1975; Martin, 1972). It has been shown that neither urine-derived factors nor cyclic mechanical changes contribute to urothelial proliferation and differentiation; however accelerated proliferation can occur in pathology. For example, streptozotocin (STZ)-induced diabetes and sucrose-induced diuresis in rodents both result in significant urothelial remodeling (Liu, 2006). In addition, using protamine sulfate, an agent that selectively damages the umbrella cell layer, it has been shown that the urothelium rapidly undergoes both functional and structural changes in order to restore the barrier in response to injury (Lavelle, 2002). The initiation of urothelial proliferation is thought to involve up-regulation of growth factors such as fibroblast growth factor and nerve growth factor (NGF) (Bassuk, 2003; de Boer, 1996). Increased NGF levels have been identified in urothelium in animals with bladder outlet obstruction, spinal cord injury and cyclophosphamide-induced inflammation. The increased target NGF may be linked with alterations in urinary frequency and unstable bladder contractions (Steers et al., 2006).

Though the urothelium maintains a tight barrier to ion and solute flux, a number of local factors such as tissue pH, mechanical or chemical trauma, or bacterial infection can modulate the urothelial barrier (Anderson et al., 2003; Hicks, 1975). A number of pathologies including diabetes have been shown to increase the susceptibility of the urothelium to infections, in particular by *Escherichia coli*. Urinary tract infections produced by uropathogenic *Escherichia coli* (UPEC) are initiated by bacterial adherence to uroplakin proteins on the apical surface of

umbrella cells (Figure 1E)(Anderson et al., 2003). The UPEC express filamentous adhesive organelles (type 1 pili) that mediate both bacterial attachment and invasion of the urothelial cells. Internalization of UPEC in the umbrella cells and formation of intracellular colonies (biofilm-like pods) of UPEC in umbrella cells has been implicated in the mechanism of recurrent urinary tract infections (Billips, 2008; Klumpp, 2006; Mulvey, 1998). Recent evidence suggests that induction of differentiation markers such as uroplakin III may play an important role in UPEC-induced cell death (Thumbikat, 2009).

Other conditions such as bladder pain syndrome/interstitial cystitis (BPS/IC) or spinal cord injury are also associated with changes in urothelial barrier. When the barrier is compromised, water, urea and toxic substances can pass into the underlying tissue (neural/muscle layers) resulting in urgency, frequency and pain during bladder filling and voiding. In some pathological conditions, the disruption of the urothelial barrier is associated with ultrastructural changes and alterations in the levels of chemical mediators such as nitric oxide and ATP that may alter epithelial function and/or integrity. Disruption of urothelial barrier integrity has also been linked to the expression of substances such as antiproliferative factor (APF), which also slows urothelial cell growth (Keay et al., 2004). APF, a frizzled 8 protein detected in the urine of patients with BPS/IC, is secreted by bladder epithelial cells obtained from these patients. Treatment of urothelial cells from normal patients with purified APF decreases the expression of adhesion and tight junction proteins. Disruption of urothelial function can also be induced by more remote pathological conditions that influence neural or hormonal mechanisms. For example, both physical and psychological stresses have been associated with alterations in epithelial permeability and even mucosal inflammation (Mayer et al., 2003; van de Merwe, 2007). Short-term ischemia can result in decreased blood flow and oxygenation and may occur with conditions that produce urinary retention or detrusor instability. The urothelium is highly sensitive to ischemic alterations, such that 1 hour of ischemia in an *in vivo* animal model can produce a reversible loss of tight junction integrity and increased permeability with detachment and desquamation of urothelial cells (Korosec, 2000). It has also been shown that estrogen deficiency is linked to urinary bladder dysfunctions including urgency, frequency, unstable bladder contractions and even underactive bladder (Robinson, 2003; Zhu, 2001). Some of these symptoms may be associated with alterations in the epithelial lining of the urinary bladder as estrogen deficiency can lead to epithelial shedding or mucosal atrophy (Hass, 2009; Imamov, 2007). Alteration in the mucosal barrier can be a result of spinal cord transection, which in rats has been shown to result in a rapid alteration in the urothelial barrier including ultrastructural changes and increased permeability (Apodaca et al., 2003). These changes are blocked by pretreatment with a ganglionic blocking agent, suggesting the involvement of the efferent autonomic pathways in the acute effects of spinal cord injury on bladder urothelium. Other types of urothelial-neural interactions are also likely, based on recent reports that various stimuli induce urothelial cells to release chemical mediators that can, in turn, modulate the activity of afferent nerves. This has raised the possibility that the urothelium may have a role in sensory mechanisms in the urinary tract.

Clinical Significance of the Sensory Web

Defects in urothelial sensor molecules and urothelial-cell signaling are likely to contribute to the pathophysiology of bladder diseases. For example, a number of bladder conditions (e.g., BPS/IC, spinal cord injury (SCI), chemically-induced cystitis) are associated with augmented release of urothelial-derived ATP, which is likely to result in altered sensations and changes in bladder reflexes induced by excitation of purinergic receptors on nearby sensory fibers (Birder et al., 2003; Chopra, 2005; Girard, 2008; Salas, 2007). ATP can also act in an autocrine manner to facilitate its own release from urothelial cells (Wang et al., 2005). Once released, ATP can alter the threshold for activation of ion channels such as TRPV1. This novel mechanism, which most likely reflects activation of intracellular protein kinases and

phosphorylation of the TRPV1 channel, represents a means by which large amounts of ATP released from damaged or sensitized cells, in response to injury or inflammation, could trigger the sensation of pain. Changes in epithelial signaling/barrier function are not unique to the urinary bladder. For example, airway epithelia in asthmatic patients as well as keratinocytes in certain types of skin diseases also exhibit a number of similar abnormalities and compromised repair processes (Bosse, 2008; Holgate, 2007; Holgate, 2008; Proksch, 2008). This is particularly relevant given the high incidence of associated diseases that can include both visceral and somatic conditions, many of which exhibit a shared loss of epithelial barrier function. Taken together, epithelial cells can respond to a number of challenges (including environmental pollutants and mediators released from nerves or nearby inflammatory cells) resulting in altered expression and/or sensitivity of various receptor/channels as well as changes in release of mediators, all of which could impact function.

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Figure 1.

A: Hypothetical model depicting possible interactions between bladder afferent and efferent nerves, urothelial cells, smooth muscle and myofibroblasts. Stimulation of urothelial receptors and channels can release mediators that target bladder nerves and other cell types; urothelial cells can also be targets for neurotransmitters released from nerves or other cell types. Urothelial cells can be activated by either autocrine (i.e. autoregulation) or paracrine (release from nearby nerves or other cells) mechanisms. Abbreviations: ACh, acetylcholine; AdR, adrenergic receptor; BR, bradykinin receptor; H^+ , proton, MR, muscarinic receptor; NE, norepinephrine; NGF, nerve growth factor; NR, neurokinin receptor; NicR, nicotinic receptor; NO, nitric oxide; P2R, purinergic 2 receptor unidentified subtype; P2X and P2Y; purinergic receptors; PG, prostaglandin; SP, substance P; Trk-A, receptor tyrosine kinase A, high affinity receptor for nerve growth factor; TRPs, transient potential channels. (Birder et al., 2007). **B:** Confocal image of the urothelium depicts TRPV1-positive afferent nerve fibers (cy3, red) located in close proximity to basal (FITC, green) urothelial cells; **C:** Image is a 3-dimensional reconstruction (taken with a confocal microscope) depicting localization of P2X3 (green; nuclei blue) in the urothelium. (Wang et al., 2005); **D:** TRPV1 expression detected within the rat urinary bladder urothelium. Image depicts basal cell TRPV1 (cy3, red) and cytokeratin 17 (FITC, green) immunoreactivity. (Birder, 2001); **E:** Image depicts an intracellular bacterial "pod" on the surface of a C3H/H3J mouse bladder infected with UT189. (Anderson et al., 2003)