McCloskey, K. D., Vahabi, B., & Fry, C. (2017). Is electrolyte transfer across the urothelium important? *Neurourology and Urodynamics*, 36(4), 863-868. <https://doi.org/10.1002/nau.23085>

Peer reviewed version

License (if available):
Unspecified

Link to published version (if available):
[10.1002/nau.23085](https://doi.org/10.1002/nau.23085)

[Link to publication record in Explore Bristol Research](#)
PDF-document

This is the author accepted manuscript (AAM). The final published version (version of record) is available online via Wiley at <https://onlinelibrary.wiley.com/doi/full/10.1002/nau.23085> . Please refer to any applicable terms of use of the publisher.

University of Bristol - Explore Bristol Research

General rights

This document is made available in accordance with publisher policies. Please cite only the published version using the reference above. Full terms of use are available:
<http://www.bristol.ac.uk/red/research-policy/pure/user-guides/ebr-terms/>

Is electrolyte transfer across the urothelium important?

Karen D McCloskey*¹, Bahareh Vahabi² and Christopher H Fry²,

¹Centre for Cancer Research and Cell Biology, School of Medicine, Dentistry and Biomedical Sciences, Queen's University Belfast, BT9 7AE

² School of Physiology, Pharmacology and Neuroscience, University of Bristol, BS8 1TD.

Author for correspondence*

Karen D McCloskey PhD

Centre for Cancer Research and Cell Biology,
School of Medicine, Dentistry and Biomedical Sciences,
Queen's University Belfast,
Belfast, BT9 7AE
k.mccloskey@qub.ac.uk

Abstract

AIMS

This article summarizes discussion at the International Consultation on Incontinence Research Society (ICI-RS) 2015 meeting of urine modification in the urinary tract by the urothelium. It considers the literature and proposes pertinent questions that need to be addressed to understand this phenomenon within a physiological context.

METHODS

Following the ICI-RS meeting, publications in PubMed relating to urine modification in the renal pelvis, ureter, and bladder were reviewed.

RESULTS

Historically, the urothelium has been simply considered as a passive, impermeable barrier, preventing contact between urine and the underlying cells. In addition to the ability of the umbrella cells to modify the surface area of the urothelium during bladder filling, the urothelium may also be involved in modifying urine composition. Several lines of evidence support the hypothesis that electrolytes and water can be reabsorbed by the urothelium and that this may have physiological relevance. Firstly, urothelial cells express several types of aquaporins and ion channels; the membrane expression of which is modulated by the extracellular concentration of ions including Na⁺. Secondly, studies of urine composition in the renal pelvis and bladder demonstrate urine modification, indicating that water and/or electrolyte transport has occurred. Thirdly, hibernating mammals, with urothelial and bladder wall histology similar to non-hibernating mammals are known to produce and reabsorb urine daily, during long periods of hibernation.

CONCLUSIONS

The phenomenon of urine modification by the urothelium may be physiologically important during normal bladder filling. Research should be focused on investigating how this may change in conditions of urinary dysfunction.

INTRODUCTION

The urinary tract comprising renal pelvis, ureter, bladder, and urethra has traditionally been regarded as a conduit and storage system for urine before it is expelled from the body during voiding. The epithelial lining of the bladder, known as the urothelium, provides a high-resistance barrier between the urine and the cells/interstitium of underlying tissues, yet, there is substantial evidence that the urothelium represents a dynamic tissue with additional physiological functions.^{1, 2} The urothelium is now known to be important in the transduction of information on, for example, bladder fullness to the underlying lamina propria, mucosal neuronal plexus, and the underlying detrusor smooth muscle.³ The urothelium typically comprises large umbrella (surface) cells, up to 250 μm diameter, several layers of intermediate cells, and a layer of basal cells on a basement membrane. Much is known of the ultrastructural properties of the urothelium including the molecular identity and the physiological roles of structural and intercellular proteins, reviewed extensively elsewhere.^{1, 2, 4} The luminal umbrella cells face a unique challenge with their basolateral surfaces exposed to plasma which has a different composition to the urine which bathes the apical luminal surface. These cells have the crucial role of increasing the urothelial surface area during bladder filling through insertion of membrane from apical membrane endosomes followed by endocytosis after voiding. In addition, it is known that uroplakin proteins (UP) on umbrella cells are involved in the pathogenesis of urinary infections, for example, UP1a which is co-opted by uropathogenic bacteria as a receptor enabling cellular adhesion and subsequent colonization.²

The urothelium is increasingly recognized as having the potential to modify the composition of stored urine.^{5, 6} This is counter-intuitive to its role as a passive barrier where it is assumed to prevent contact between the urine and urinary tract cells, thereby preserving urine composition. This is the essential basis of clinical urinalysis where urine expelled from the bladder is considered as being representative of urine excreted by the kidney. Yet, it has been known for several decades that the bladder, renal pelvis,⁷ and ureters⁸ have the potential to modify urine via transport of Na^+ , K^+ , urea, water, and creatinine across the urothelium.⁹ Furthermore, the Na^+ -conserving hormone, aldosterone, increases Na^+ transport across the bladder wall¹⁰ and anti-diuretic hormone (ADH) induces net movement of water across the frog bladder wall.¹¹

The aim of the present article is to review what is known of urine modification in the urinary tract and to highlight areas of research needed to advance our understanding of this area within a clinically relevant context.

1 FUNCTIONAL EXPRESSION OF AQUAPORINS IN UROTHELIUM

Aquaporins (AQP) are integral membrane proteins forming pores, which transport water, glycerol, and some solutes.¹² AQPs are known to be involved in fluid homeostasis, water transport, glycerol, and fat transport in kidney epithelial cells, vascular endothelial cells, adipocytes, and epidermal cells. At least seven AQPs, AQP-1, 2, 3, 4, 6, 7, 8, are functionally expressed in the kidney for water transport and urine concentration.¹³ Their somewhat surprising expression in urothelial cells suggests that these cells may also be capable of water transport in the urinary tract, particularly the bladder. Gene expression of AQP-3, 4, 7, 9, and 11 has been demonstrated in fresh human urothelium, moreover, protein expression of AQP-3, 4, 7, and 9 was confirmed with immunofluorescence.¹⁴ AQP-3 protein

has also been reported in human bladder from tissue microarray immunohistochemistry studies.¹⁵ Recently, AQP-1, 3, 9, and 11 have been identified in another mammalian model, pig bladder (B. Vahabi, M. Manso, M. Drake, C.H. Fry, unpublished data). Interestingly, the urothelium of the American black bear, a mammal that typically hibernates for 4–5 months and does not eat, drink, urinate, or defecate during that time but is known to produce and reabsorb urine in equal amounts daily,¹⁶ also expresses AQP-1 and AQP-3.¹⁷

The cellular localization of AQPs in urothelial cells is an interesting indicator of functionality. AQP-2 and AQP-3 are located on the basolateral (not apical) membrane of umbrella cells and the plasma membranes of neighboring intermediate and basal cells in rat bladder.¹⁸ A similar distribution of AQP-3 has been reported for dog bladder urothelium.¹⁹ It has been proposed that specific AQPs are expressed on the apical membrane of umbrella cells, for initial water influx and subsequent egression through basolateral AQPs, for example, AQP-2 or AQP-3 to the intermediate and basal cells.² This would appear to be an elegant mechanism for water transport from the bladder lumen, through the urothelial cell layers toward the interstitial space and capillaries in the lamina propria.

Functional experiments using a model of in vitro differentiated normal human urothelial cell cultures showed that expression of AQP-3 and AQP-9 was upregulated by increased osmolality through changes in NaCl concentration. Interestingly, blockade of AQPs with mercuric chloride reduced water and urea flux showing AQP functionality under these conditions,²⁰ consistent with urothelium sensing and responding to hyperosmotic conditions to maintain water and salt homeostasis. In vivo experiments on rats in conditions of water deprivation or water loading showed that expression of AQP-2 and AQP-3 was significantly increased in the water deprivation conditions. The above studies are consistent with the hypothesis that AQPs impact urine osmolality through water transport under conditions of water deprivation/dehydration.

It is interesting to note that roles of AQPs other than water transport may also occur in the urothelium. The ability of AQPs to transport cations may also contribute to urine modification, moreover, their regulation of the expression of other proteins, involvement in cell adhesion, and control of cell volume²¹ suggest extensive involvement in urothelial physiology.

3 ION CHANNELS ON UROTHELIAL CELLS FOR ELECTROLYTE TRANSPORT

Urothelial cells express a large number of ion channels and transport proteins, in keeping with the ability to modify urine ionic composition and osmolality by absorption or secretion. These have been reviewed by others^{1, 2, 4, 22-24} and will be briefly summarized here.

3.1 ENaC and Na⁺/K⁺-ATPase

The epithelial sodium channel, ENaC, also known as the amiloride-sensitive sodium channel is expressed on the apical membrane of umbrella cells, facilitating transurothelial Na⁺ flux along with Na⁺/K⁺-ATPase on the basolateral membrane, which provides the driving force for Na⁺ absorption. In vitro preparations of urothelium in Ussing chambers in experimental conditions to mimic bladder filling exhibit significant increased ENaC activity, potentially from apical membrane incorporation of ENaCs via vesical fusion.^{25, 26} Such findings

demonstrate the mechanisms available for Na⁺-transport from the urine, across the urothelial layer, eventually to lamina propria capillaries. Determining whether this occurs in vivo in healthy bladders during filling represents a challenge. The use of transgenic models, for example, with conditional urothelial ENaC knockdown, would be an interesting approach enabling dissection of the involvement of ENaC-mediated Na⁺-absorption and its impact on urine composition.

3.2 K⁺ channels

Several mechanisms for K⁺ transport are reportedly present in urothelial cells including non-selective cation channels (NSCC) expressed on the apical membrane. NSCC channels are mechanosensitive and also transport Ca²⁺ across the apical surface of umbrella cells. Their molecular identity is not yet certain. Other K⁺ channels in the urothelium include Kir1.1 (inwardly rectifying K⁺ channel), which is reportedly expressed on umbrella cells' apical membrane²⁷ and the Kir2.1 channel, also known as the heparin-binding EGF channel. While the function(s) of Kir1/Kir2.1 channels in umbrella cells are not fully known, they may be involved in modification of urinary [K⁺]. Members of the Ca²⁺-activated potassium channel family are also expressed in urothelium; gene expression of the large-conductance Ca²⁺-activated K⁺ channel (KCNMA1, BK channel) and the small/intermediate-conductance Ca²⁺-activated K⁺ channel (KCNN1-4, SK/IK) have been reported.²⁸ In an earlier study, Wang et al.²⁶ reported that the SK channel blocker apamin, and the BK/IK blocker, charybdotoxin reduced K⁺ transport, whereas the selective BK blocker, iberiotoxin had no effect; overall indicating that SK and IK but not BK channels functionally transport K⁺ in urothelial cells. In the same study, contribution of ATP-sensitive K⁺-channels (KATP) to K⁺ secretion from urothelial cells was shown by reduction of K⁺-transport with the KATP blocker, glibenclamide. Consistent with this, gene expression of the KATP channels, Kir6.1 (KCNJ8) and Kir6.2 (KCNJ11) and protein expression of Kir6.1 at the basolateral membrane of umbrella cells and on other urothelial cells has been reported.²⁸

3.3 TRP channels

TRP channels are non-selective cation channels permeable to calcium, magnesium and sodium. Several of these are known to be expressed by urothelial cells (reviewed in ref.²³) and information on their physiological roles is emerging. Expression of TRPV1 on urothelial cells is still considered to be putative; similarly, the cold-sensing channel TRPM8 is not widely accepted as being expressed in the urothelium. Everaerts et al.²⁹ characterized TRPV2, TRPV4, and TRPM7 in mouse urothelium. TRPV2 and TRPV4 are known to be expressed on basal urothelial cells; the latter is located close to adherens junctions and is involved in mechanosensation. Urothelium sensing of bladder fullness or extension is also achieved by novel mechanosensitive Piezo1 channels which transduce increase of cytosolic Ca²⁺-concentrations to ATP release.³⁰ We do not currently know under which conditions TRP channels are involved in the transurothelial transport of cations; however, TRPV5 and TRPV6 are important mechanisms of Ca²⁺-transport in the epithelial cells of the renal distal convoluted tubule.^{31, 32}

3.4 Purinergic receptors

A further family of non-selective cation channels is represented by the purinergic, P2X receptors, P2X1-P2X7, all of which have been identified in the urothelium of several species. P2X5, P2X6, and P2X7 are reportedly to be expressed in rat bladder urothelial cells.³³ Vial & Evans³⁴ demonstrated P2X2, P2X4, and P2X7 on mouse bladder urothelium, moreover all P2Xs were found to be expressed in feline bladder urothelium.³⁵ P2X1, P2X2, P2X3, P2X6, and P2X7 are expressed on human urothelial cells.³⁶⁻³⁹ The potential involvement of P2X receptor/channels in ion transport in the context of urine modification is unknown; however, it is known that P2X receptors on umbrella cells may contribute to apical membrane insertion triggered by Ca^{2+} -influx.⁴⁰ Given that delivery of ENaCs to the apical membrane occurs via a similar mechanism, P2X-mediated Ca^{2+} -influx may indirectly enable Na^{+} -influx under conditions where ATP is released from urothelial cells, for example, during filling.

3.5 Cl^{-} channels

Secretion of Cl^{-} from urothelial cells has been shown in experimental conditions, yet the molecular identity of the ion channel(s) remains unknown. Wang et al.²⁶ subsequently discussed pathways for Cl^{-} entry across the basolateral membrane of umbrella cells including Cl^{-} channels and Cl^{-} /bicarbonate exchangers but noted that an apical pathway of Cl^{-} secretion had not been described in the urothelium.

3.6 Solute transporters

Briefly, transporters are present in urothelial cells for urea and creatinine,² enabling reabsorption or secretion depending on the local environment. The urea transporter A, UT-A, is located apically, whereas UT-B is primarily located on the basolateral surface of umbrella cells and the membrane of intermediate and basal cells enabling a system of urea absorption apically by UT-A, transport through the cell and exit on the basolateral side via UT-B.

4 MEASUREMENT OF URINE ELECTROLYTE CONCENTRATIONS IN ANIMALS AND HUMANS

The concept of ion and water transport across urothelial tissue has been demonstrated in both animal and human models. Na^{+} transport across ex vivo rabbit urothelial tissue⁴¹ and water permeability of human bladder⁴² were demonstrated several decades ago. Investigation of the permeability of intact rabbit bladder urothelium by Negrete et al.⁴³ showed that this was low under normal conditions, consistent with the urothelium acting as an effective barrier. In vivo studies of animals under conditions of water deprivation or water loading have also been employed to investigate the permeability of the bladder to water and several ions. Furthermore, studies of urine composition in patients undergoing renal procedures have provided physiological information on modification of urine as it travels from the kidney to the bladder.

An interesting study of rats during water deprivation, water loading, or ad libitum H_2O involved urine collection and its subsequent instillation into isolated in situ bladders from animals in the corresponding condition. Measurements of Na^{+} , K^{+} , and Cl^{-} indicated that (1) the instilled urinary ion concentrations and (2) animal hydration status influenced the

degree of water reabsorption across the urothelium leading the authors to conclude that urine composition was modified in the lower urinary tract.⁴³

Studies of the American black bear, a mammal that typically hibernates for 4–5 months and does not eat, drink, urinate, or defecate during that time,¹⁶ may provide a helpful perspective. The fact that the bear produces urine daily without urination indicates that urine is produced and reabsorbed in similar quantities. The composition of the bladder wall of the black bear was reportedly remarkably similar in histology and ultrastructure to that of non-hibernating mammals including humans. Interestingly, expression of urothelial proteins including uroplakins, occludin, and claudin was similar to other mammals, moreover, expression and localization of AQP-1, AQP-3, and Na⁺/K⁺-ATPase were not remarkably different.¹⁷ These observations coupled with the fact that the bear bladder also operates a filling/emptying cycle in non-hibernating conditions raises the possibility that under certain (patho) physiological conditions, the human bladder might also modify urine composition. Spector et al.¹⁷ noted that it is challenging to obtain bladder tissue from bears during the hibernation period, therefore limiting our ability to determine the functional expression of urothelial proteins and transport mechanisms during this time. They speculated that dietary changes (known to change expression of uroplakins, tight junctions, and ion channels) prior to hibernation, significantly increased consumption of food and subsequent storage of fat may cause upregulation of AQPs and ion channels/transporters and downregulation of the membrane barrier proteins, which together would facilitate urine reabsorption during hibernation.

Evidence for post-kidney urine modification in humans was reported by Cahill et al.⁴⁴ who compared urine composition from the renal pelvis and bladder in patients undergoing renal procedures. Measurements of urine pH, osmolality, Na⁺, and K⁺ revealed that these differed between bladder and renal pelvis, supporting the hypothesis that urine was modified in the human lower urinary tract in vivo. Recently, an unpublished study of paired urine samples from renal pelvis and bladder taken from 21 well-hydrated patients undergoing percutaneous nephrolithotomy or ureteric stent insertion prior to lithotripsy corroborates the findings of Cahill et al.⁴⁴ Samples were analyzed for [Na⁺] and [K⁺] with flame photometry; osmolality with an automatic micro-osmometer; and pH with a combination glass electrode. Urine osmolality was increased by a median of 1.6-fold in bladder compared to renal pelvis samples. Na⁺ and K⁺ were similarly increased in bladder samples with 1.4-fold and 1.9-fold increases, respectively. The initial, renal pelvis [Na]:[K] was about 4.0, indicating that approximately twice the quantity of Na⁺ is absorbed compared to K⁺. Bladder urine pH was around 0.35 pH units less acidic compared to that from renal pelvis; however, the alkaline shift was greater as renal pelvis' acidity increased, and at approximately pH 7.0, pH was unchanged in the two compartments. This suggests a net acid transporter across the urothelium under most conditions. The increase of [Na⁺] and [K⁺] in bladder compared to renal pelvis urine suggests significant movement of free water compared to electrolytes across the urinary tract urothelium. Although urine is stored in the bladder for long periods of time, the large surface-to-volume ratio of the ureteric lumen may enable it to have a significant role in regulating water transport. The particular routes for water movement require evaluation; paracellular or transcellular; as do the pathways that regulate such a flux. However, net Na⁺ and K⁺ transport is also likely as bladder urine [K⁺] increases more than [Na⁺], suggesting preferential electrolyte transport routes.

5 PHYSIOLOGICAL AND CLINICAL RELEVANCE

Physiologically, the movement of both water and electrolytes across the urinary tract urothelium indicates a supplementary route to conserve these important moieties and also allows bladder filling not to become excessive with long micturition intervals. It also advises caution in that measurement of bladder urine electrolyte concentrations is not a pure measure of renal function, but also reflects (upper and lower) urinary tract activity. A better understanding of the mechanisms involved in mediating water and solute transport across urinary tract urothelium should aid development of novel therapeutic interventions for treatment of debilitating conditions such as nocturia and overactive bladder syndrome (OAB). Urine concentration and volume is known to affect urge sensation of bladder fullness in patients with lower urinary tract disorders and together may initiate sensory information at level of the urothelium.⁴⁴

6 FUTURE RESEARCH PRIORITIES

Several pertinent questions need to be addressed to enable us to understand the clinical relevance of urine modification by the urinary tract at the level of the urothelium and the conditions under which this occurs.

Does urine modification occur physiologically at baseline levels, representing normal homeostasis of water and particular ions?

In mild dehydration, for example, during exercise or fasting prior to surgery, is there upregulation of urothelial permeability to water and ions?

Are compensatory mechanisms for “baseline” modification evoked after urothelium damage, for example, from bacterial infection, spinal cord injury, radiation therapy?

Is urine modification downregulated or augmented in lower urinary tract disease? If so, is it a cause or consequence of lower urinary tract symptoms?

Can urine modification mechanisms be harnessed to mitigate bladder sensation?

Does salt and water movement occur primarily across the bladder wall or also across the wall of the ureters?

Clearly, the development of appropriate experimental platforms including both *ex vivo* cellular systems and *in vivo* models is necessary to advance this area. A major challenge will lie with investigation of physiological baseline urinary modification and elucidating the conditions which change this with pathophysiological effects in patients. In animal models, advanced ion-selective imaging techniques and implantation of micro-scale ion-sensing electrodes at the level of the renal pelvis and bladder seems to be feasible in the short-term. Such an approach in animal models of lower urinary tract disease would be a powerful means of understanding the extent to which urine modification contributes to lower urinary tract symptoms.

References

- 1 Apodaca G. The uroepithelium: not just a passive barrier. *Traffic* 2004 Mar;5(3):117-128.
- 2 Khandelwal P, Abraham SN, Apodaca G. Cell biology and physiology of the uroepithelium. *Am J Physiol Renal Physiol* 2009 Dec;297(6):F1477-501.
- 3 Andersson KE, McCloskey KD. Lamina propria: the functional center of the bladder? *Neurourol Urodyn* 2014 Jan;33(1):9-16.
- 4 Birder L, Andersson KE. Urothelial signaling. *Physiol Rev* 2013 Apr;93(2):653-680.
- 5 Shafik A, El Sibai O, Shafik AA, Ahmed I. Do vesical and voided urine have identical compositions? *Scand J Urol Nephrol* 2004;38(3):243-246.
- 6 Shafik A, Ahmed I, El Sibai O, Shafik AA. Does the composition of voided urine reflect that of the renal pelvis? *Urol Res* 2006 Aug;34(4):261-264.
- 7 Schmidt-Nielsen B. Excretion in mammals: role of the renal pelvis in the modification of the urinary concentration and composition. *Fed Proc* 1977 Oct;36(11):2493-2503.
- 8 Levinsky NG, Berliner RW. Changes in composition of the urine in ureter and bladder at low urine flow. *Am J Physiol* 1959 Mar;196(3):549-553.
- 9 Wickham JE. Active Transport of Sodium Ion by the Mammalian Bladder Epithelium. *Invest Urol* 1964 Sep;2:145-153.
- 10 Burton TJ, Cooper DM, Dunning-Davies B, Mansour D, Masada N, Ferguson DR. Aldosterone stimulates active Na⁺ transport in rabbit urinary bladder by both genomic and non-genomic processes. *Eur J Pharmacol* 2005 Mar 14;510(3):181-186.
- 11 Komissarchik YY, Snigirevskaya ES. Giant vacuoles arising during ADH-induced transcellular bulk water flow across the epithelium of the frog urinary bladder. *Cell Biol Int* 2002;26(10):873-883.
- 12 Zeidel ML, Nielsen S, Smith BL, Ambudkar SV, Maunsbach AB, Agre P. Ultrastructure, pharmacologic inhibition, and transport selectivity of aquaporin channel-forming integral protein in proteoliposomes. *Biochemistry* 1994 Feb 15;33(6):1606-1615.
- 13 Nielsen S, Frokiaer J, Marples D, Kwon TH, Agre P, Knepper MA. Aquaporins in the kidney: from molecules to medicine. *Physiol Rev* 2002 Jan;82(1):205-244.
- 14 Rubenwolf PC, Georgopoulos NT, Clements LA, Feather S, Holland P, Thomas DF, et al. Expression and localisation of aquaporin water channels in human urothelium in situ and in vitro. *Eur Urol* 2009 Dec;56(6):1013-1023.

- 15 Mobasheri A, Wray S, Marples D. Distribution of AQP2 and AQP3 water channels in human tissue microarrays. *J Mol Histol* 2005 Feb;36(1-2):1-14.
- 16 Nelson RA. Winter sleep in the black bear. A physiologic and metabolic marvel. *Mayo Clin Proc* 1973 Oct;48(10):733-737.
- 17 Spector DA, Deng J, Coleman R, Wade JB. The urothelium of a hibernator: the American black bear. *Physiol Rep* 2015 Jun;3(6):10.14814/phy2.12429.
- 18 Spector DA, Wade JB, Dillow R, Steplock DA, Weinman EJ. Expression, localization, and regulation of aquaporin-1 to -3 in rat urothelia. *Am J Physiol Renal Physiol*. 2002; 282:F1034–F1042.
- 19 Spector DA, Yang Q, Klopouh L, Deng J, Weinman EJ, Steplock DA, et al. The ROMK potassium channel is present in mammalian urinary tract epithelia and muscle. *Am J Physiol Renal Physiol* 2008 Dec;295(6):F1658-65.
- 20 Rubenwolf PC, Georgopoulos NT, Kirkwood LA, Baker SC, Southgate J. Aquaporin expression contributes to human transurothelial permeability in vitro and is modulated by NaCl. *PLoS One* 2012;7(9):e45339.
- 21 Kitchen P, Day RE, Salman MM, Conner MT, Bill RM, Conner AC. Beyond water homeostasis: Diverse functional roles of mammalian aquaporins. *Biochim Biophys Acta* 2015 Dec;1850(12):2410-2421.
- 22 Lewis SA. Everything you wanted to know about the bladder epithelium but were afraid to ask. *Am J Physiol Renal Physiol* 2000 Jun;278(6):F867-74.
- 23 Deruyver Y, Voets T, De Ridder D, Everaerts W. Transient receptor potential channel modulators as pharmacological treatments for lower urinary tract symptoms (LUTS): myth or reality? *BJU Int* 2015 May;115(5):686-697.
- 24 Merrill L, Gonzalez EJ, Girard BM, Vizzard MA. Receptors, channels, and signalling in the urothelial sensory system in the bladder. *Nat Rev Urol* 2016 Apr;13(4):193-204.
- 25 Lewis SA, de Moura JL. Incorporation of cytoplasmic vesicles into apical membrane of mammalian urinary bladder epithelium. *Nature* 1982 Jun 24;297(5868):685-688.
- 26 Wang EC, Lee JM, Johnson JP, Kleyman TR, Bridges R, Apodaca G. Hydrostatic pressure-regulated ion transport in bladder uroepithelium. *Am J Physiol Renal Physiol* 2003 Oct;285(4):F651-63.
- 27 Yu W, Khandelwal P, Apodaca G. Distinct apical and basolateral membrane requirements for stretch-induced membrane traffic at the apical surface of bladder umbrella cells. *Mol Biol Cell* 2009 Jan;20(1):282-295.
- 28 Everaerts W, Vriens J, Owsianik G, Appendino G, Voets T, De Ridder D, et al. Functional characterization of transient receptor potential channels in mouse urothelial cells. *Am J Physiol Renal Physiol* 2010 Mar;298(3):F692-701.

- 29 Miyamoto T, Mochizuki T, Nakagomi H, Kira S, Watanabe M, Takayama Y, et al. Functional role for Piezo1 in stretch-evoked Ca^{2+} influx and ATP release in urothelial cell cultures. *J Biol Chem* 2014 Jun 6;289(23):16565-16575.
- 30 Hoenderop JG, Bindels RJ. Epithelial Ca^{2+} and Mg^{2+} channels in health and disease. *J Am Soc Nephrol* 2005 Jan;16(1):15-26.
- 31 Mensenkamp AR, Hoenderop JG, Bindels RJ. TRPV5, the gateway to Ca^{2+} homeostasis. *Handb Exp Pharmacol* 2007;(179)(179):207-220.
- 32 Lee HY, Bardini M, Burnstock G. Distribution of P2X receptors in the urinary bladder and the ureter of the rat. *J Urol* 2000 Jun;163(6):2002-2007.
- 33 Vial C, Evans RJ. P2X receptor expression in mouse urinary bladder and the requirement of P2X(1) receptors for functional P2X receptor responses in the mouse urinary bladder smooth muscle. *Br J Pharmacol* 2000 Dec;131(7):1489-1495.
- 34 Birder LA, Ruan HZ, Chopra B, Xiang Z, Barrick S, Buffington CA, et al. Alterations in P2X and P2Y purinergic receptor expression in urinary bladder from normal cats and cats with interstitial cystitis. *Am J Physiol Renal Physiol* 2004 Nov;287(5):F1084-91.
- 35 Elneil S, Skepper JN, Kidd EJ, Williamson JG, Ferguson DR. Distribution of P2X(1) and P2X(3) receptors in the rat and human urinary bladder. *Pharmacology* 2001;63(2):120-128.
- 36 Tempest HV, Dixon AK, Turner WH, Elneil S, Sellers LA, Ferguson DR. P2X and P2X receptor expression in human bladder urothelium and changes in interstitial cystitis. *BJU Int* 2004 Jun;93(9):1344-1348.
- 37 Shabir S, Cross W, Kirkwood LA, Pearson JF, Appleby PA, Walker D, et al. Functional expression of purinergic P2 receptors and transient receptor potential channels by the human urothelium. *Am J Physiol Renal Physiol* 2013 Aug 1;305(3):F396-406.
- 38 Svennersten K, Hallen-Grufman K, de Verdier PJ, Wiklund NP, Poljakovic M. Localization of P2X receptor subtypes 2, 3 and 7 in human urinary bladder. *BMC Urol* 2015 Aug 8;15:81-015-0075-9.
- 39 Wang EC, Lee JM, Ruiz WG, Balestreire EM, von Bodungen M, Barrick S, et al. ATP and purinergic receptor-dependent membrane traffic in bladder umbrella cells. *J Clin Invest* 2005 Sep;115(9):2412-2422.
- 40 Eaton DC. Intracellular sodium ion activity and sodium transport in rabbit urinary bladder. *J Physiol* 1981 Jul;316:527-544.
- 41 Hilson AJ, Lewis CA, Harland SJ. The permeability of the human bladder to water assessed using tritiated water. *Contrib Nephrol* 1990;79:41-44.

- 42 Negrete HO, Lavelle JP, Berg J, Lewis SA, Zeidel ML. Permeability properties of the intact mammalian bladder epithelium. *Am J Physiol* 1996 Oct;271(4 Pt 2):F886-94.
- 43 Spector DA, Deng J, Stewart KJ. Hydration status affects sodium, potassium, and chloride transport across rat urothelia. *Am J Physiol Renal Physiol* 2013 Dec 15;305(12):F1669-79.
- 44 Cahill DJ, Fry CH, Foxall PJ. Variation in urine composition in the human urinary tract: evidence of urothelial function in situ? *J Urol* 2003 Mar;169(3):871-874.