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1 **The role of the mucosa in normal and abnormal bladder function**

2

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22

1 **Abstract**

2 The internal face of the detrusor smooth muscle wall of the urinary bladder is covered by a
3 mucosa, separating muscle from the hostile environment of urine. However, the mucosa is
4 more than a very low permeability structure and offers a sensory structure that monitors the
5 extent of bladder filling and composition of the urine. The mucosa may be considered as a
6 single functional structure and comprises a tight epithelial layer under which is a basement
7 membrane and lamina propria. The latter region itself is a complex of afferent nerves, blood
8 vessels, interstitial cells and in some species including humans a *muscularis mucosae*. Stress
9 on the bladder wall through physical or chemical stressors elicits release of chemicals, such
10 as ATP, acetylcholine, prostaglandins and nitric oxide, that modulate the activity of either
11 afferent nerves or the muscular components of the bladder wall. The release and responses
12 are graded so that the mucosa forms a dynamic sensory structure and there is evidence that
13 the gain of this system is increased in pathologies such as overactive bladder and bladder pain
14 syndrome. This system therefore potentially provides a number of drug targets against these
15 conditions, once a number of fundamental questions are answered. These include: how is
16 mediator release regulated; what are the intermediate roles of interstitial cells that surround
17 afferent nerves and blood vessels as; what is the mode of communication between urothelium
18 and muscle – by diffusion of mediators or by cell-to-cell communication?

19

1 **Introduction**

2 The urinary bladder has two functions: to store urine, up to 500 ml in the normal adult, and
3 to completely void its content when expeditious. Storage is associated with very little increase
4 of intravesical pressure and low bladder wall tension; whilst voiding occurs with a sustained
5 rise of pressure, sufficient to overcome outflow resistance, due to contraction of detrusor
6 smooth muscle. This two-state system is controlled by the central nervous system but
7 modulated by interaction between different cell types in the layers of the bladder wall. In
8 pathological conditions such as overactive bladder this on-off process may be disrupted by
9 uncontrolled activity that could elicit unpleasant sensations of urinary urgency or pain and
10 also contractions that may be powerful enough to cause involuntary loss of urine. It is
11 therefore important to understand how storage and voiding modalities of the bladder are
12 controlled to provide therapies that minimize these pathologies.

13

14 **Structure of the bladder wall**

15 The smooth muscle (detrusor) of the bladder wall is protected by an external serosa and on
16 the vesical face overlain by a mucosa that itself consists of a tight transitional epithelium
17 (urothelium), basement membrane and lamina propria (LP; Figure 1). The urothelium itself
18 is covered by a mucopolysaccharide glycocalyx that offers protection for the urothelium from
19 the hostile medium of urine. The urothelium is made up of three layers: a basal cell layer
20 attached to a basement membrane, an intermediate layer and a superficial or apical layer
21 composed of large hexagonal cells known as the “umbrella cells”. An essential function of the
22 urothelium is to offer an effective barrier between urine and underlying tissues, achieved by
23 tight junctions between umbrella cells, severely limiting solute and water movement across
24 the barrier [1,2]. Damage to the urothelium, evident on exposure to noxious agents or
25 associated with pathologies such as spinal cord injury [3], are accompanied by irritative lower
26 urinary tract symptoms. However, the urothelium has transport functions as evidenced by
27 the development of a finite membrane potential, solute and water movement and the presence
28 of aquaporins, urea transporters, ion channels (eg ENaC) and mineralocorticoid receptors [4-
29 6]. Moreover, the different composition of urine sampled from the bladder lumen and renal
30 pelvis is consistent with post-renal urinary tract salt and water exchange [7].

31

32 The LP that separates the urothelium from the detrusor layer is composed of an extracellular
33 matrix containing interstitial cells, fibroblasts, adipocytes, afferent and efferent nerve

1 endings, blood vessels and, in some species including humans, a more ill-defined muscular
2 layer – the *muscularis mucosae*. The functional interaction of these different cells and how
3 they communicate with the urothelium and detrusor layers is crucial to understand how this
4 layer has essential roles to sense bladder filling as well as exert control over detrusor
5 contractile activity.

6

7 The detrusor layer itself constitutes the mass of the bladder wall and consists of smooth
8 muscle bundles separated by connective tissue and interstitial cells. Parasympathetic
9 postganglionic nerves provide the excitatory input.

10

11 **The release of mediators and sensations arising from the bladder wall**

12 Physical or chemical stressors applied to the bladder itself, isolated sections of the bladder
13 wall, strips of mucosa dissected free of detrusor, or isolated urothelial cells evoke release of
14 several small molecules including: ATP, acetylcholine (ACh), prostaglandins or nitric oxide
15 [4,8-10]. The fact that all these preparations release these compounds assumes that the
16 source is the urothelium, although the contribution from other cells has not been
17 systematically evaluated. Physical stressors include longitudinal strain or tension; the rate of
18 change of these variables; transmural pressure changes; osmotic swelling; or shear stresses
19 to cells; chemical or cellular stressors include extracellular acidosis [11], noxious compounds
20 such as doxorubicin [12] and inflammatory conditions [13]. Primary sensory neurons also
21 release several neuropeptides such as CGRP and substance-P that may mediate local
22 inflammatory responses [14]. However, this is beyond the scope of this article and will not be
23 considered further.

24

25 The pathways for release and their signaling roles have been mostly investigated for ATP and
26 ACh. Overall, their action will be largely autocrine or paracrine as extracellular ATPases
27 (eNTPDases) and cholinesterases will limit their half-time. In principle, these mediators can
28 either affect local afferent nerves to convey sensations of filling to the central nervous system,
29 regulate local blood flow by affecting vessel resistance, or modulate detrusor contractile
30 function. Mucosa afferents express a number of receptors that include: P2X and P2Y
31 purinergic families; TRP –V, -M, and -A families; as well as PAC1 (pituitary adenylate cyclase-
32 activating polypeptide (PACAP))-selective receptors. P2X_{2/3} receptors are understood to
33 mediate the excitatory effects of locally released ATP. P2X₃ knock-out mice showed a

1 diminished micturition reflex whereby greater stretch of the bladder wall was required to
2 elicit a given degree of afferent signaling. However, activity was not abolished [15]
3 completely, which may suggest additional roles for CGRP, TRPV1 and PACAP receptors [15,16]
4 although their functional ligands are yet to be fully elucidated. The lifetime and extent of the
5 effect for ATP released from the urothelium will be limited due to the presence of ectoATPases
6 (E-NTPDase3) on the basal surfaces of urothelial cells [17]. This would be anticipated for a
7 dynamic sensory modulator but also raises the question of the roles of ADP, AMP and
8 adenosine in also modulating signalling responses.

9

10 The quantity of ATP released during imposition of stressors alters with the age and the
11 pathology of the parent tissue, suggesting an underlying cause of pathological lower urinary
12 tract sensations. Thus, ATP release is raised in bladder wall tissue from: old animals and
13 humans compared to younger counterparts [18], tissue biopsies of patients with overactive
14 bladders [19] and cultured urothelial cells of patients with painful bladder syndrome/
15 interstitial cystitis [20].

16

17 Urothelial cells also have the capacity to synthesise and exhibit stretch-activated release of
18 acetylcholine (ACh) [21,22]. There is inconsistent evidence as to whether release is enhanced
19 [22] or diminished [18] with age, but several other agents including the cytotoxic drug,
20 doxorubicin, and lipopolysaccharide reduced ACh release stimulated by cell stretch [23,24].
21 Comparison of ACh and ATP release reveals some interesting differences: stretch-activated
22 release of ACh is much greater than ATP per unit mass of tissue; the magnitude of stresses
23 required to release ACh is much smaller, as is the dynamic range of stresses that release ACh
24 [25]. Moreover, the release of ATP is modulated by muscarinic receptor activation
25 independently of physical stressors; muscarinic receptor agonists increase ATP release whilst
26 antagonists, particularly to M2 but not M3 receptors, inhibit it. Thus, it has been suggested
27 that ACh release is the first step in a sensory transducer system that itself regulates the further
28 release of ATP with consequent downstream effects [25,26]. Two observations follow which
29 question perceived wisdom about the use of antimuscarinic agents to manage overactive
30 bladder (OAB) symptoms: firstly their site of action may not solely be on detrusor M3
31 receptors at the efferent nerve/smooth muscle junction, as assumed, but also on the mucosa;
32 secondly drugs with a mixed M2/M3 profile may be more effective than selective M3 receptor
33 antagonists. Certainly, antimuscarinic agents increase cystometric capacity in patients with

1 OAB, which can be explained by their action on storage rather than solely on voiding
2 mechanisms.

3 Stretch-induced prostaglandin (PGE2) from the mucosa has also been measured and may
4 exert direct effects on detrusor contractile function or, via an EP1 receptor, enhance local ATP
5 release to increase afferent activation [27]. Moreover, a positive feedback process is
6 suggested by the ability of ATP to augment PGE2 release [28]. Urothelial cells contain the
7 enzymatic machinery to synthesise nitric oxide (NO) [29] and there is evidence that it
8 suppresses afferent nerve activity [30]. Increase of NO production, as occurs for example in a
9 cat model of bladder pain syndrome, is also associated with a loss of barrier function [31] that
10 in turn will augment afferent activity by allowing noxious components of urine more direct
11 access to suburothelial structures.

12

13 **Pathways for mediator release**

14 Significant effort has been expended to identify the cellular routes for mediator release and
15 suggests the involvement of several pathways. ATP release has been identified via hemi-
16 channels of connexin or pannexin proteins, or even through vesicles [32,33]. However, these
17 conclusions are generally based on inhibitors of hemichannel proteins or vesicular transport
18 and there is debate about the specificity of these agents. In addition, release is enhanced by
19 an increase of intracellular $[Ca^{2+}]$ that may underlie the augmentation of release by TRPV1
20 channel activation and extracellular acidosis [11] and is attenuated by extracellular Ca^{2+} that
21 is consistent with involvement of connexin hemichannels. However, the mode of action of P2Y
22 receptor agonists that increase release and adenosine (A1) receptor agonists that reduce
23 release has not been clarified. Of interest is that ATP release is reduced from the tissue of
24 patients who have received botulinum toxin type-A (BnTx-A) injections to reduce overactive
25 bladder symptoms [34]. Moreover, direct application of BnTx-A attenuates stress-dependent
26 ATP release and the binding targets for BnTx-A has been identified on urothelial cells [35].
27 This also raises the question whether BnTx-A as an agent to reduce OAB contractions, does so
28 by reducing transmitter release from efferent nerves, as it has assumed to work, or by
29 dampening the sensory responses to bladder filling, as suggested by these observations.
30 Release of ACh is via different routes: it is unaffected by reduction of vesicular formation,
31 blockade of hemichannels or botulinum toxin. The only effective modulator identified was an
32 inhibitor of CFTR channels, which reduced release by about 50% [25].

33

1 **The mucosa and contractile functions of the bladder**

2 Contractile function in the bladder exists in two modalities: phasic contractions initiated by
3 transmitters released from efferent parasympathetic fibres that evoke large contractions to
4 void urine; spontaneous contractions that are not primarily initiated by motor nerves. The
5 origin and function of the latter remain unclear but they have several properties that
6 distinguish them from nerve-mediated contractions and imply they have a physiological and
7 pathological role:

- 8 • they are unaffected by neurotoxins, but are Ca^{2+} -sensitive;
- 9 • they are greatly augmented by the mucosa overlaying the detrusor;
- 10 • they can manifest as micromotions – localised, non-propagating contractions on the
11 bladder wall – that are mirrored as small intravesical pressure fluctuations;
- 12 • they are enhanced in pathologies that manifest as overactive bladders.

13 Their normal function may be to maintain a significant tone in the bladder wall during filling
14 to ensure it maintains a roughly spherical shape but not enough to reduce the natural
15 compliance of the bladder in this phase. Several, not mutually exclusive, theories have been
16 proposed that might also contribute to the large spontaneous contractions associated with a
17 subtype of OAB called detrusor overactivity:

- 18 • a myogenic theory, due to intrinsic spontaneous activity of detrusor myocytes
- 19 • a neurogenic hypothesis whereby spontaneous nervous activity initiated in the central or
20 peripheral nervous system drives contractions.
- 21 • spontaneous release of neurotransmitters
- 22 • a urotheliogenic theory whereby the mucosa drives spontaneous detrusor contractions.
- 23 • the mucosa itself has significant, independent contractile function

24 Of these the urotheliogenic theory and an independently contractile mucosa are the most
25 consistent with experimental evidence, although a neurogenic origin is likely in a subset of
26 patients. However, the questions arise about the nature of the interaction between mucosa
27 and detrusor, as well as how the mucosa itself generates significant contractile activity.

28

29

30 **The contractile properties of the mucosa**

31 The mucosa, in most species, may be readily separated from the detrusor layer by blunt
32 dissection and *in vitro* generates spontaneous contractions, as well as tonic responses to
33 electrical field stimulation and cholinergic agonists [36-38]. Several origins, not mutually

1 exclusive, have been proposed including: interstitial cells with a contractile phenotype
2 (myofibroblasts); pericytes around blood vessels or the *muscularis mucosae*. It is evident that
3 the pharmacological profile of mucosa spontaneous contractions is different from that of the
4 detrusor layer, for example capsaicin augments detrusor activity whilst suppressing mucosal
5 activity [37]. This would argue against the possibility that in dissecting the preparations there
6 is residual contamination of detrusor smooth muscle. This phenomenon is of significance as
7 the mucosa thickens in several conditions associated with overactive bladder [39] and this
8 activity may be especially significant in these pathologies. There is also evidence that such
9 contractility activity may be influenced by mucosal ATP release. Under resting conditions
10 mucosal ATP release is cyclical with a periodicity of about 10 minutes and this is reflected in
11 a similar periodicity of the integral of spontaneous contractility but with a delay of a few
12 minutes [37]. It might be suggested that ATP release from urothelium diffuses within the
13 mucosa to modulate contractility activity. It does not identify the cellular targets except that
14 they probably have a receptor phenotype to ATP or its metabolites. The contractile behavior
15 of the mucosal layer under various pathological conditions has not yet been investigated:
16 however, there is a change in the characteristics of spontaneous contractions of this layer with
17 ageing [40].

18 19 **Functional interactions between the mucosa, detrusor and associated vasculature**

20 There is also convincing evidence of mucosa-detrusor interaction in generating spontaneous
21 activity – the urotheliogenic theory. The most straightforward observation is that an *in vitro*
22 bladder wall preparation of detrusor and attached mucosa generates substantial spontaneous
23 contractions and these are dramatically reduced when the mucosa is removed [41,42]. This
24 is complicated by the fact that an intact mucosa overlaying detrusor muscle also exerts a tonic
25 negative inotropic effect [43]. This complex interaction can be by diffusion of mediators
26 between the two layers or from a cellular interaction. The observation that simply placing a
27 mucosa layer over previously denuded detrusor restores some contractile activity supports a
28 role for a diffusive interaction. However, if this was the sole mode of interaction it would be
29 expected that the pharmacological profile of spontaneous contractions would be solely
30 determined by the phenotype of detrusor and this is not the case. Apart from the opposite
31 actions of capsaicin on mucosa and detrusor activity (above), the same is true of P2Y receptor
32 agonists such as ADP, UDP and UDP. These agonists generally suppress or are at least neutral
33 on detrusor function but they increase mucosa activity [37]. Moreover they greatly enhance

1 spontaneous contractions of bladder wall preparations when mucosa and detrusor or
2 attached [44]. Optical imaging experiments that map intracellular $[Ca^{2+}]$ and membrane
3 potential propagated waves across the bladder wall reveal not only is an intact mucosa
4 required for such activity but it is augmented by the above P2Y agonists. Moreover, these
5 experiments also show that such propagated activity is initiated in the sub-urothelium of the
6 mucosa and actually propagates to the detrusor – again augmented by P2Y agonists [44].
7 These mapping experiments also suggest that local diffusion of agents is insufficient alone to
8 explain mucosa-detrusor interaction as the propagation velocity of such waves is too rapid
9 and moreover too extensive over the bladder wall and suggests cellular interaction is also
10 likely.

11
12 One potential cellular mediator of mucosa-detrusor interaction is the dense network of
13 interstitial cells in the suburothelium – a network substantially increased in pathologies
14 associated with enhanced spontaneous activity such as spinal cord injury [38]. These cells
15 tend to have their cell bodies in the suburothelium nearest to the urothelium, but projections
16 run towards the detrusor layer where much of the immunoreactivity to the gap junction
17 protein connexin-43 is found. These cells also have the attributes of forming an electrical
18 functional syncytium: they are connected by connexin-43 gap junctions; and also generate
19 spontaneous depolarisations due to activation of a large density Ca^{2+} activated Cl^- current, $I_{Cl,Ca}$
20 [44]. Moreover, $I_{Cl,Ca}$ is enhanced by interventions that accelerate Ca^{2+} wave propagation both
21 across the bladder wall and between mucosa and detrusor, namely P2Y agonists and local
22 reduction of pH. It may be proposed therefore that a function of suburothelial interstitial cells
23 is to provide a cellular communication between the mucosa and detrusor that will augment
24 contractile activity of the latter. The cells are ideally located below the urothelium to respond
25 to mediators released from this layer, as well as their metabolites and their excitable nature
26 means they can effectively propagate responses.

27
28 Moreover, interstitial cells might be involved in the local control of bladder tissue perfusion
29 as a subpopulation of these cells is associated with the microvessels in the LP [45]. It is
30 postulated that adjacent perivascular interstitial cells have a role in generating spontaneous
31 vasoconstrictions of venules, which might be beneficial in maintaining blood flow during the
32 filling phase of the micturition cycle [46]. Inadequate perfusion of the bladder and the
33 resultant ischemia can readily affect the urothelium and suburothelial cells, leading to altered

1 urothelial signaling/barrier function and detrusor smooth muscle overactivity [47]. The
2 relationship between suburothelial microvessels, interstitial cells and the urothelium needs
3 to be further studied.

4

5 **Conclusions.**

6 The mucosa lining the inner surface of the detrusor smooth muscle layer of the bladder has
7 essential roles other than providing an essential barrier function to protect detrusor from the
8 unphysiological environment of urine. The urothelium acts as a sensor to bladder filling,
9 although it has to be determined what is the actual physical stressor: wall stress, transmural
10 pressure, acidosis from ischaemia, etc. The urothelium responds by releasing chemical
11 mediators that eventually activate afferent nerves and/or locally influence muscle function.
12 The role of intermediate cells, such as interstitial cells, remains to be determined. However,
13 their electrically excitable nature gives them the capacity to modulate the function of nerves,
14 detrusor muscle and even local blood vessels. Overall, the mucosa offers a dynamic sensory
15 structure that allows the bladder to respond directly to the volume and composition of urine
16 and thus optimise bladder contractile function. A major unanswered question is whether
17 pathological changes to bladder function, such as overactive bladder and bladder pain
18 syndrome, are determined by alterations to mucosa behaviour.

19

20

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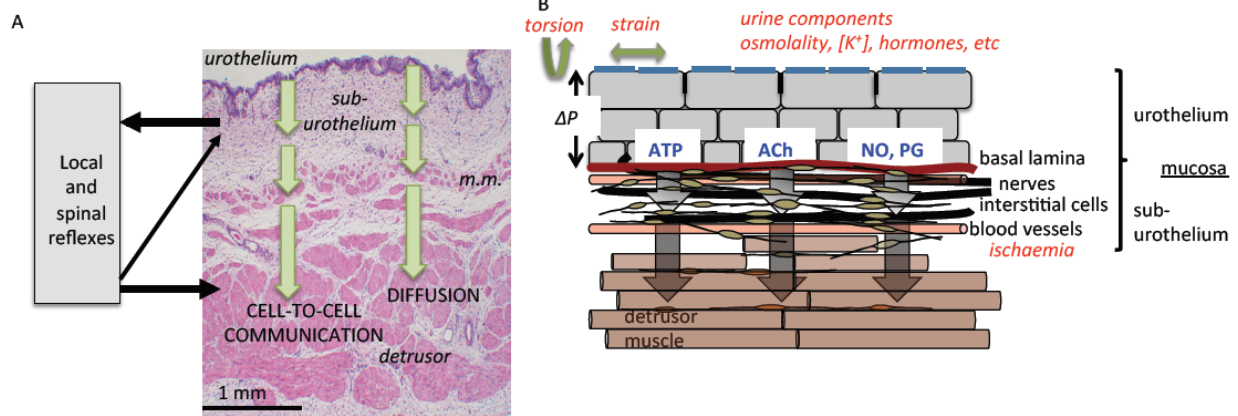
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 2 Figure 1. Section of the sheep bladder wall. The section shows the urothelium, sub-
 3 urothelium and detrusor smooth muscle layers. The suburothelium is a complex structure of
 4 blood vessels, interstitial cells, afferent nerves and in this species a *muscularis mucosae* (m.m.).
 5 External physical and chemical agents can cause release of mediators (arrows) from the
 6 urothelium that could influence suburothelium structures to elicit nervous responses,
 7 changes to blood vessel tone and contractile responses of detrusor and possibly *muscularis*
 8 *mucosae*. Contractile responses could be mediated either by diffusion of mediators and/or by
 9 cell-to-cell communication.

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