W28: Do we understand the impact of lower urinary tract disorders on the urethra?

Workshop Chair: Karen McCloskey, United Kingdom

Start	End	Topic	Speakers
		Overview of the session: what will we learn?	Karen McCloskey
		Dysfunctional neurophysiology of the outflow in congenital bladder disease	Neil Roberts
		The urethra in continence and sensation: from fibres to cells	Celia Cruz
		Open discussion and summary of putative urethra biotargets	Karen McCloskey Celia Cruz Neil Roberts

Aims of Workshop

Coordination of bladder and urethra function is essential in lower urinary tract physiology yet, many studies focus solely on bladder when investigating relevant disorders. There is emerging evidence that in addition to cellular remodelling and pathophysiology within the bladder, there are concurrent changes in the bladder outlet and urethra. This workshop will review our current knowledge of urethra cellular composition including paraneurons that are absent from bladder but present in urethra, and basic urethral physiology. We will then consider recent data from models of dysfunction including spinal cord injury and urofacial syndrome.

Learning Objectives

1. To update current knowledge of urethra cellular composition and physiology
2. To learn about urethral pathophysiology in models of spinal cord injury and congenital peripheral neuropathy.
3. To identify promising biotargets in urethral translational urology.

Target Audience

Urology, Pure and Applied Science

Advanced/Basic

Intermediate

Suggested Learning before Workshop Attendance

Manak I, Gurney AM, McCloskey KD, Woolf AS, Roberts NA. Dysfunctional bladder neurophysiology in urofacial syndrome Hpse2 mutant mice. Neurourol Urodyn. 2020;39(7):1930-1938.

Roberts NA, Woolf AS. Heparanase 2 and Urofacial Syndrome, a Genetic Neuropathy. Adv Exp Med Biol. 2020; 1221:807-819.

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Coelho A, Charrua A, Oliveira R, Cavaleiro H, Cruz CD, Cruz F. Underactive bladder in aging rats is associated with a reduced number of serotonin-expressing cells in the urethra and is improved by serotonin application to the urethra. Low Urin Tract Symptoms. 2019;11(4):248-254.

Coelho A, Oliveira R, Cavaleiro H, Cruz CD, Cruz F. Evidence for an urethro-vesical crosstalk mediated by serotonin. Neurourol Urodyn. 2018;37(8):2389-2397.

Frias B, Santos J, Morgado M, Sousa MM, Gray SM, McCloskey KD, Allen S, Cruz F, Cruz CD. The role of brain-derived neurotrophic factor (BDNF) in the development of neurogenic detrusor overactivity (NDO). J Neurosci. 2015 ;35(5):2146-60.

Workshop summary

1. Introduction

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

The physiology of the bladder and urethra represent remarkable synergy. During filling, the bladder smooth muscle is relaxed, and the urethra remains contracted, whereas during emptying, the roles are reversed with the urethra circular smooth muscle actively relaxing while the detrusor vigorously contracts. With central nervous control and local sensing working together, this system is elegantly controlled in normal physiology; however, in many lower urinary tract disorders, defects in bladder, urethra or both lead to urinary storage and voiding symptoms.

Many studies have focussed on the bladder and its control mechanisms, to understand the cellular and molecular basis of urinary dysfunction. In contrast, the bladder outlet and urethra have received comparatively limited attention. The proximal urethra is lined by a urothelium, supported by a lamina propria that is surrounded by circular and longitudinal smooth muscle. Urethral smooth muscle displays phasic background activity, considered to contribute to sphincteric tone, and underpinned by ion channel activity, particularly Ca^{2+}-activated Cl^- channels (Sergeant et al, 2006). It has been proposed that this activity is either intrinsic within a population of urethral smooth muscle cells and/or within neighbouring interstitial cells (Sergeant *et al*, 2019). Contraction of the urethral smooth muscle is largely mediated by adrenergic and to a lesser extent, cholinergic nerves and relaxation is due to nitric oxide signalling (Sergeant *et al*, 2019).

Uniquely, the urethral urothelium contains brush cells (Hashimoto *et al*, 1999), known as paraneurons, which mediate serotoninergic signalling (Hanyu *et al*, 1987; Coelho *et al*, 2018; 2019). These cells are absent in the bladder. Serotonin, released by urethral paraneurons, activates urethra-vesical signalling that enhances bladder reflex contractions (Coelho *et al*, 2018). Paraneurons may participate in urethral sensing (Hanyu *et al*, 1987) and may be differentially expressed in conditions including the streptozotocin-diabetic rat bladder (Cao *et al*, 2019). Emerging evidence shows that remodelling of urethral structure and function occur, contributing to dysfunction either in a causal or a consequential fashion. This may occur in relevant pathological conditions that course with impaired urinary dysfunction, including spinal cord injury (SCI) and urofacial syndrome.

In SCI, the bladder undergoes remarkable histological and functional time-dependent changes (Vizzard, 2006; Johnston *et al*, 2012;) reflecting early inflammation and late fibrosis. While denervation, smooth muscle hypertrophy and fibrosis has been studied in the bladder, the impact of SCI has on the urethra is incompletely understood. Dr Cruz will present recent data showing that the SCI-urethra undergoes remodelling which may, at least partially, explain SCI detrusor-sphincter dyssynergia (DSD) where the urethra and bladder simultaneously, leading to potentially life-threatening renal failure from urinary retention.

Urofacial syndrome (Ochoa and Gorlin, 1987) characterised by neuropathic bladder and distinctive distortions in facial expressions is reportedly associated with defects in detrusor-sphincter coordination i.e., functional obstruction (Kutlu *et al*, 2010) in the absence of physical/anatomical obstruction. This rare, autosomal recessive condition has heterogeneity of genetic basis, including mutations in HPSE2, the gene for heparanase 2 (Daly *et al*, 2010) and in LRIG2, both of which are expressed in nerves of the bladder wall (Stuart *et al*, 2013). Dr Roberts will share recent work from his research group on murine genetic models of urofacial syndrome (Roberts *et al*, 2019; Manak *et al*, 2020).

2. Dysfunctional neurophysiology of the outflow in congenital bladder disease
Dr Neil Roberts PhD, Research Fellow, University of Manchester, UK.

Of the roughly 7000 known rare diseases, which affect up to 7 % of the population, approximately 80 % are caused by single-gene defects. Congenital kidney and urinary tract diseases are individually rare but are collectively the main cause of end-stage renal disease in children and young adults, with abnormalities of the lower urinary tract (LUT) a leading contributor to this. As our understanding of the molecular and tissue pathophysiology of these diseases improves, it raises the prospect of using advanced technologies to cure, rather than treat the symptoms, of these diseases.

In urofacial syndrome, a high-pressure bladder and dyssynergia between bladder contraction and outflow relaxation can lead to vesicoureteral reflux and, eventually, kidney failure. Using whole-tissue imaging, we identified an autosomal recessive basis of the disease, with affected families having pathogenic variants in either *HPSE2* or *LRIG2* and used these insights to establish faithful mouse models of the bladder dysfunction. By studying the urofacial syndrome mice, we identified nerve-patterning defects in the bladder body (an increase in nerve fascicles) and in the outflow (reduced nerve fascicles). Interestingly, when we studied live tissues by physiology, we found that the nerve function in the bladder body and outflow was perturbed to a similar extent, despite the differences in the appearance of their nerves. Further, the bladder body was hyper-contractile to cholinergic receptor stimulation. To understand the aetiology of the disease we have begun to analyse embryonic bladders. This data indicates that even at the earlier stage of bladder innervation, there are fewer nerves in the urofacial mouse bladder neck and outflow region. The precise mechanism linking nerve patterning defects in the embryonic bladder neck and outflow with the loss of neuronal input, and changes in bladder contractility, is still being determined. However, the compelling evidence of a neurogenic defect has promoted us to initiate a pre-clinical trial of viral vector mediated gene therapy. We found that the adeno-associated virus vector 9 (AAV9) is able is able to target transgenes to the bladder nerves of neonatal mice, following systemic

injection. Urofacial syndrome gene addition into neonatal bladder nerves is now being assessed for efficacy in ameliorating or preventing the bladder pathophysiology, with the hope that this can be translated to human patients.

3. The urethra in continence and sensation: from fibres to cells

Professor Celia Cruz PhD, University of Porto, Portugal

The lower urinary tract (LUT) is composed of a urine reservoir, the bladder, and a tubular organ for urine excretion, the urethra, operating in a coordinated switch-like manner for continence and micturition. LUT function is highly dependent on intricate neuronal pathways involving neurons located in the peripheral and central nervous system. Therefore, it comes as no surprise that SCI massively disrupts those neuronal circuits resulting in loss or severity impairment of voluntary control over the urinary bladder. Urinary dysfunction after SCI causes a significant reduction in quality of life and dignity. SCI is typically followed by spinal shock, a period of reduced or absent bladder contractility. With time, neuroplastic mechanisms operating at the spinal cord level catalyse the emergence of neurogenic detrusor overactivity (NDO), often accompanied by detrusor-sphincter-dyssynergia (DSD), resulting in urinary incontinence. Changes in the bladder after SCI have been well characterized and, for that reason, the majority of NDO treatments are aimed at this organ. The contribution of the urethra to SCI-induced urinary impairment is less clear and it is only beginning to be addressed.

Using a well-established model of largely incomplete SCI, we investigated tissue changes in the urethra. We found remarkable time-dependent changes. In animals with bladder dysfunction, we found signs of denervation in the urethral mucosa. Expression of markers of sensory fibres were decreased, while parasympathetic innervation was not affected. We also identified changes in the sphincter innervation. The internal urethral sphincter showed signs of atrophy, and this was accompanied by a reduction of the sympathetic innervation. In the external sphincter, there was also loss of sensory innervation. These results provide evidence of massive tissue reorganization that may contribute to urinary dysfunction.

In addition to peripheral-central pathways controlling LUT function, local communication between the urethra and the bladder is also critical for normal LUT activity. We recently demonstrated that serotonin, produced by specialized epithelial cells present in the urethral lining, is an important mediator of urethra-vesical crosstalk. These epithelial cells present both in humans and experimental animals, are located in the vicinity of sensory and parasympathetic fibres coursing in the urethral mucosa. Administration of serotonin to the bladder does not affect its reflex activity, while urethral serotonin instillation produces strong bladder contractions. These contractions are blocked by urethral anaesthesia or pre-treatment with specific antagonists for serotonin receptors. Interestingly, in animal models of bladder underactivity, urethral serotonin signalling is significantly reduced.

Overall, these observations suggest that the urethra, traditionally seen as a mere conduit, might play a more prominent role in LUT function than previously estimated. We found that the urethra undergoes considerable tissue reorganization in cases of urinary impairment, as seen in animal models of SCI. SCI might also affect a recently described serotonin-dependent urethrovesical reflex. We have demonstrated the presence of serotonergic cells in the urethral lining and showed that peripheral serotonin is a potentiator of bladder contractions. Although further studies are warranted, it is possible to conclude that the urethra is a promising target to develop new therapies for LUT dysfunction.

4. Summary

To fully understand the pathophysiology of urinary dysfunction, investigation of defects in the bladder and the outflow/urethra should be included. Hypothesis-driven research should explore bladder-urethral crosstalk and consider the role of sensing mechanisms, including the novel urethral paraneurons, innervation and smooth muscle function. Key summary points include:

- the urethra undergoes substantial changes in structure and innervation post-SCI, likely involved in LUT dysfunction
- urethrovesical reflex is dependent on peripheral serotonin this is impaired in underactive bladder models and likely affected by other conditions
- female outflow contraction is not understood; mouse models do not have sympathetic innervation and have a low response to phenylephrine
- cystometric measurements do not distinguish between smooth muscle and the external sphincter, making it difficult to pinpoint defects and evaluate the contribution of these tissue types to maintaining tonic continence vs anti-stress mechanisms.

5. References

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