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Is the Urothelium Intelligent?

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Abstract

The urothelium separates the urinary tract lumen from underlying tissues of the tract wall. Previously considered as merely an effective barrier between these two compartments it is now recognized as a more active tissue that senses and transduces information about physical and chemical conditions within the urinary tract, such as luminal pressure, urine composition, etc. To understand this sensory function it is useful to consider the urothelium and suburothelium as a functional unit; containing uroepithelial cells, afferent and efferent nerve fibers and suburothelial interstitial cells. This structure responds to alterations in its external environment through the release of diffusible agents, such as ATP and acetylcholine, and eventually modulates the activity of afferent nerves and underlying smooth muscles. This review considers different stresses the urothelium/suburothelium responds to; the particular chemicals released; the cellular receptors that are consequently affected; and how nerve and muscle function is modulated. Brief consideration is also to regional differences in the urothelium/suburothelium along the urinary tract. The importance of different pathways in relaying sensory information in the normal urinary tract, or whether they are significant only in pathological conditions is also discussed. An operational definition of intelligence is used, whereby a system (urothelium/suburothelium) responds to external changes, to maximize the possibility of the urinary tract achieving its normal function. If so, the urothelium can be regarded as intelligent. The advantage of this approach is that input–output functions can be mathematically formulated, and the importance of different components contributing to abnormal urinary tract function can be calculated.

Keywords

chemical transmitters; lower urinary tract; sensory transduction; urinary tract dysfunction; urothelium

INTRODUCTION AND BARRIER FUNCTION OF THE UROTHELIUM

The uroepithelium (urothelium) is the interface between the lumen of the urinary tract and underlying tissues. The most superficial layer is composed of large, hexagonal umbrella cells, covered with uroplakins and connected by tight junctions.[1,2]. Below is an intermediate

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cell layer and a basal cell layer connected to a basement membrane. The region between the urothelium and the underlying smooth muscle layers is called the suburothelium and is richly supplied by blood vessels, nerves and fibroblasts (including myofibroblasts or interstitial cells (IC)) and embedded in a collagen matrix.[3,4] Although the passive barrier function which separates luminal contents from the muscle layers is principally a function of the true urothelium there is accumulating evidence that the urothelium and suburothelium (henceforth called UsU and referred to also as the mucosa) forms a functional transduction unit and will be considered below.

The ability of the urothelium to function as an effective barrier to the passage of small molecules and ions has been considered previously[5] and apart from effective umbrella cell tight junctions,[1] several other important features contribute such as: the presence of uroplakins and an effective glycosaminoglycan layer covering the luminal face[6,7] and the ability to incorporate additional membrane into the superficial layer when it is stretched, as for example when the bladder fills with urine.[8]

INTELLIGENCE: ITS APPLICATION TO THE UROTHELIUM

Recent studies suggest that the UsU also responds to external physical and chemical stimuli by secreting agents that in turn influence afferent nerve signaling and more directly the function of underlying tissues in the wall of the urinary tract. Moreover, the transfer function between stimulus strength and output may vary and thus can explain how the transducer function of the urothelium adapts to different conditions, or contributes to pathological lower urinary tract conditions. Can we therefore consider the urothelium to be “intelligent?” Intelligence may be defined in several ways, including.

- *Cognitive intelligence*: the ability to reason, plan, solve problems, think abstractly, comprehend complex ideas, learn quickly, and learn from experience.[9]
- *Machine intelligence*: in a changing environment a system acts to increase the probability of achieving its ultimate goal.[10,11]
- *Operational intelligence*: the mere ability to collect and sort information, as appropriate to the intelligence services.

The second of these is perhaps the most valuable as it is amenable to exact mathematical formulation.[10] Moreover it expresses the idea that a system (here the urothelium) responds to different inputs (stresses) to achieve control over a goal (the micturition cycle): moreover, lower urinary tract dysfunction may be explained by physical changes to the system. It is required to identify the particular environmental changes; how they are sensed; how the information is integrated; what are the effectors (targets); what changes occur that are associated with urinary tract dysfunction.

RESPONSE OF THE UROTHELIUM TO STRESSES

The urothelium can come under many forms of stress that include: physical stress (during bladder filing); ischemia as occurs also during bladder filling;[12] contact with urine of varying ionic composition and osmolality; changes to the endocrine environment; as well as abnormal situations such as urinary tract infections and radiation damage during radiotherapy treatments.

Several histological and functional observations suggest that the urothelium can indeed respond to external stresses.

- The urothelium and suburothelial ICs possess many receptors to potential signaling molecules.[13]

- The UsU is densely innervated, with unmyelinated fibers.[14,15]
- The urothelium secretes many molecules that can have a transmitter function.[13]
- Stimulation or removal of, or damage to, the UsU changes the activity of underlying tissues, including smooth muscle.[16,17]

RECEPTORS IN UROTHELIUM AND SUBUROTHELIUM CELLS

Numerous receptors/ion channels have been identified on urothelial cells and suburothelial ICs including those to purines (ionotropic P2X, metabotropic P2Y subtypes), adenosine, catecholamines (α,β), acetylcholine (muscarinic, nicotinic), neurotrophins (p75, trkA, EGF family ErbB1-3), growth factors (VEGF); as well as different TRP channels (that can respond for example to altered local pH), amiloride- and mechanosensitive-epithelial Na^+ channels (ENaC) and the intracellular machinery that can respond to steroid hormones such as aldosterone.[13,18–26] The presence of these receptors and ion channels will enable the urothelium to respond to stresses from a variety of sources (see below).

INNERVATION OF THE UROTHELIUM/SUBUROTHELIUM

Nerve fibers immunoreactive to P2X, TRPV-1 and peptidergic receptors are present throughout the suburothelium and even penetrate the urothelium. Moreover, some fibers also have the machinery to synthesize nitric oxide with as yet unknown function.[13,27,28] In addition, adrenergic (tyrosine hydroxylase-positive) and cholinergic (choline acetyltransferase, ChAT-positive) nerves have been identified in close proximity to the urothelium.[29] Thus nerves may not just respond to neurotransmitters (see below), but also act as a source of modulators and exhibit efferent function. The density of innervation and associated receptors varies in biopsies taken from patients and animal models exhibiting detrusor overactivity (DO) and is further modulated after treatment with agents that reduce DO. Thus, in humans with neurogenic DO (NDO) there is an increase in the density of nerves immunoreactive to $TRPV_1$ and $P2X_3$ receptors. Furthermore, intravesical administration of resiniferatoxin, a C-fiber afferent neurotoxin that attenuates the incidence of DO in these patients, reduces also the density of TRPV1 and P2X3 immunoreactive suburothelial nerves.[30,31] Botulinum toxin has also been used to reduce the incidence and severity of NDO and biopsies from these patients also exhibited fewer $P2X_3$ and $TRPV_1$-staining nerve fibers.[32]

Within the suburothelium there is a network of cells with morphological characteristics of myofibroblasts or interstitial cells connected by gap junctions containing connexin 43.[33] These cells also make intimate connections with nerves and can also respond to exogenous ATP and a decrease of extracellular pH. Furthermore, the number of these cells is significantly increased in animal and human biopsies taken from bladders exhibiting DO.[34] It has been proposed that these cells are intermediaries in signal transduction between the urothelium and afferent nerves. This is of particular interest as the receptors (e.g., P2Y6) and intracellular pathways that evoke excitatory responses in these cells are different from those in other cells in the bladder wall.[23,35] They therefore offer a relatively selective target if they are involved in signal-transduction, especially in bladders exhibiting DO.

SECRETION OF TRANSMITTERS AND ACTIVATOR PATHWAYS

The release of molecules by the urothelium that are ligands to the receptors described above implies that the UsU layer possesses considerable integrative activity, as well as influencing outputs of associated nerves and nearby muscular tissue. Several molecules and receptors have been particularly well-investigated including: ATP/purinergic/adenosine receptors; TRPV channels; and acetylcholine/muscarinic receptors.

ATP and Adenosine Pathways

A key observation about the sensory role of the urothelium was the finding that physical stress caused a release of ATP, with the involvement of epithelial Na^+ channels.[36] The expression of $P2X_3$ receptors on UsU nerve fibers[32] permitted a sensory transduction to be proposed whereby bladder filling stretched the urothelium, causing basolateral ATP release and subsequent activation of bladder wall afferents. The functional syncytium of suburothelial interstitial cells, also responsive to exogenous ATP, would permit greater integration of the signaling mechanism across larger regions of the bladder wall.

The importance of this as a sensory pathway is also suggested by the fact that urothelial ATP release was augmented in tissue from patients with NDO, and this augmentation was in turn reduced after successful treatment with botulinum toxin.[37] Stretch-induced urothelial ATP release from human biopsies is also associated with increasing age,[38] itself associated with a greater prevalence of idiopathic DO. Augmented ATP release has also been demonstrated in tissue from bladders with bladder pain syndrome, or equivalent animal models and suggests that this is a fundamental sensory mechanism that when augmented leads to several lower urinary tract disorders. Furthermore, ATP can lower the threshold for generating ionic currents through $TRPV_1$ receptors (below), again suggesting considerable integration of activity to increase the gain of the sensory system.

A relatively unexplored aspect of the UsU purinergic signaling system is the role of adenosine. The bladder wall expresses ectoATPases that breakdown extracellular ATP to AMP and adenosine. The urothelium expresses adenosine receptors,[22] thus offering a further route to regulate this sensory pathway.

Transient Receptor Potential (TRP) Pathways

There are many TRP channels, but the best characterized in the bladder is $TRPV_1$ which has been described not only in bladder afferents, but also in the urothelium, suburothelial ICs, and smooth muscle cells.[13,39] The ion-channel protein is activated by moderate heat and protons, as well as agents such as capsaicin, analogues such as resiniferatoxin and lipid metabolites such as anandamide. The channel may have role in normal bladder function as $TRPV_1$-null mice exhibit more low-amplitude, non-voiding bladder contractions suggesting a role in urine storage, as well as reducing stretch-induced ATP release and bladder afferent discharge.[18]

$TRPV_1$ channels have also been implicated in bladder disorders, such as NDO and inflammation-induced models of cystitis,[30,40] when there is a greater expression of $TRPV_1$ receptors on nerves and urothelium. Furthermore, they could be involved in evoking responses during bladder filling when bladder wall blood flow is reduced,[12] leading to local hypoxia and acidosis, especially in hypertrophied bladders. Intravesical instillation of vanilloids, such as capsaicin or resiniferatoxin, improves urodynamic parameters in patients with NDO and reduces pain in patients with hypersensitivity disorders. It has been presumed that these agents target bladder nerves, but they could also influence urothelial cells by desensitizing their receptors or depleting their stores of transmitters.

Less is known about the role of other TRPs in bladder function or disease. $TRPV_4$ is a non-selective cation channel activated by heat, shear stress, changes in osmolarity and lipid ligands and is expressed within the uroepithelium.[41] $TRPV_4$-null mice show impaired voiding and in the rat, intravesical instillation of a $TRPV_4$ agonist triggers a voiding reflex which could regulate the late phase of micturition.[41]

Some TRP channels are cold-sensing ($TRPM_8$ and $TRPA_1$), so that they are of interest in the light of the ice-water test that may evoke involuntary detrusor contractions in patients with

chronic spinal cord lesions or following bladder outlet obstruction.[42,43] Intravesical instillation of menthol augments the bladder cooling reflex and suggests that $TRPM_8$, expressed in both nerves and the urothelium, may be involved.[44] $TRPA_1$ receptors are also expressed in C-fiber afferents and urothelium and agonists induce detrusor overactivity.[45]

Acetylcholine Receptor Pathways

Acetylcholine, like ATP, can be released from the urothelium when stretched[46] indicating an additional sensory mechanism that can augment acetylcholine released from local afferents. Furthermore, pelvic nerve electrical stimulation, or reflex activation of the autonomic nervous system by spinal cord injury elicits changes to urothelial permeability,[47] possibly through a cholinergic mechanism. The urothelium expresses all muscarinic receptor subtypes as well as nicotinic receptors.[19,48] This again suggests the possibility of localized integration as exogenous muscarinic and nicotinic agonists elicit an increase of intracellular Ca^{2+} concentration and release of NO and ATP from cultured urothelial cells.[49]

The mechanism and role for acetylcholine released through these routes however, remains unclear. Whether agents such as botulinum toxin also affect cholinergic routes in the UsU as they affect purinergic pathways remains to be identified.

LOCAL INTERACTION WITH SMOOTH MUSCLES

Much of the above discussion has concentrated on the role of the UsU in regulating afferent nerve activity. It is hypothesized therefore that the UsU acts as a receptor system: transducing and integrating sensory signals, whilst the nervous system encodes the information for relay to the central nervous system. However, it is also apparent that the UsU can also influence directly the underlying tissue of the urinary tract wall, in particular the smooth muscle layers. This offers the possibility that the UsU can also regulate urinary tract function independently of nervous reflexes that leave the urinary tract. Whether this local control is mediated by local nervous reflexes, by propagation via other excitable cells, such as the interstitial network of the suburothelium and detrusor layer, or via the physical diffusion of chemicals between the UsU and smooth muscle needs to be identified. However, there is evidence that the interstitial cell network and chemical diffusion are both involved.

An original observation was that the urothelium exerted a negative inotropic effect on detrusor contractility through a diffusible factor, because the effect persisted even when the mucosa was dissected away from the detrusor muscle layer but kept in close proximity.[50] The factor remains to be identified but can be released selectively by muscarinic receptor activation, as contractions evoked by raised extracellular [K] or neurokinin A were unaffected by removal of the mucosa.[51] By contrast, the presence of an intact mucosa seems to increase the incidence of spontaneous activity in isolated detrusor preparations unstimulated by nerve activation or the action of exogenous agonists. Furthermore, spontaneous activity evoked by agents such as UTP or ADP was especially prominent in mucosa-intact preparations.[52] The latter observation is of significance because UTP and ADP are ineffective in generating contractile responses in pure detrusor muscle and suggests an effect mediated by an intact mucosa. In fact, these agents are as effective as ATP in generating excitatory responses from suburothelial interstitial cells (ICs).[52] It may be hypothesized that the UsU can exert a dual effect on detrusor muscle, a negative inotropic influence mediated by a diffusible agent and a stimulatory effect mediated by ICs that requires physical contact between the two layers. This hypothesis could explain observations such as: an increase of spontaneous activity in isolated whole bladders that have an increased population of ICs (e.g., in spinal-cord injured adult rats or neonatal rats); the increased spontaneous activity is greatly attenuated by gap junction blockers; and

attenuation of increased spontaneous activity by agents such as glivec that target c-kit receptors present on ICs, but not on detrusor muscle cells.[34]

Interaction between the UsU and detrusor layers may also be observed with imaging of membrane potential and intracellular Ca^{2+} waves in isolated sheets of bladder or segments of the bladder wall.[16,17] Stimulation of the UsU layer with mechanical stimuli or very low concentrations of muscarinic agonists initiates propagating waves that spread across this layer. Propagation into the detrusor layer is slow and relatively ineffective in normal bladders but is more effective in bladders that exhibit enhanced spontaneous activity and increased IC numbers. Thus, the functional interaction between UsU and the detrusor layer may be especially important in generating large, spontaneous bladder contractions reminiscent of those in patients with DO.

REGIONAL DIFFERENCES IN UROTHELIAL FUNCTION

Most studies on urinary tract epithelium have concentrated on that from the dome of the bladder. That from the trigone appears to be comparable and similar modulatory effects over smooth muscle spontaneous activity by different purines are observed.[53] In the urethra there is a change from the transitional epithelial cells to a stratified or columnar epithelium that lack many of the characteristic markers of the bladder urothelium.[54] Furthermore, the distribution and immunoreactive labels in nerves and ICs are different in the bladder proper, bladder neck and urethra suggesting different mechanisms that regulate the integrative functions of these tissues,[55] but there is as yet to real understanding of the significance of such differences. One important reason for more work in understanding the morphology and function of urethral urothelium is to generate effective artificial urethral implants,[56] that ideally would have a lining of differentiated urethral cells. The morphological and functional characteristics of upper tract urothelium are also less-well understood compared to the bladder dome. In this instance the desire would be to understand how dilatation of the ureter, as when upper tract intraluminal pressures are raised, will affect urothelial function. Some studies demonstrate that many of functional phenomena, such as urothelial control over smooth muscle contractility and release of ATP upon physical stress are present in the upper urinary tract.[57,58]

CONCLUSIONS AND RECOMMENDATIONS

The urothelium is recognized to be more than a simple barrier separating the luminal contents from the deeper tissues of the urinary tract. As a functional unit it may be more appropriate to consider the urothelium and suburothelium as a functional unit which responds to external stresses by the release of modulator agents that regulate the activity not just of nearby afferent nerves but also underlying smooth muscle. If intelligence is considered to be a reflection of a biological system which alters its output in response to changing surroundings, with the object of allowing the organism to adjust to this altered environment, then the urothelium, like any sensory system may be considered intelligence. It does not presuppose its ability to plan ahead but merely to effectively respond to stimuli. The interest in this approach is to understand how the urothelium can respond to more abnormal stimuli and how derangements to this complex system may contribute to urinary tract disorders. A number of particular research goals can be proposed that may have more immediate impact.

- To understand the cellular mechanisms whereby the urothelium secretes activators in response to physical and chemical stresses.
- To understand the significance of different membrane receptors and ion channels in transducing the effects of activators.

- To understand the cellular and tissue actions of agents such as botulinum toxin and antimuscarinics that modulate effectively lower urinary tract symptoms.
- To understand the interaction between different cell types (urothelium, ICs, nerve fibers) in the urothelium/suburothelium to enable the integrated function of this structure to be better understood.
- To characterize immunoreactive labels in the urothelium/suburothelium of different regions of the urinary tract to enable artificial implants to be generated more effectively.

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