

# The human bladder urothelium: new concepts and future perspectives

**A. Giannantoni<sup>1</sup>,  
E. Costantini<sup>1</sup>,  
S.M. Di Stasi<sup>2</sup>,  
A. Zucchi<sup>1</sup>,  
F. Santaniello<sup>1</sup>,  
F. Palladino<sup>1</sup>, and  
M. Porena<sup>1</sup>**

<sup>1</sup>Department of Urology,  
University of Perugia,  
Perugia, <sup>2</sup>Department of  
Urology, "Tor Vergata"  
University, Roma, Italy

*ABSTRACT. The bladder urothelium has long been considered as having a purely passive role by providing a barrier protecting the underlying smooth muscle from irritative substances stored in the urine. Recent reports support the idea that urothelial cells represent more than a protective layer. They exhibit a number of dynamic sensory properties that allow them to respond to either chemical and physical environments. These properties include the expression of nicotinic, muscarinic, tachykinin and adrenergic receptors, as well as vanilloid receptors. Furthermore, urothelial cells exhibit responsiveness to some neurotransmitters (ATP, NKA, Substance P) released from afferent nerves, and they are able to release chemical mediators such as ATP and NO, which regulate the activity of underlying nerves. Moreover, the concept that the bladder urothelium can participate in non-barrier functions is supported by the recent observation that in some species, this tissue produces and releases proteolytic enzymes.*

Urodinamica 15: 45-49, 2005

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## INTRODUCTION

The urothelium of the bladder has long been considered a protective layer acting as a barrier between the underlying smooth muscle of the bladder (detrusor) and urine within the bladder wall. However, in recent years this layer has been shown to have more than a passive role in bladder function and it is now understood that it plays an active role in its regulation (1, 2). Recent evidences suggest that bladder urothelium exhibits neuron-like properties, expresses various receptors and ion channels and releases neurotransmitters in response to various stimuli.

These evidences allow a more comprehensive understanding of the pathophysiology of different bladder disorders as well as to establish better pharmacologic modulation (1, 2).

### Correspondence

D.ssa Antonella Giannantoni,  
Department of Urology,  
University of Perugia,  
Policlinico Monteluce,  
Via Brunamonti 51,  
06100 Perugia, Italy.

### E-mail

uropg@unipg.it

## UROTHELIUM AND BLADDER INNERVATION

The urothelium shows 3 distinct layers: the basal layer, with cells of small dimensions, the intermediate layer with moderate sized cells, and the superficial layer with particular "umbrella" cells. The superficial layer contains tight junctions that prevent the passage of substances and ions from the urine to the bloodstream. Umbrella cells are covered by "uroplakins", which are crystalline proteins organized into hexagonal plaques, probably to contribute to the permeability barrier of the urothelium (3).

The urothelium and underlying detrusor smooth muscle receive rich innervation from various population of nerves, which varies throughout the lower urinary tract. The innervation of the bladder dome is predominantly parasympathetic in contrast to the bladder trigone region, which receives a more significant sympathetic innervation. There is also a non-adrenergic non-cholinergic control of smooth muscle that may involve nitric oxide and peptides, such as substance P, calcitonin gene-related peptide (CGRP) and vasoactive intestinal polypeptide (4).

The sensation of bladder fullness is the primary step in the initiation of the micturition reflex. Afferent innervation of the bladder, producing sensations of fullness which lead to detrusor contraction, is conveyed by the pelvic and the hypogastric nerves, which contain myelinated (A $\delta$ ) and unmyelinated (C) axons. The C-axons generally have endings in the suburothelial layer of the bladder wall immediately below the basal lamina, but actually we know that they also penetrate into the urothelium (5). A $\delta$ -fibers and some C-afferent fibers are mechano-sensitive and respond to bladder filling by providing information about bladder wall tension and volume, possibly via stretch of the mucosal layer. A subset of these fibers is silent and does not respond to normal bladder filling, but it can respond to more noxious stimuli (6).

Removal of the urothelium is able to change the responses of the detrusor in both animal and human bladder and can significantly increase the contractile response of the detrusor to carbachol and field stimulation. This inhibitory effect of the urothelium appears to be due to the release of a diffusible factor released from the urothelial cells after stimulation of muscarinic and histamine receptors. Stimulation of  $\beta$ -adrenoceptors in the bladder induces detrusor relaxation. Although this effect clearly involves a direct action on smooth muscle, it may also be mediated by release of "epithelium-derived relaxing factors" (which include Nitric Oxide) from the urothelium. There are several possible sources of Nitric Oxide (NO) production, including endothelial cells, nerves, smooth muscle, and urothelium, but recent studies demonstrated that major sites of NO release were the urothelium and afferent nerves (7). Other authors retain that the inhibiting factor may be distinct from NO and that it persists in the presence of  $\beta$ -adrenoceptor blockade or cyclooxygenase inhibition (8). Although this urothelium-derived diffusible factor remains elusive, it appears to be a key player not only in the cholinergic control of bladder function, but also in regard to the interaction and coordination of cholinergic, adrenergic and possibly other systems controlling muscle tone in the bladder trigone of the pig (9). Muscarinic receptors are abundant in the detrusor muscle and cholinergic innervation via muscarinic receptors has been considered for long time as having a primary role in detrusor contractility.

Recently, it has been observed that muscarinic receptors are also located into urothelial cells and, in some experimental models, muscarinic receptor density is higher in the urothelium than in the detrusor muscle (2). It has been observed that basal acetylcholine release from the urothelia of patients aged over 65 years is higher than that obtained in younger patients (2).

## BLADDER UROTHELIUM AND PURINERGIC RECEPTORS

On the basis of recent experimental observations, a chemically based mediation of afferent activation caused by mucosal stretch has been proposed. The urothelium releases ATP at the basolateral surface when the hydraulic gradient across the bladder wall is altered, which ultimately mediates afferent excitation (2, 10). Muscarinic receptors can regulate ATP release. The ATP release observed in the urothelium of animals affected by interstitial cystitis is a calcium-dependent process which includes at least 2 intracellular calcium component (IP<sub>3</sub> and ryanodine-sensitive channels) (2). Probably, the upregulated release of ATP in interstitial cystitis is mediated by an influx of extracellular calcium combined with the muscarinic receptors-mediated increase (2). Urothelial Na<sup>+</sup> transport via epithelial Na<sup>+</sup> channels is central to ATP release, as the process is modulated by amiloride, an inhibitor factor of these channels. ATP release has been shown to increase in urothelial cells cultured from patients with interstitial cystitis and probably underlies the enhanced sensation seen in this group (11).

Several receptors are believed to mediate afferent nerve fibers excitation by generating depolarizing responses. Several investigators have shown that, among the 7 subtypes of purinergic (P2X) receptors that have been identified, P2X<sub>3</sub> receptors are selectively expressed predominantly on small-diameter nociceptive sensory neurons in the dorsal root, trigeminal, and nodose ganglia (2, 12, 13). In human and rat bladders, P2X<sub>3</sub> receptors have been located adjacent to nerve fibers, in the suburothelial space and urothelium in particular. P2X<sub>3</sub> knockout mice have shown reduced voiding frequency and increased bladder volume probably due to a decreased sensation of bladder filling. Intravesical application of a P2X<sub>3</sub> receptor agonist into mouse bladder induces high-threshold (nociception-associated) and low-threshold (non nocicep-

tion-associated) fibers to discharge at a reduced threshold, whereas the administration in a P2X<sub>3</sub>-deficient mice has significantly reduced micturition frequencies and significantly increases bladder capacities compared with P2X<sub>3</sub> wild-type mice, consistent with decreased afferent sensitivity to filling (2).

Anesthetized P2X<sub>3</sub>-deficient mice also have micturition hyporeflexia, and they do not show detrusor contractions in response to saline infusion-induced distension. These evidences suggest that purinergic receptors may be potential targets for novel therapeutic intervention for detrusor overactivity.

## BLADDER UROTHELIUM AND VANILLOID RECEPTORS

Painful sensations induced by capsaicin, the pungent substance in hot peppers, are caused by stimulation of a vanilloid receptor, which is a transient proteic receptor potential channel (TRPV1) expressed by nociceptive primary afferent neurons (1, 2). TRPV1 participates in the detection of at least two additional noxious stimuli, acid (pH<6) and heat (>43°C). The urinary bladder is rich with capsaicin-sensitive afferent fibers that respond to bladder distension or to the presence of irritant chemicals, and in turn trigger reflex bladder activity. It has been demonstrated that TRPV1 is expressed not only by afferent nerves that form close contacts with bladder epithelial (urothelial) cells but also by the urothelial cells themselves (1, 2). Furthermore, it has been demonstrated that the exogenous application of vanilloids (capsaicin and resiniferatoxin) into the bladder increases intracellular Ca<sup>2+</sup> and evokes NO release in urothelial cells, and that these responses require TRPV1 (1).

The same authors observed that vanilloid-evoked release of ATP and NO was significantly decreased (72%) following mechanical removal of the urothelium, and that NO release was similarly diminished (91%) following

treatment of the tissue by protamine sulphate, which has been demonstrated to selectively disrupt the epithelium (1). Recently, presence and distribution of TRPV1 has been described also in the human bladder urothelium (14).

Recent studies have shown that high levels of Nerve Growth Factor (NGF), a neurotrophin produced by target tissues, can accelerate the expression of TRPV1, and that a state of NGF deprivation arises in bladder sensory neurons after intravesical administration of vanilloids (15). After the uptake by high-affinity tyrosine kinase receptors (TrkA) in sensory neurons, excess NGF is retrogradely transported to the cell nucleus, where NGF increases translation of TRPV1 through activation of a mitogen-activated protein kinase. The TRPV1 so synthesized is thereafter transported by the anterograde axonal flow to the peripheral sensory endings (14).

Recently, endogenous vanilloid-like receptor ligand, similar to capsaicin has been identified in experimental animals (15). Anandamide, which is devoid of a homovanillic ring, shows chemical properties very similar to capsaicin, and it has been observed to induce reflex detrusor activity (15). Intravesical administration of vanilloids causes a long-lasting suppression of sensory activity in type C primary afferent fibers, by means of a down-regulation of TRPV1. As an up-regulation of vanilloid receptors occurs in inflamed tissues or in the overactive detrusors, vanilloid agents have been used in the treatment of these pathologic conditions (16).

#### **BLADDER UROTHELIUM AND TACHYKININ RECEPTORS**

It is well known that bladder sensory afferent nerve fibers contain substance P, neurokinin (NK) A, and calcitonin-gene related peptide (CGRP), which interact with tachykinin NK-1 and NK-2 receptors and CGRP receptors, respectively (2, 13). NK-1 receptors were found over the endothelium of arterial blood vessels

within the detrusor muscle and lamina propria and over small vessels in the subepithelium. NK-2 receptors were seen over the detrusor muscle and CGRP receptors are usually located over the smooth muscle layer of arteries and venules. Thus, the general distribution of this kind of receptors is in agreement with the known actions of Substance P and CGRP as vasodilator agents, and of NKA in contracting detrusor muscle. Actually, there is evidence that also the urothelium can express tachykinin receptors (NK-2); NKA may initiate micturition by stimulation of NK-2 receptors in the urothelium (1, 2, 12).

#### **SUBUROTHELIAL MYOFIBROBLASTS: POSSIBLE INTERMEDIARIES IN THE SENSORY PROCESS?**

It has been recently demonstrated the presence of a suburothelial layer of particular cells resembling myofibroblasts, which differ from flat epithelial cells and detrusor cells (8). In the literature, the terms "myofibroblast" and "interstitial cell" are used to define the same group of cells. Myofibroblasts have a number of electron microscopic characteristics, such as fine cytoplasmic filaments associated with dense bodies, subsurface vacuoles and an interrupted basal lamina. In the human bladder, myofibroblasts stain for vimentin, and  $\alpha$ -smooth muscle actin, but not for desmin (7). Electron microscope studies have shown that these cells make close apposition with C-fiber nerve endings, a feature observed also in other tissues. Observations in similar cells from other tissues have shown that they may be modulated by a NO-cGMP system. When stimulated by ATP, these cells generate inward currents and large increases in intracellular calcium concentration. These morpho-functional evidences suggest that there is a network of functionally connected myofibroblasts immediately below the urothelium that may be modulated by other nerve fibers. This is probably the point-con-

nection between the urothelium and sub-urothelial nerves (17).

## CONCLUSIONS

The bladder urothelium has long been considered as having a purely passive role providing a barrier protecting the underlying smooth muscle from irritative substances stored in the urine. Recent observations support the idea that urothelial cells represent more than a protective layer, and that they exhibit specialized sensory and signalling properties that allow them to respond to either chemical and physical environments. Bladder urothelial cells seem to be able to establish reciprocal communication with neighbouring nerves in the bladder wall. The specific properties of urothelial cells include the expression of nicotinic, muscarinic, tachykinin and adrenergic receptors, as well as vanilloid receptors. Furthermore, they exhibit responsiveness to some neurotransmitters (ATP, NKA, Substance P) released from afferent nerves, and they are able to release chemical mediators such as ATP and NO, which regulate the activity of underlying nerves. Moreover, the concept that the bladder urothelium can participate in non-barriers functions is supported by the recent observation that, in some species, this tissue produces and releases proteolytic enzymes.

Further researches about the sensory role of urinary bladder epithelial cells will provide better understanding of some pathologic conditions and will allow us to correctly treat these diseases.

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