

## IDENTIFICATION OF DIFFERENT TYPES OF PELVIC AFFERENT ENDINGS IN THE MOUSE BLADDER USING ANTEROGRADE TRACING FROM DORSAL ROOT GANGLIA *IN VIVO*

### Hypothesis / aims of study

Sensory stimuli within the bladder are detected by spinal afferent neurons, whose cell bodies lie in dorsal root ganglia (DRG). Unmyelinated C fibres and thinly myelinated Ad axons that project (i) via the sacral-pelvic nerves, and have cell bodies in L6 and S1 DRG or (ii) via lumbar-hypogastric nerves, with their cell bodies primarily in L1-L2 DRG. In functional electrophysiological studies four major classes of sensory neurons were identified in the mouse bladder: (i) low threshold stretch-sensitive muscular and (ii) muscular-mucosal; (iii) stretch-insensitive mucosal and (iv) so-called serosal afferents [1]. In the urinary bladder, the terminal endings of spinal afferents that detect noxious and innocuous stimuli have never been identified in any species.

The main objective of the study was to identify the different morphological types of spinal afferent nerve endings within the mouse urinary bladder wall.

### Study design, materials and methods

In C57BL/6 (n=15) mice, left L6 – S2 dorsal root ganglia were exposed *in vivo* and injected with 1 ul dextran biotin (Molecular Probes). Animals were then allowed to recover for a period of 7-8 days. Whole mount bladder preparations were fixed overnight in 4% paraformaldehyde solution before being stained with Streptavidin CY3 to visualise anterogradely labelled sensory fibers. Once anterograde labelling was determined, specimens were stained with antisera against CGRP. In separate series of experiments, single unit extracellular recordings were made from fine nerve trunks of pelvic nerves entering the mouse bladder in urothelium-free, flat sheet preparations *in vitro* [2]. Stretch-sensitivity of bladder afferents was determined by their responses to isotonic stretch by 1-30 g of increasing load. All experiments *in vitro* were performed in the presence of nicardipine (4 uM).

### Results

From 15 mice studied *in vivo*, 160 discrete spinal afferent nerve endings were identified in the bladder wall. These endings were classified into four distinct classes, based on their morphology and location in the bladder wall. The endings were identified as simple endings in the sub-urothelium, simple endings in the detrusor, complex endings in the detrusor and branching endings in the detrusor.

Simple-type endings were identified in the sub-urothelium, which accounted for 19% of all anterogradely labelled nerve endings and were all immunoreactive to CGRP. One quarter (25%) of all anterogradely labelled nerve endings were identified as simple-type endings, that were located in the detrusor muscle, 78% of which were CGRP positive. These endings always ran along the smooth muscle fibres, with little or no branching. Complex-type endings in the detrusor muscle were the most common ending located in the mouse bladder, accounting for 44% of all endings located, 75% of which were CGRP immunoreactive. Complex-type endings consisted of a single axon branching into many fibres with no obvious directionality to one another. Branching-type endings in the detrusor accounted for 12% of all endings identified in the mouse bladder, 89% of which were CGRP positive. These endings consisted of a single axon that branched into many varicose fibres which lay parallel to one another and to the smooth muscle fibres.

In electrophysiological recordings from urothelium-free bladder preparations, based on sensitivity to stretch, two major group of afferents were distinguished: (i) low threshold stretch-sensitive afferents (threshold 1-3 g) with mean firing rate of  $6.52 \pm 1.45$  Hz (n=7) at 30 g load and high threshold afferents (threshold 10 g and more) with mean firing rate of  $3.35 \pm 0.75$  Hz (n=6).

### Interpretation of results

The data demonstrate the morphological complexity of the sacral-pelvic innervation of the bladder with at least three distinct types of endings: simple-type endings in both the sub-urothelium and muscle layers and complex and branching-types endings in the detrusor. In electrophysiological experiments in detrusor preparations, where the urothelium and most of the sub-urothelium were sharp dissected away, two distinct types of afferents: low and high threshold stretch-sensitive mechanoreceptors were recorded. It is tempting to speculate that two distinct morphological types of endings identified in the muscle layers (branching- and complex-type), correspond to low and high threshold stretch-sensitive afferents determined by functional electrophysiological studies in urothelium-free bladder preparations.

### Concluding message

This is the first identification of spinal afferent nerve endings in the urinary bladder wall. The data shows the complexity of the sensory innervation of the urinary bladder, with three distinctly different morphological endings in two different layers of the bladder. Ongoing studies will reveal which specific morphological types of endings relate to the known functional classes of bladder mechanoreceptors. This would be greatly beneficial for understanding mechanisms of both normal and pathological sensation from the bladder.

### References

1. Xu L, Gebhart G (2008). Characterization of mouse lumbar splanchnic and pelvic nerve urinary bladder mechanosensory afferents. *J Neuropysiol* 99: 244-53.
2. Zagorodnyuk V et al (2007). Properties of major classes of bladder mechanoreceptors of the guinea pig *in vitro*. *J Physiol* 585.1: 147-63.

Disclosures

**Funding:** National Health and Medical Research Council of Australia grant #1046881 **Clinical Trial:** No **Subjects:** ANIMAL  
**Species:** mouse **Ethics Committee:** Animal Welfare Committee of Flinders University