

## A NOVEL CELL TYPE (RIBBON CELLS) ASSOCIATED WITH THE SUB-EPITHELIUM OF THE PROXIMAL URETHRA OF THE FEMALE RAT

### Hypothesis / aims of study

The physiology of the urethra has been neglected in recent years. However, its central role in preparing for and co-ordinating the initiation and termination of voiding is clear. The microanatomy of the urethra involving epithelial folding and possible smooth muscle sphincter placements suggest complex systems. In addition, the possibility of different afferent nerve types in the proximal and distal urethra further adds to this complexity. During investigation of this complexity it became apparent that there were further complexities with the appearance of unusual cells in the proximal urethra. The aim of this study was to locate and characterise these cells.

### Study design, materials and methods

Urethras (n=10) were dissected from Sprague-Dawley female rats (200-250 grams): 5 anesthetized with Pentobarbital (60mg/kg, intraperitoneal) and 5 killed by cervical dislocation. 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) and visualized with fluorescent secondary Abs.

### Results

Cgrp-IR has been routinely used to identify afferent nerves. During an analysis to determine the diameter of cgrp-IR nerves in the proximal and distal urethra, it became apparent that there were structures that were not easily identifiable as nerves. Figure 1 shows an size frequency distribution illustrating the diameter of cgrp-IR structures in the proximal and distal urethra (Figure 1 A and B). In the distal urethra, nerve fibres were easily identifiable with axons and varicosities (median diameter of axons and varicosities were 0.35 and 1.2 µm respectively). In contrast, in the proximal urethra, in addition to axons with varicosities (median diameters 0.3 and 0.9 µm respectively), there appeared to be a population of larger cgrp-IR structures (median diameter 2.4 µm, range 1.5 to 6.5 µm). In mammalian systems structures of such dimensions are unlikely to be nerve axons.

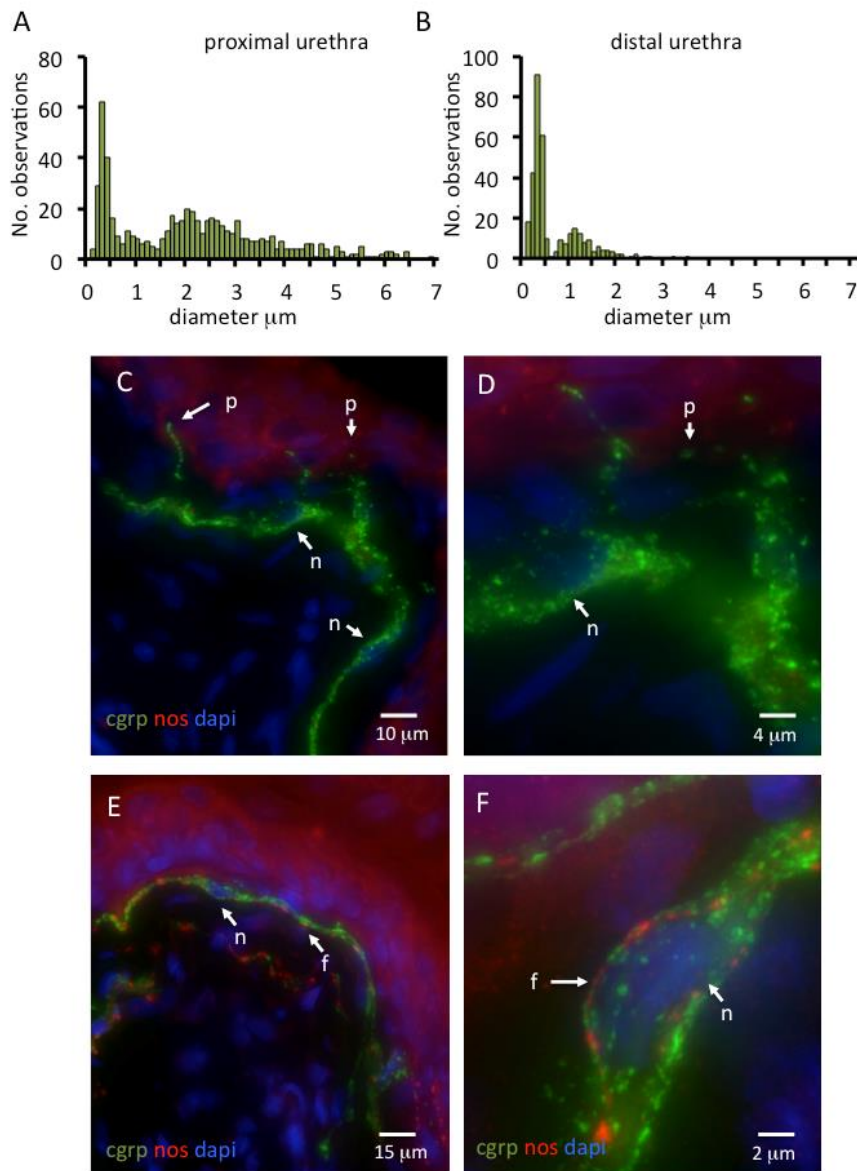
Figure 1, C-F, illustrate the key structural features. (i) the structures were associated with nuclei (C-F, labelled n). Thus, these structures must be considered to be cells. (ii) the distribution of cgrp-IR was evenly distributed over the cell surface including over the nucleus. No evidence of cgrp-IR fibres was seen, indicative that the structure is not a collection of axons (see panels D and E). (iii) the structures were long often up to 200 µm. The structure in panel E is over 160 µm and has the appearance of a ribbon, hence the description of the structures as 'ribbon cells'. (iv) many cells extended processes that made contact with the urethral epithelium ((p) panels C and D). (v) nerve fibres, nos-IR, were also observed to be in close proximity to these structures ((f) panels E and F), running over and around the cells.

### Interpretation of results

The present observations demonstrate the presence of a structure, located primarily in the proximal urethra that is immunoreactive for cgrp. The expression of cgrp-IR would lead to speculation that this might be a sensory axon. However, its diameter and the presence of a nucleus, clearly identifies it as a cell. There are several immediate possibilities. These cells are not neuronal and are some form of interstitial cell with cgrp-IR. Sub-epithelial interstitial cells are found in the rat bladder but these typically are short, fine spindle shaped cells. However, this possibility exists. Secondly they could be neuronal. Afferent neurones, with cell bodies in the periphery have been described in the gut: Dogiel cells. It is possible that these cells are similar to Dogiel cells and could send processes to the CNS. Finally, the close proximity of these cells to the epithelium and the close association with afferent nerves suggest that they could be intermediate accessory receptor cells receiving input from the epithelium and activation from the afferent fibre. All of this is speculation and the function of this system remains unknown.

### Concluding message

The finding of cgrp-IR structures that are clearly cells in the sub-epithelial space is wholly unexpected. The observations and possibilities regarding their function must now be debated and discussed. In addition, methods and approaches need to be identified to study the possible role that these cells may play in epithelia/nerve physiology in the proximal urethra.



**Figure 1.** Illustration of large cgrp-IR structures in the urethra of the female rat. A and B show size frequency distributions for the diameters of these cgrp-IR structures in the proximal and distal urethra respectively. In the proximal urethra a population of large diameter structures is clearly seen. C-F show example of fluorescent images (D is a magnification of C) demonstrating the basic properties of these structures that, because of the presence of nuclei, can be identified as cells. (see text for details).

Disclosures

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