The detrusor-free bladder - it can still hold its water

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Local physiological processes in the bladder wall are typically studied with techniques that include *in vitro* myography of bladder strips combined with electrical field stimulation and/or pharmacological modulators of membrane receptors or ion channels; recordings of afferent nerve activity perhaps in combination with pressure-volume recordings in *ex vivo* bladders; Ussing chamber measurements of urothelial physiology and live-cell Ca^{2+} imaging in tissue sheets or isolated bladder cells. A recent paper (Durnin *et al.*, 2018) has developed an enhanced version of pressure-volume recordings in *ex vivo* mouse bladder, enabling investigation of modulator release from the urothelium into the lumen *and* the lamina propria. The paper reports a methodology for removal of the outer detrusor layer from the bladder, leaving a detrusor-denuded bladder comprising only the mucosal layer (confirmed through histological and immunofluorescence observations including AQP-3^{+} urothelial cells and PDGFRα^{+} lamina propria interstitial cells).

Pressure-volume recordings from intact and denuded bladders were remarkably similar, revealing that the mucosa layer alone was sufficient to generate typical pressure-volume relationships during physiological filling conditions. This finding revealed that the presence and activity of detrusor smooth muscle had little impact on the shape of the pressure-volume curve, which instead appeared to be almost exclusively generated by the mucosa. Interestingly, spontaneous contractions (transient changes in intravesical pressure) were a property of intact bladders during filling, not of denuded preparations, indicating that the murine bladder mucosa did not contain contractile elements capable of generating spontaneous contractions e.g. muscularis mucosae. The physiological relevance and cellular origin of detrusor smooth muscle and spontaneous activity has been a research focus for many years, in particular, the contribution of the mucosa (Drake *et al.*, 2018). Durnin and colleagues did not exclude the possibility that mucosal-detrusor cellular signalling was necessary for the generation or modulation of detrusor spontaneous contractions but importantly, demonstrated that such activity was not necessary for optimal pressure-volume relationships during physiological bladder filling (Durnin *et al.*, 2018). Spontaneous activity is considered to be an important determinant of bladder tone during filling, placing the detrusor in an optimal structural position for emptying, therefore requiring less effort than initiating coordinated contraction from a low-tone baseline (Turner and Brading, 1997). This tone may in fact be more dependent on the mucosa than the detrusor given that there was no difference in pressure-volume relationships whether or not the detrusor was present.

The denuded bladder preparation was demonstrated to be superior to existing protocols used to study modulator release from the urothelium. Purines were detected in both the bladder lumen and the lamina propria interstitium; moreover, the actual concentration was higher in the lamina propria. This observation could also be explained by (non-urothelial) lamina propria cells releasing ATP, however the study was not designed to test this directly. The results raise questions around interpretation of modulator release data from intact preparations, which may have been considered to be comparable between the lumen and lamina propria compartments. The ability to measure purines directly at the lamina propria in the denuded preparation is therefore preferable to using intravesical measurements as a surrogate; the methodology may therefore be attractive to investigators studying the nature of modulators released from the urothelium to the lamina propria space during *physiological filling*. As little is known of excitatory or inhibitory modulators acting on afferent nerves, lamina propria cells e.g. interstitial cells and vascular smooth muscle, this enhanced methodology facilitates both detection of released substances and the use of pharmacological drugs (or genetically modified mice) to examine the underlying signalling mechanisms.

The use of the denuded bladder preparation will be applicable to pathophysiological studies where permeability of the urothelium is particularly relevant. The authors showed increased transurothelial transport in denuded bladders treated with intravesical lipopolysaccharide (LPS). The combination of physiological filling conditions and LPS advances studies in other inflammatory model systems in that transport can now be assessed at low/high bladder volumes and during the development of intravesical pressure during bladder filling. It would be interesting to know whether LPS instillation itself changed the pressure-volume curve in intact or denuded preparations and how this might affect afferent nerve activity.

As with all *ex vivo* techniques, there may be limitations with the denuded bladder model. Removal of detrusor smooth muscle may trigger stress responses in neighbouring lamina propria cells, or, damaged cells at the dissection site may release purines (Burnstock, 2016). The use of Krebs-Ringer-Bicarbonate buffer for intravesical filling may introduce pH changes within the bladder lumen in the absence of carbogen bubbling, an effect which could affect modulator release and transport (this could be addressed using alternative physiological solutions for filling). It will be interesting to learn from future studies if/how changes in intravesical pH (mimicking pH range of urine in health and pathophysiology) affects transmitter release in the lamina propria compartment. Also, if, as seems to be the case, cross talk between detrusor smooth muscle, lamina propria, urothelium and afferent nerves occurs during filling (Heppner *et al.*, 2016) then investigators may wish to interpret results from the denuded preparation with some caveats. It is remarkable that absence of the

detrusor has little impact on the ability of the bladder to hold its 'water' in physiological pressure-volume parameters. Adoption of this preparation in bladder research will undoubtedly enable better elucidation of urothelial-lamina propria signalling mechanisms and advance our understanding of bladder physiology.

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COMPETING INTERESTS

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