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AFFERENT NERVE DISTRIBUTION IN THE BLADDER WALL OF YOUNG MICE.

Hypothesis / aims of study

In the urinary bladder, the cholinergic innervation is an essential component of a motor-sensory mechanism. Recent studies show that anti-muscarinics may decrease the afferent activity of sensory nerves [1]. It is therefore important to investigate were sensory nerves in the bladder are located. This study describes the different localizations of afferent nerves throughout the bladder wall in normal mice.

Study design, materials and methods

We used 8 six months old C57BL6 mice. Bladders were removed and incubated in carboxygenated KREBS solution at 36°C. Tissues were fixed in 4% paraformaldehyde and processed for immunohistochemistry. We used a sheep antibody against the vesicular acetylcholine transporter (VAChT) as a marker for the cholinergic innervation and a rabbit antibody against calcitonin gene related peptide (CGRP) was used to visualize afferent nerves. Sheep or rabbit neuronal Nitric Oxide Synthase (nNOS) was used to visualise ganglia.

Results

We observed $VAChT^+$ cholinergic innervation of the muscle layers (Fig. 1). In the lateral wall virtually no $VAChT^+$ innervation was seen in the lamina propria. $CGRP^+$ nerves were present throughout the muscle and the lamina propria (Fig. 1). At the base the number of $VAChT^+$ nerves in the superficial layers of the bladder wall is increased. Close apposition of $VAChT^+$ and $CGRP^+$ nerves was observed (Fig. 2, panel A). Ganglion cells were present adjacent to the serosal side of the outer muscle at the bladder base (Fig. 2, panel B and C). $VAChT^+$ and $CGRP^+$ nerves are neighbouring the ganglia.

Interpretation of results

Since no efferent system has yet been described for the lamina propria these $VAChT^+$ and $CGRP^+$ fibres are presumed to be afferent. In mice afferent nerve fibres from the urinary bladder are relayed to ganglia located outside the bladder wall at the bladder base. The increased number of afferent nerves as well as the specific localization of the ganglia could indicate the main site for afferent processing in a healthy mouse bladder located at the bladder base.

Concluding message

As anti-muscarinics inhibit afferent activity our data suggests that these could work on ganglia located in the base of the bladder.

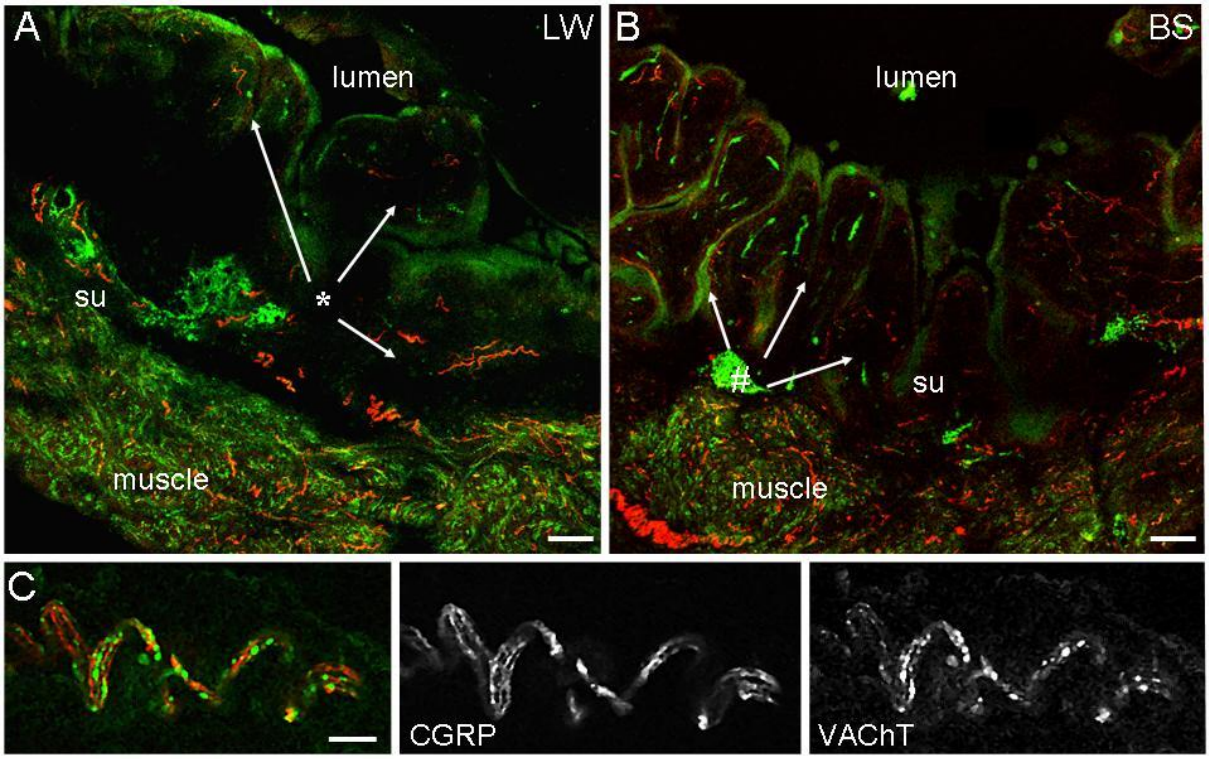

Figure 1. Regional cholinergic and CGRP innervation of the mouse bladder afferent nerve fibres.
A: Lateral wall with sparse cholinergic innervation (VAChT green) in the lamina propria but CGRP (red) fibres are present(* arrows). B: Bladder base, VAChT+ and CGRP+ fibres in the lamina propria (# arrows).
C: Magnification of the suburothelium CGRP+ nerves and VAChT+ nerve running in close proximity to each other.
Calibration bars at 50 µm in A and B and 30 µm in C.

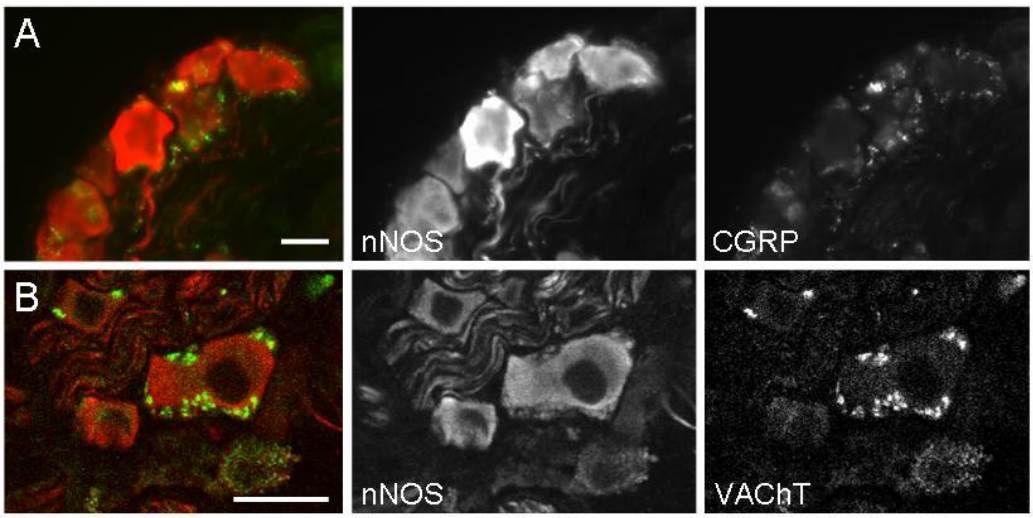

Figure 2. External ganglia found on the outside of the mouse bladder wall.
A: nNOS+ ganglion cells colocalizing with VAChT+ and associated with cholinergic terminals. B: nNOS+ ganglion cells and CGRP+ varicosities associated with these cells. Calibration bars at 30 µm.

References
1. De Laet, K., S. De Wachter, and J.J. Wyndaele, Systemic oxybutynin decreases afferent activity of the pelvic nerve of the rat: new insights into the working mechanism of antimuscarinics. Neurourol Urodyn, 2006. 25(2): p. 156-61.

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What were the subjects in the study?	**ANIMAL**
Were guidelines for care and use of laboratory animals followed or ethical committee approval obtained?	**Yes**
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