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HETEROGENEITY AND COMPLEXITY OF PUTATIVE AFFERENT NERVES IN THE SUB-EPITHELIAL AND MUSCLE LAYERS OF THE PROXIMAL URETHRA OF THE FEMALE RAT

<u>Hypothesis / aims of study</u>

The urethra plays a key role in generating afferent information. Electrophysiological studies have classified afferents, primarily by conduction velocity, as typically Aδ and C fibres. However, the location and nature of these nerves in the urethral wall is uncertain. The present study investigated the location of putative fibres in the proximal urethra of the female rat.

<u>Study design, materials and methods</u>

Twelve urethras were dissected from Sprague-Dawley female rats (200-250 grams) anesthetized with Pentobarbital (60mg/kg, intraperitoneal). The urethras were fixed with 4% paraformaldehyde, frozen and sections (8μm) were prepared for immunohistochemistry. Antibodies (Abs) were used to detect immunoreactivity (IR) to calcitonin gene related peptide (cgrp-IR), vesicular acetylcholine transporter (vacht-IR), nitric oxide synthase (nos-IR) and visualized with appropriate fluorescent secondary Abs.

<u>Results</u>

The proximal urethra, the initial 5-7 mm, demonstrates extensive folding. In this region the rhabdosphincter is also present and the smooth muscle becomes progressively thicker. The bulk of the smooth muscle being more distal to the folds (Figure 1A). The smooth muscle is densely innervated with vacht-IR (putative motor) fibres and in addition cgrp-IR fibres (putative sensory). As shown in the panels Figure 1B the number of cgrp-IR fibres becomes progressively less along the urethral length. Figure 1C shows a quantification of the cgrp-IR density relative to muscle thickness. Few cgrp-IR fibres were seen in the rhabdosphincter. Figure 1 D shows an example of nerve staining close to the epithelium. Distinct cgrp-IR and nos-IR fibres are seen (left panel) and distinct cgrp-IR and vacht-IR fibres (right panels). (The nos-IR and vacht-IR fibres may be the same). These regions of complex innervation appeared as dense arrays, with fibres in the lamina propria and adjacent to the epithelium. Sub-epithelial fibres seldom penetrated the epithelium. Note, that regions of high density are found adjacent to regions of lower density suggesting ‘hot spots’ of afferent outflow. The side panels illustrate, at higher magnifications, cgrp-IR and vacht-IR fibres. Both fibre types have axons and varicosities. The varicosities of the cgrp-IR fibres are larger than the vacht-IR fibres (1.5 $\pm$ 0.3 μm compared to 1.1 $\pm$ 0.2 μm respectively). The cgrp-IR and vacht-IR fibres are distinct and show complex intertwining.

<u>Interpretation of results</u>

The observations suggest structural and functional complexities in the proximal urethra of the female rat. Extensive folding of the epithelium and a distal high density of smooth muscle suggests that it would be possible to dilate the proximal urethra with a closed smooth muscle sphincter. The high density of putative afferent nerves in the vicinity of the epithelium in this region would also suggest significant afferent outflow detecting such change. The sensory role that this arrangement might play leading to a void is totally speculative but may be akin to imminent voiding with strong desire to void. There also appears to be at least two sub-types of fibre associated with the sub-epithelium. Although there is no direct evidence, it is likely that these are afferent fibres. The different nerve fibre types suggests that they could respond to different stimulus modalities. The observation of ‘hot spots’ of innervation suggests possible regions of increased sensitivity to input stimuli. The intertwining and close proximity of the different fibre types may be a micro-anatomical curiosity but it may also allow axon-axon interactions. A functional role for such an axon-axon interaction is unknown but may allow peripheral alteration of afferent nerve outputs. The progressive loss of cgrp-IR fibres in the muscle towards the bulk of the smooth muscle, the sphincter, suggest that this afferent outflow may underlie a different physiological system from that of the sub-epithelium.

<u>Concluding message</u>

The afferent outflow from the proximal urethra cannot now be considered as a simple system responding to a single modality. The observations, demonstrating putative muscle and sub-epithelial fibres suggests at least two afferent systems. With respect to the sub-epithelial system there is additional complexity: at least two fibre types, ‘hot spots’ and intertwined fibres. The physiological systems underpinned by this complexity are unclear. However, complexity must be taken into account in investigations of urethral function and, in particular, electrophysiological characterization of urethral afferent outflow.

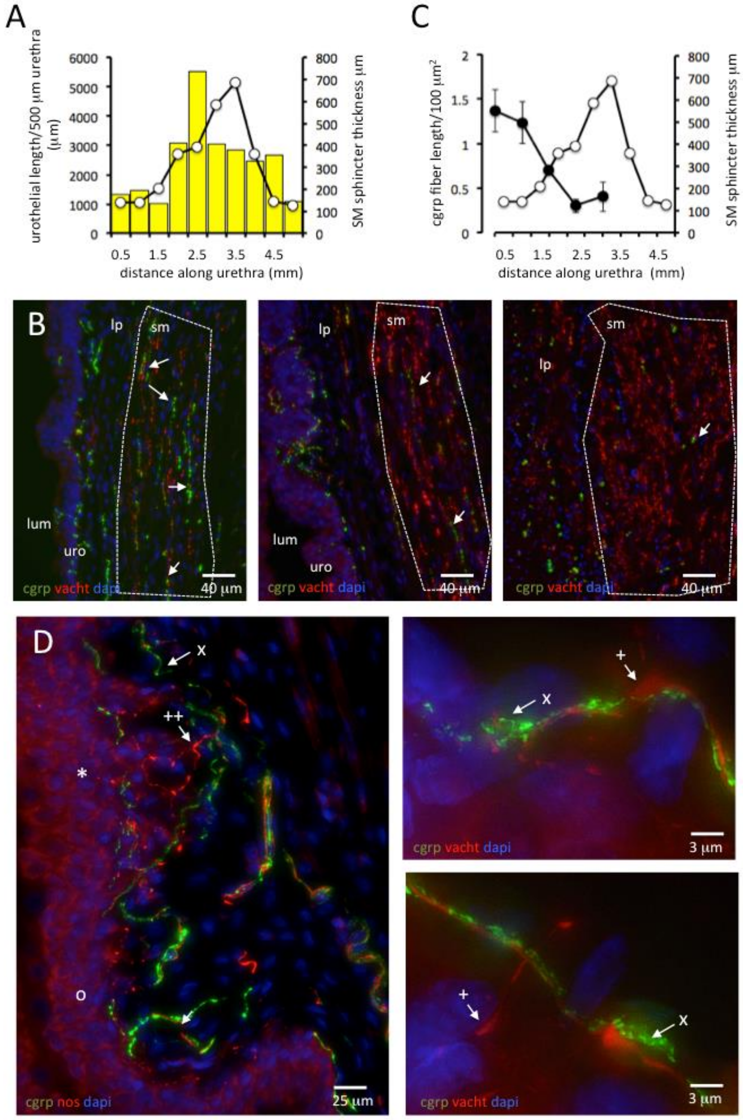

Figure 1. A illustrates measurements of urothelial folding (histogram) and smooth muscle thickness (open symbols) in the proximal urethra. B shows three images of the smooth muscle sphincter of the urethral wall illustrating blocks of smooth muscle (dotted box). cgrp-IR, representing sensory (arrows), and vacht-IR, motor, are illustrated. C, plots again the smooth muscle thickness and superimposes measurements of cgrp-IR fibre density (fibre length (µm)/100 µm^2 muscle cross-section (●)). D illustrates cgrp-IR (x) and nos-IR (++) fibres close to the epithelium and in the lamina propria. Note regions of dense innervation (*) adjacent to a region with few nerves (o). The panels on the right show images at higher magnification showing distinct cgrp-IR (x) and vacht-IR fibres (+), with no co-localisation. These fibres have distinct varicosities and wrap around each other.

Disclosures

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