W24: Recognising the Pioneering Work of Professor Geoffrey Burnstock: Purinergic Signalling in Disorders of Bladder Function

Workshop Chairs: Karen McCloskey, United Kingdom and Christopher Fry, United Kingdom

Start	End	Topic	Speakers
		Professor Geoffrey Burnstock's legacy on purinergic signalling	Karen McCloskey
		Purines and urothelial sensing	Christopher Fry
		ATP and afferent nerves in the bladder	Nicholas Spencer
		Purinergic signalling and bladder dysfunction	Toby Chai
		Discussion	Karen McCloskey Christopher Fry Toby Chai Nick Spencer

Aims of Workshop

Professor Geoffrey Burnstock (1929–2020) was a neurobiologist who advanced biomedical knowledge through his pioneering work on purinergic signalling. Professor Burnstock's illustrious career was recognised by accolades including Fellow of the Royal Society (1986), Australian Academy of Science's Macfarlane Burnet Medal (2017) and Companion of the Order of Australia (2018). He is best known for demonstrating that extracellular ATP acts as a signalling molecule across many areas. This workshop will review what we currently know of bladder purinergic signalling in the bladder and pathophysiological changes. Translational impacts of Professor Burnstock's legacy will be discussed in the context of clinical urology.

Learning Objectives

1. To review purinergic signalling in the urinary tract recognising Prof Burnstock's legacy
2. To understand the importance of purinergic signalling in bladder sensory afferent nerves, urothelial function, bladder pain and detrusor contraction
3. To discuss translational impacts of purinergic targets in bladder therapeutics.

Target Audience

Urology, Pure and Applied Science

Advanced/Basic

Intermediate

Suggested Learning before Workshop Attendance

https://www.theguardian.com/science/2020/jun/19/geoffrey-burnstock-obituary

https://www.ucl.ac.uk/biosciences/news/2020/jun/professor-geoffrey-burnstock-1929-2020

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1. Introduction: Professor Geoffrey Burnstock's legacy on purinergic signalling

Professor Karen McCloskey PhD, Queen's University Belfast, UK

Professor Geoffrey Burnstock was a neurobiologist, with Australian nationality, whose pioneering work on purinergic signalling was considered controversial at the time, challenging accepted paradigms in the field. It is now well established that ATP is released from nerves and other cells, mediating autocrine and paracrine signalling in many settings (Sui et al, 2014), including the urinary bladder. The story of this work is inspirational and instructive to students, early career researchers and established researchers alike. It is reported that Burnstock never disregarded unexpected results and was motivated to undertake detailed investigations to answer challenging research questions.

ATP is now known to be a key player in urinary tract physiology (Andersson, 2015; Burnstock, 2018). Release of ATP from bladder urothelial and other mucosal cells occurs upon distension (Ferguson et al, 1997; Knight et al, 2002; Durnin et al, 2016) along with increased afferent nerve firing (Bahns et al, 1987) potentially mediating a sensory transduction system, to locally sense bladder filling status. A wide array of chemical and biological agents, including ATP itself (Sun et al, 2001; Birder et al, 2003), act to modulate ATP release from the urothelium and many are candidate targets for reducing urgency/aberrant sensation in conditions of overactive bladder and pelvic pain.

In non-human mammals, ATP is co-released with acetylcholine from intramural nerves, as demonstrated in electrical field stimulation studies in *ex vivo* bladder preparations, where it acts post-synaptically to contract detrusor smooth muscle. Interestingly, ATP plays little or role in neurogenic contraction of human detrusor from normal bladders (Tagliani et al, 1997); however, it apparently develops as a cause or consequence of pathology and is observed in diseased bladder (Bayliss et al, 1999). Furthermore, the atropine-resistant, ATP-mediated contraction in non-human mammalian bladder is upregulated in models of obstruction, providing further evidence of participation in pathological mechanisms (deGroat et al, 2015).

Study of the array of purinergic receptors expressed in bladder cells and nerves has shone a light on the diversity of ATP signalling and has nominated candidates, attractive in symptom treatment development (Burnstock and Kennedy, 2011; Andersson, 2015). Recently, the P2X7 purinergic receptor has received attention as a therapeutic target in interstitial cystitis/bladder pain (Taidi et al, 2019) and in urothelial cancer (Gilbert et al, 2019).

The workshop reviews the importance of purinergic signalling in urinary tract physiology. Presenters also consider how purinergic signalling changes in bladder dysfunction through the dual lenses of 'cause' and 'consequence'.

2. Purines and urothelial sensing

Professor Christopher Fry PhD, University of Bristol, UK

The algogenic (pain-producing) properties of purines, such as ADP and ATP, were inferred from their ability to increase sensory nerve firing in a number of tissues. This included work at the Middlesex Hospital in London (Bleehen, 1978) and literally five minutes' walk from the anatomy department at University College London where Geoffrey Burnstock was based. He furthered this work (Bodin et al., 1991), working with blood vessels and showed that their distention increased ATP in the extracellular space raising the potential

for it to work as a signalling molecule between different tissues. Moreover, he demonstrated the ATP originated not from smooth muscle but from endothelial cells. This work was extended to urinary tract tissue where it was demonstrated that distension of the bladder or ureter lumen also caused ATP release. This led to two conceptual advances: Burnstock (1999) proposed that distension of visceral organs in general had such an ability to release purines when distended, and might be a basis of visceral pain if release was excessive; secondly Lori Birder, Gerard Apodaca and their colleagues (Apodaca et al 2007) introduced the concept of a sensory web in the bladder wall centred around urothelial ATP release and consequent activation of sensory nerves and modulation of detrusor function. Thus, the paradigm developed that ATP release from urinary tract (and other) tissues under altered stresses might not just be a pathological algogenic activity, but also a physiological mechanism for measuring the extent of urinary tract filling.

Subsequent work has further established some details of this sensory process and a description of afferent fibres arising from the bladder will be given by Nick Spencer. ATP release is initiated by a large number of chemical mediators, as described by Karen McCloskey and Toby Chai, but also by physical stresses. In the latter context, lateral tension seems to be the physical variable so that stiffer tissues would release more ATP. This is of significance as fibrosis increases tissue stiffness and is a structural remodelling that increases with age and some pathologies, each associated with increased prevalence of overactive bladder in humans. Urothelial ATP also appears to demonstrate a circadian rhythmicity and current data are consistent with the possibility that if desynchronised ATP release may be locked into the 'on-state' in the sleep phase, providing a potential contributor to nocturia. This contribution to the workshop will provide more detail of signalling pathways that elicit urothelial ATP release and hopefully offer insight into the mode of action of current therapies for overactive bladder, as well as identify further drug targets. This remains a very active subject of research as is a testimony to the insight provided by Geoffrey Burnstock and others who played such a great role in initiating the topic.

3. ATP and afferent nerves in the bladder

Professor Nick Spencer PhD, Flinders University, Australia

We are deeply indebted to Professor Burnstock for his great wisdom, energy and vision during his research of the urinary bladder and for stimulating the field of autonomic neuroscience into further studies of the role of ATP, particularly in sensory function in the bladder. Vlaskovska et al (2001) in Professor Burnstock's laboratory proposed a "..major sensory role for urothelially released ATP acting via P2X3 receptors on a subpopulation of pelvic afferent fibers." This work ignited numerous laboratories to turn their attention to purinergic signaling in bladder sensation.

This presentation will describe the new advances that have been made recently with regards to the sensory nerve endings in the bladder wall, which have until recently, remained poorly understood. Spinal afferent neurons underlie the transduction and transmission of noxious (painful) stimuli, (including innocuous stimuli that do not reach conscious sensations) from visceral organs to the spinal cord. Although the location of the nerve cell bodies of spinal afferents is well known to reside in dorsal root ganglia (DRG), the location of nerve endings of spinal afferents that transduce sensory stimuli into action potentials is poorly understood. Until recently, the nerve endings of spinal afferents had never been identified in the bladder of any species. We developed an anterograde tracing technique to selectively label only spinal afferents in mice. This meant that we could follow the course of a single spinal afferent axon and identify the nerve endings emanating from these single DRG neurons. Four distinct morphological types of spinal afferent axons have been identified. Three types existed in the detrusor muscle and one major type in the urothelium. Most nerve endings were located in detrusor muscle where the three types could be identified as: "branching", "simple" or "complex" morphology. Most spinal afferent nerve endings were CGRP-immunoreactive. Single spinal afferent axons bifurcated many times after upon entering the bladder and developed varicosities along their axon terminal endings. This work demonstrates that spinal afferents do innervate the urothelium.

Some, or potentially all of the spinal afferent nerve endings recently identified must contribute to the sensation of "fullness" and/or "pain" from the bladder. Which specific types of nerve endings these are

remains unclear. We speculate that the nerve endings activated by low levels of innocuous stimuli likely encode into the noxious range. This is based on findings from our laboratory that show the prevalence of low threshold, wide dynamic range spinal afferents in this organ and the very high abundance of expression of the capsaicin receptor (Trpv1) in bladder innervating spinal afferents.

4. Purinergic signalling and bladder dysfunction

Professor Toby Chai MD, Boston University, USA

Physician-scientists advance healthcare by harmonizing clinical expertise with investigative approaches to discover advances that promote health and treat conditions. Our laboratory team was fortunate enough to receive encouragement from Professor Geoffrey Burnstock during our initial explorations into whether human urothelial release of ATP could be clinically relevant in human hypersensory bladder disorders. We found that human clinical urothelial specimens obtained for patients with IC/BPS (interstitial cystitis / bladder pain syndrome), had upregulation of purinergic signalling in response to mechanical stretch. Both ATP release by urothelial cells and P2X3 receptor upregulation in urothelial cells were augmented in IC/BPS compared to asymptomatic controls. These findings were subsequently supported by findings from other investigators. Human urothelial purinergic signalling has also been found to play a role in other bladder hypersensory conditions including overactive bladder and bacterial cystitis. Although currently there is no regulatory approved pharmacologic agent with purinergic action to treat bladder conditions, gefapixant, a P2X3 receptor antagonist named in honour of Professor Burnstock, has completed a positive phase 2b trial for refractory or unexplained chronic cough, a hypersensory condition of the respiratory tract. It is hoped in the near future treatments for bladder hypersensory conditions will successfully leverage purinergic signalling as the mechanism of action.

5. Summary

The workshop has reviewed knowledge in the field and presented recent research on the important roles of ATP in bladder physiology, recognising the influence of the pioneering work of Professor Burnstock and his team. ATP's role as a neurotransmitter and the identification of purinergic receptors - fast ligand-gated ion channels, underlying spontaneous excitation (EJPs) in bladder smooth muscle is now well established. The modulation of bladder afferent mechanosensation by purinergic mechanisms is being understood in the context of recent identification of spinal afferent nerve endings in the bladder. The findings that most spinal afferent endings (~80%) innervate bladder detrusor smooth muscle and that spinal afferent axon terminals in mouse detrusor muscle and urothelium are almost exclusively peptidergic (CGRP+) support further research endeavours in bladder sensation. Pre-clinical research on bladder and clinical trials in respiratory medicine indicate that development of treatments for bladder dysfunction based on purinergic mechanisms may emerge in coming years.

The presenters would like to thank you for your participation in this workshop and your support of the ICS 2020 meeting.

6. References

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