The role of the bladder urothelium as a chemical-neural transducer via purinergic signalling in the rapid defence against bacterial infection

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Commentary

The urinary bladder is exposed to bacteria due to its anatomical location, and its physiological structures and functions enable inherent defence mechanisms that protect against infection. The urothelium is the epithelial tissue that lines the distal portion of the urinary tract, including the bladder, and forms a highly resistant physical barrier which prevents invasion of bacteria into the body. Also, the mucosa is a specialised epithelial lining that protects any body structure which is continuous with the external environment, except for the skin itself. It produces a lubricating fluid containing the sticky protein mucin, and this prevents bacterial attachment [1]. In addition, the innate immune response of the bladder provides an essential active defensive mechanism against bacterial infection, where the bladder urothelium and resident leukocytes respond to bacterial activity to ultimately result in a neutrophil response [2].

The urothelium also has sensory functions and plays an important role in the relay of bladder stimuli to the central nervous system. As previously discussed in *Purinergic Signalling*, urothelial ATP signalling via purinergic receptors has an important role in bladder function [3]. ATP plays a key role in bladder sensory functions [4], and altered purinergic sensory pathways are involved in bladder pathologies, such as interstitial cystitis and overactive bladder [5, 6]. A common symptom of bacterial cystitis is increased frequency of urination, which is termed urinary frequency. This is an effective defensive response that rapidly flushes out the bacteria from the bladder to limit infection. It has previously been suggested that ATP may modulate urinary function in

bacterial infection; however, the precise mechanism is not known and this was the focus of this study.

To induce infection, the authors used LPS, a major component of the Gram-negative bacterial cell wall and a common virulence factor that is widely used, to induce acute bacterial cystitis in mice [7]. After the bladder was exposed to LPS for 12 h, neutrophil infiltration, interstitial oedema, vasodilation and microhaemorrhages were observed, all characteristic of inflammatory histological changes in the bladder. Neutrophils were also detected in the urine. Cystometry was performed on anaesthetised mice for functional analysis and intercontraction intervals (ICIs) were measured. Intravesical LPS exposure resulted in urinary frequency (decreased ICIs) within 1 h, prior to any inflammatory changes detected in the bladder or urine. Thus, LPS induced urinary frequency, with acute functional changes occurring more rapidly than inflammatory responses in the bladder and urine. The mechanisms underlying the ability of the bladder to respond to bacterial infection were then explored.

Toll-like receptors (TLRs) play a critical role in immune systems and the mechanism of action of LPS involves TLR4. In this study, TLR4 immunoreactivity was observed on the apical side of the umbrella urothelium, indicating that TLR4 is in direct contact with urine, suggesting a role in detecting luminal bacteria. Intravesical infusion of TAK-242, a TLR4 selective antagonist, inhibited the effect of LPS on urinary frequency in functional cystometry. ATP release was measured using a luciferin-luciferase assay, and LPS treatment resulted in rapid ATP release towards the mucosal side in isolated bladders in an Ussing chamber, which was entirely blocked by TAK-242 treatment. These results suggest that urinary frequency and urothelial ATP release induced by LPS is mediated by TLR4 in the urothelium.

Sensory information is transmitted from the bladder by primary afferent neurons to neurons in the spinal cord, predominantly the L6-S1 spinal neurones. Neuronal

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activity generates c-Fos expression, which can be measured by immunohistochemical analysis in the spinal cord [8]. Intravesical infusion of LPS increased c-Fos expression in the L6-S1 spinal neurons of wild-type mice, demonstrating the involvement of bladder afferent nerves. Purinergic P2X2 and P2X3 receptors are expressed on some bladder afferent nerves [9, 10] and urothelial cells. Attenuation of LPS-induced urinary frequency in P2X2 and P2X3 knockout mice (P2X2^{-/-} and P2X3^{-/-}, respectively) has previously been reported [11], and in this study, the increase in LPS-induced c-Fos positive cells in the L6-S1 spinal cord was lower in the knockout mice compared to wild-type mice. This suggests a role for ATP signalling via P2X2 and P2X3 receptors in LPS-induced activation of L6-S1 spinal neurons.

Ultrasonographic bladder images were obtained from anaesthetised mice to measure bladder function—the maximum sagittal cross-sectional area (CSA) of the bladder just before micturition reflected maximum bladder capacity, and the minimum CSA just after micturition was reflective of post-voiding residual volume. Intravesical infusion of ATP (5–50 mM) decreased the largest CSA in a concentration-dependent manner but did not affect the smallest CSA. Intravesical ATP infusion (5–100 mM) also decreased ICIs in a concentration-dependent manner in cystometry experiments. Intravesical ATP infusion also induced c-Fos expression in the L6-S1 spinal cord, similar to LPS infusion. In the P2X2^{-/-} and P2X3^{-/-} mice, the reduction in ICIs and the increase of c-Fos positive cells in the L6-S1 spinal cord after ATP infusion was lower in comparison to wild-type mice. Intravesical application of a non-selective purinergic receptor antagonist, pyridoxal phosphate-6-azophenyl-2,4-disulfonic acid (PPADS), inhibited the effects of ATP. This demonstrates that intravesicular ATP stimulates purinergic receptors to activate L6-S1 spinal neurons and induce urinary frequency.

This study identifies a novel mechanism of how the detection of bacterial LPS in the urothelium triggers urinary frequency, a common symptom in the rapid defence in bacterial cystitis. Luminal LPS is detected through TLR4 in the urothelium which results in urothelial ATP release, and this transmits sensory information via afferent nerve fibres to ultimately decrease ICIs. This is the first study demonstrating that TLR4 on the urothelium couples neural signalling via a purinergic-mediated pathway, and further studies are required to elucidate the specific cellular mechanisms involved in the ability of TLR4 to modulate release of urothelial ATP. This is of particular interest as purinergic sensory pathways are implicated in bladder pathologies. In addition, luminal ATP is unlikely to cross the barrier towards the basolateral side, and directly activate afferent nerves, and further studies are required to clarify potential mechanisms of how an increase in luminal

ATP exerts its actions on bladder afferents. This study demonstrates that the urothelium plays an essential role as a chemical-neural transducer via purinergic signalling to induce urinary frequency, a rapidly effective response against bacterial infection.

Declarations

Ethical approval This article does not contain any studies with human participants or animals performed by any of the authors.

Conflicts of interest Basu Chakrabarty declares that he has no conflict of interest.

References

1. Birder LA, Andersson KE (2013) Urothelial signalling. *Physiol Rev* 93(2):653–680. <https://doi.org/10.1152/physrev.00030.2012>
2. Abraham SN, Miao Y (2015) The nature of immune responses to urinary tract infections. *Nat Rev Immunol* 15(10):655–663. <https://doi.org/10.1038/nri3887>
3. Burnstock G (2014) Purinergic signalling in the urinary tract in health and disease. *Purinergic Signal* 10(1):103–155. <https://doi.org/10.1007/s11302-013-9395-y>
4. Birder LA (2005) More than just a barrier: urothelium as a drug target for urinary bladder pain. *Am J Physiol Renal Physiol* 289(3):F489–495. <https://doi.org/10.1152/ajprenal.00467.2004>
5. Sun Y, Chai TC (2006) Augmented extracellular ATP signalling in bladder urothelial cells from patients with interstitial cystitis. *Am J Physiol Cell Physiol* 290(1):C27–34. <https://doi.org/10.1152/ajpcell.00552.2004>
6. Kumar V, Chapple CR, Rosario D, Tophill PR, Chess-Williams R (2010) In vitro release of adenosine triphosphate from the urothelium of human bladders with detrusor overactivity, both neurogenic and idiopathic. *Eur Urol* 57(6):1087–1092. <https://doi.org/10.1016/j.eururo.2009.11.042>
7. Ueda N, Kondo M, Takezawa K, Kiuchi H, Sekii Y, Inagaki Y, Soda T, Fukuhara S, Fujita K, Uemura M, Imamura R, Miyagawa Y, Nonomura N, Shimada S (2020) Bladder urothelium converts bacterial lipopolysaccharide information into neural signaling via an ATP-mediated pathway to enhance the micturition reflex for rapid defense. *Sci Rep* 10(1):21167. <https://doi.org/10.1038/s41598-020-78398-9>
8. Ueda N, Kondo M, Takezawa K, Kiuchi H, Sekii Y, Inagaki Y, Soda T, Fukuhara S, Fujita K, Uemura M, Imamura R, Miyagawa Y, Nonomura N, Shimada S (2018) Intravesical ATP instillation induces urinary frequency because of activation of bladder afferent nerves without inflammatory changes in mice: a promising model for overactive bladder. *Biochem Biophys Res Commun* 506(3):498–503. <https://doi.org/10.1016/j.bbrc.2018.10.106>
9. Cockayne DA, Hamilton SG, Zhu QM, Dunn PM, Zhong Y, Novakovic S, Malmberg AB, Cain G, Berson A, Kassotakis L, Hedley L, Lachnit WG, Burnstock G, McMahon SB, Ford AP (2000) Urinary bladder hyporeflexia and reduced pain-related behaviour in P2X3-deficient mice. *Nature* 407(6807):1011–1015. <https://doi.org/10.1038/35039519>
10. Cockayne DA, Dunn PM, Zhong Y, Rong W, Hamilton SG, Knight GE, Ruan H, Ma B, Yip P, Nunn P, McMahon SB,

- Burnstock G, Ford APDW (2005) P2X2 knockout mice and P2X2/P2X3 double knockout mice reveal a role for the P2X2 receptor subunit in mediating multiple sensory effects of ATP. *J Physiol* 567(Pt 2):621–639. <https://doi.org/10.1113/jphysiol.2005.088435>
11. Takezawa K, Kondo M, Kiuchi H, Ueda N, Soda T, Fukuhara S, Takao T, Miyagawa Y, Tsujumura A, Matsumoto-Miyai K, Ishida Y, Negoro H, Ogawa O, Nonomura N, Shimada S (2016) Authentic role of ATP signaling in micturition reflex. *Sci Rep* 6:19585. <https://doi.org/10.1038/srep19585>
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