

🏆 Best in Category Prize – Urethra Male / Female

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URETHRAL SEROTONERGIC PARANEURONS PLAY KEY ROLE IN URETHRAL INSTABILITY AND PAIN

Hypothesis / aims of study

Bladder pain, frequent micturition, and urgency are symptoms commonly encountered in patients with painful bladder syndrome/interstitial cystitis, incontinence, and overactive bladder syndrome. A major underlying mechanism for these symptoms is abnormal sensory processing due to alterations in the pathways that detect, process, and transmit sensory stimuli from the periphery to the central nervous system (CNS). Sensory information from the urethra, including flow of fluid, distension, irritation, infection or pH, is critical for bladder function, triggering and/or amplifying bladder contractions and contributing to efficient emptying (1-3). In pathologies, such as infections or incontinence, it is believed that increased excitatory input from the urethra contributes to bladder overactivity and symptoms of urgency, frequency and pain. However, how sensory information from the urethra is detected and transmitted to the CNS is not understood.

We put forth the novel hypothesis that specialized cells within the urethral epithelium (called paraneurons) are part of a 'sensory web', which can detect, shape and transmit specific modalities of sensory information.

The aim of this study is to characterize the morphology and putative function of a class of paraneurons, distinguished from other cells by their serotonergic (5-HT) content.

Study design, materials and methods

Immunohistochemistry and confocal microscopy in whole mount urethrae from adult female C57Bl/6 mice (~25g, 6-8 weeks old) were used to investigate morphological features of 5-HT⁺ cells (paraneurons) and their relationship with nerve fibers. Functional assessments were performed using fura-2AM calcium imaging in acutely dissociated primary afferent neurons labelled by retrograde axonal transport of the fluorescent dye FAST Dil injected into the urethral wall. Activation of nociceptive pathways was assessed using immunostaining for pERK (a marker for neuronal activation) in the lumbosacral spinal cord (L6-S1) and visceromotor reflexes (VMR; a surrogate method for assessing nociception in animals).

Results

Immunohistochemical studies revealed 5-HT⁺ cells of non-epithelial origin (negative for the epithelial markers cytokeratin 5 and 17) that were confined to the epithelium and were characterized by bipolar and multipolar dendritic-like processes (Fig. 1A-C). These cells were located in close proximity (< 3 μm) to sensory nerve fibers positive for calcitonin gene-related peptide (CGRP), substance P (SP) and TRPV1 (Fig. 1A-C). Synapsin I, a presynaptic marker, was present in 5-HT⁺ cells as well as in afferent nerves. In contrast, there was no evidence for a close relationship with either efferent parasympathetic (positive for vesicular ACh transporter or nitric oxide synthetase) or sympathetic nerves (positive for tyrosine hydroxylase).

In vitro 5-HT (1 μM) stimulation of acutely dissociated urethral primary afferent neurons was used to mimic the effect of 5-HT released from paraneurons on afferent nerve activity. We found that this elicited an increase in intracellular calcium concentration ([Ca²⁺]_i) in ~80% of FAST Dil positive neurons (Fig. 1D). These responses were blocked by antagonists of 5-HT₂ and 5-HT₃ receptors (ritanserin and Y-25130 hydrochloride, 1 μM). Approximately 50% of 5-HT responding neurons also responded to capsaicin (0.5 μM).

In vivo, urethral distension up to 40 cmH₂O (pressures which are detected by the urethra during voiding when bladder pressure is equal to urethral pressure) had no effect on VMRs (Fig. 1E). However, intra-urethral 5-HT (1 μM) application, as well as the positive control, acetic acid (1%), significantly increased VMRs triggered by similar urethral distension (Fig. 1E). Intra-urethral 5-HT also activated pERK in the L6-S1 spinal cord neurons known to receive visceral afferent input (*i.e.*, located in the superficial dorsal horn, sacral parasympathetic nucleus and dorsal commissure) (Fig. 1F).

Interpretation of results

These key experiments identified 5-HT⁺ paraneurons that are anatomically positioned to sense the urethral epithelial environment and transmit this information to underlying nerves. Our findings suggest a complex bidirectional communication network with underlying urethral nerves, which may also involve urethral epithelial cells (Fig. 1G).

The results also suggest a crucial role of 5-HT⁺ paraneurons in detection and transmission of noxious information. This is supported by: (1) close proximity of 5-HT⁺ cells to TRPV1⁺ nerves, (2) evidence for expression of presynaptic machinery necessary to release 5-HT, (3) 5-HT induced changes in [Ca²⁺]_i in capsaicin sensitive urethral primary afferent neurons, and (4) activation of pERK in spinal cord neurons. Furthermore, visceral hyperalgesia developed in response to urethral distension after intra-urethral 5-HT perfusion.

Additionally, 5-HT increased [Ca²⁺]_i in non-capsaicin sensitive primary afferent neurons, suggesting that 5-HT can alter the excitability of other non-nociceptive afferents.

It is conceivable that changes in the properties of paraneurons resulting in increased availability of 5-HT could increase afferent input from the urethra. This can trigger or augment ongoing bladder contractions, contributing to urgency and overactivity, influencing lower urinary tract function and visceral sensations including pain.

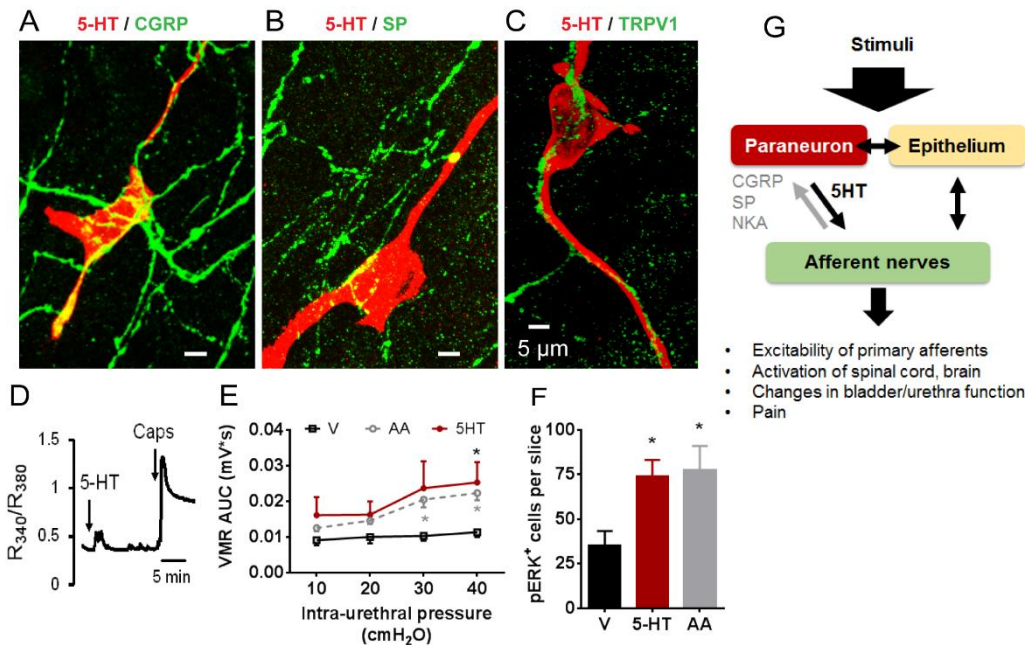


Figure 1. A-C. 5-HT⁺ paraneurons (red) are in close proximity (yellow indicates possible contacts) to nerves fibers (green). **D.** Example of transient [Ca^{2+}]_i increases in a urethral primary afferent neuron in response to 5-HT (1 μM) and capsaicin (0.5 μM). **E.** VMRs to urethral distension in the presence of intra-urethral saline (V), acetic acid (AA, 1%) and 5-HT (1 μM) (n=4 mice per group; * ANOVA p<0.05). **F.** pERK⁺ cells in the L6-S1 spinal cord after intra-urethral perfusion of saline (V), AA and 5-HT (n=5-9 mice per group; * ANOVA p<0.05). **G.** Schematic of urethral 'sensory web' involving paraneurons and epithelial cells that detect mechanical and/or chemical stimuli (e.g., flow, distension, inflammation, pH), and transmit this information to the afferent nerves via release of transmitters (5-HT, others). Signals are then transmitted to the spinal cord and brain. Changes in peripheral 5-HT signalling can increase the excitability of afferent neurons, alter bladder/ urethral function and result in pain.

Concluding message

These morphological and functional findings provide novel insights into a putative paraneuron-neural network within the urethra that utilizes 5-HT signalling to modulate primary sensory pathways carrying nociceptive and non-nociceptive information to the central nervous system. We further postulate that urethral paraneuron-neural interactions could lead to a 'urethral instability' that can influence storage and voiding reflexes and result in symptoms such as urgency and pain. Thus, increased understanding of the paraneuron-sensory web will provide insight into new targets and approaches to modulate signalling pathways for management of lower urinary tract disorders.

References

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Disclosures

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