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INNERVATED MYOFIBROBLASTS IN THE URINARY BLADDER? FUNCTIONAL AND ULTRASTRUCTURAL EVIDENCE.

Hypothesis / aims of study

It is now clear that the urinary bladder mucosa (urothelium and lamina propria) has contractile properties independent of the detrusor [1]. Dense smooth muscle actin staining on the suburothelial interstitial cells or myofibroblasts suggested that these cells are contractile, and mediate the contraction to exogenously applied NKA in mucosal strips [1]. However, the functional role of endogenous NKA in the mucosa is unclear. We hypothesised that endogenous NKA might originate from suburothelial afferent nerves. Our aims were to examine the ultrastructure of the porcine bladder mucosa and characterize the relationship of the suburothelial myofibroblasts with adjacent cells including suburothelial nerves, and to examine contractility of mucosal strips to nerve stimulation and to potential mediators.

Study design, materials and methods

Functional studies: Pig bladders were obtained on ice from an abattoir, approximately 2 h after death. Portions of the dome were separated into mucosa and detrusor. Mucosal strips (2 x 8mm) were cut and mounted in organ baths in Krebs-Henseleit solution at 37^{o}C under 1 g tension [1]. Some detrusor strips were also prepared for comparative purposes. After equilibration for 1 h, with periodic washing, contractile responses were elicited to a maximum concentration of carbachol (10^{-4}M). This was used to assess the viability of the strips, and standardise their contractility.

On some strips, electrical field stimulation (EFS) was elicited using a range of frequencies (1 - 20Hz), 0.1ms and 60V, for 10s. Other strips were used to examine the concentration-response relationship of the mucosal strips to other agents, including the peptides bradykinin and endothelin and mediators serotonin and histamine. The strips were exposed to increasing concentrations of these agents, at time intervals of 30-60 min, to avoid tachyphylaxis. Tension was monitored using isometric force transducers and recorded; responses were measured in g tension and expressed as percent of the maximal contraction to carbachol [1]. Data were expressed as mean ± SEM.

Microscopic studies: Portions of dome were fixed in Zamboni's solution and processed for immunohistochemistry, or fixed in glutaraldehyde and processed for electron microscopy.

Results

Functional studies: As expected, carbachol (10^{-4}M) elicited increases in tension of both mucosal and detrusor strips. EFS produced substantial contractions of detrusor strips as shown previously in this preparation [2]. In contrast, EFS elicited only very small increases in tension of mucosal strips (n=4) from the same bladders.

Pig mucosal strips also contracted in response to bradykinin and endothelin (Figure 2), with EC_{50} values 319 nM and 220 nM, respectively. However, endothelin elicited a contraction only at concentrations of 100 nM and above. The mucosal strips also contracted weakly in response to serotonin and histamine, with EC_{50} values of 530 nM and 1.4 μM, respectively.

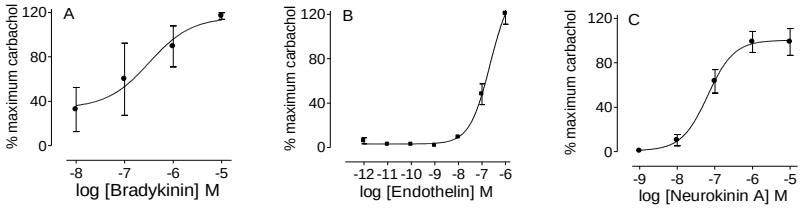

Figure 1. *(A) Bradykinin and (B) endothelin (n=3-4) elicited contraction of pig mucosal strips, compared with (C) neurokinin A, EC_{50} 65 nM (n=6) [from 1]. Data are shown as mean ± SEM.*

Immunohistochemical studies: In low resolution studies (n=4), antibodies to synaptophysin (mouse, Dako) and tachykinins (rabbit, Peninsula) showed positive immunostaining of discrete nerve fibres located within the suburothelial nerve plexus close to the basal urothelial cells and in association with myofibroblasts. Antibodies to the tachykinin NK2 receptor (goat, Santa Cruz) produced unsatisfactory results even in the positive control tissue (human colon).

Electron microscopic studies: The suburothelial population of porcine bladder myofibroblasts (suburothelial interstitial cells) possessed prominent rough endoplasmic reticulum and elongated processes (Figure 2A). Extensive nerve bundles with varicosities were located below the urothelium and in close proximity to the myofibroblasts (Figure 2B). These nerve bundles contained individual varicosities with discrete regions containing synaptic vesicles (Figure 2C). The dense-cored synaptic vesicles were 100-250 nm in diameter, indicating a primarily peptidergic content. Some regions of these nerve endings were bare of Schwann cells and faced the myofibroblast cell, satisfying the definition of neuroeffector junctions, as neurotransmitter release sites.

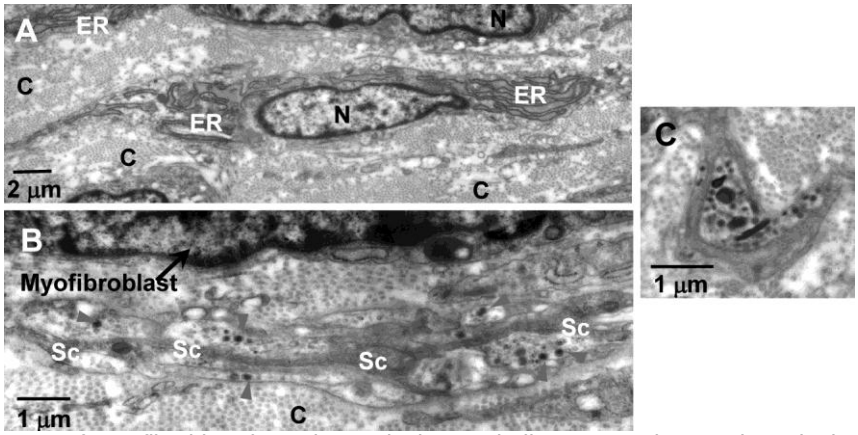

Figure 2. *(A) Ultrastructure of myofibroblast layer beneath the urothelium. Note the rough endoplasmic reticulum (ER). N, nucleus; c, collagen. (B) High magnification of nerve bundle, showing several nerve fibres with synaptic vesicles (arrowheads) and associated Schwann cells (Sc). (C) Individual varicosities contain multiple peptidergic vesicles.*

Interpretation of results

Myofibroblasts are considered to be key cells in afferent signalling in the bladder [3]. In ultrastructural studies, the appearance of the suburothelial cells was similar to published descriptions of human myofibroblasts. The size of the dense-cored synaptic vesicles in adjacent nerves suggests a primarily peptidergic content. The synaptophysin and tachykinin-like immunoreactivity present under the urothelium and the strategic location of suburothelial myofibroblasts between the urothelium and (putative) afferent nerve plexus suggests that they are involved in sensory processing and cross talk between the two cell layers. We originally hypothesised that peptides such as NKA would be released from these dense-cored synaptic vesicle-containing varicosities, to act directly on suburothelial myofibroblasts, resulting in contraction of the porcine bladder mucosa. However, this putative efferent role was not supported by our results showing negligible contractions of the mucosal strips to EFS.

The physiological roles of endothelin and other agents, in this tissue, is unclear. Although able to contract the mucosal strips (Figure 1), they were less potent as contractile mediators compared with NKA. At present it is unknown if the mucosal strip contractions are due to the presence of specific receptors for these peptides on the suburothelial myofibroblasts. Alternatively, receptors may be present on other cell types, causing the release of as yet unidentified mediators, which subsequently cause myofibroblast contraction.

Concluding message

Porcine bladder mucosal strips are able to contract to a number of peptide and non-peptide mediators. Ultrastructural studies showed that cells identified as myofibroblasts, containing prominent rough endoplasmic reticulum and elongated processes, were in close proximity to peptide-containing nerve terminals. These nerves may have a sensory rather than a direct efferent function.

References

1. 1. Br J Pharmacol 153: 1465-1473, 2008.
2. 2. Br J Urol 60: 337-342, 1987.
3. 3. Am J Physiol Renal Physiol. 295: F688-697, 2008.

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Is this a clinical trial?	**No**
What were the subjects in the study?	**ANIMAL**
Were guidelines for care and use of laboratory animals followed or ethical committee approval obtained?	**No**
Statement that no ethical approval was needed	**No ethical approval needed. Bladders obtained from pig carcasses after slaughter.**