

CULTURED CELL MODEL FOR CHARACTERIZING UROTHELIAL, INTERSTITIAL AND DETRUSOR SMOOTH MUSCLE CELL COMMUNICATION

Hypothesis / aims of study

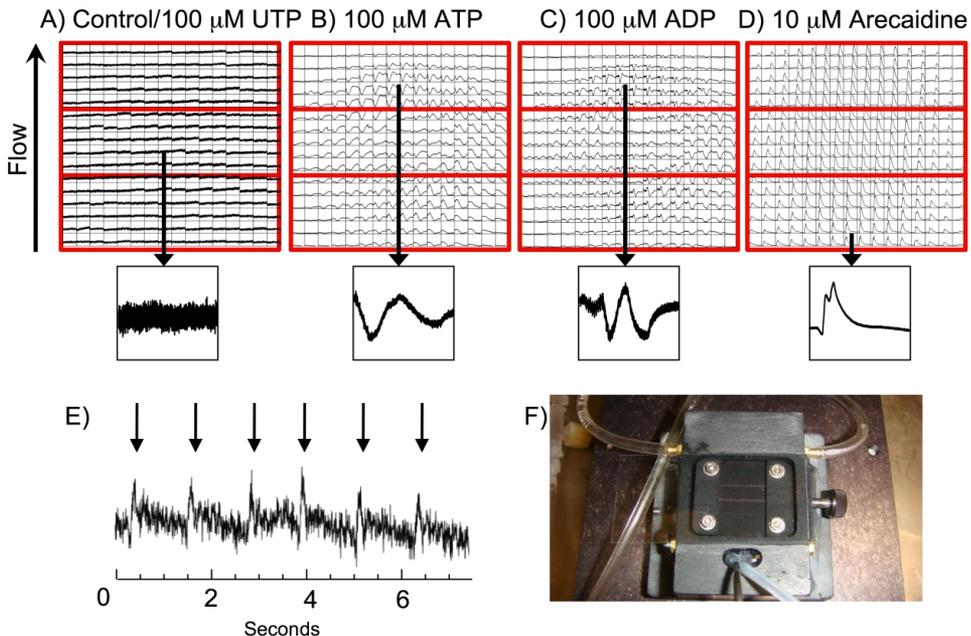
It is well established that urothelial cells release signaling molecules such as ATP or acetylcholine in response to mechanical distension (1). It is believed that this urothelial release plays a role in detecting bladder fullness during filling. However, the targets and mechanisms of action for these signaling molecules from the urothelium are still not entirely clear. One possible target is the network of interstitial cells (IC) that extend throughout the bladder wall. These may act as a communication network to transduce signals from the urothelium to modulate bladder activity by acting upon smooth muscle and/or sensory nerves. In this study, we cultured suburothelial interstitial cells and detrusor smooth muscle and optically mapped Ca^{2+} transients through confluent cultures. This methodology will enable the study of the interactions and the signalling mechanisms involved between urothelial, IC and smooth muscle in the absence of other cell types.

Study design, materials and methods

Isolation of urothelial, suburothelial interstitial and detrusor smooth muscle cells. Adult female C57BL/10 mice were humanely sacrificed and the bladder excised. The bladder was cut open and urothelial layer was scraped, washed off and collected with sterile salt solution. The remaining mucosa was peeled away from the detrusor layer, both mucosa and detrusor tissue were then digested in a collagenase based dissociation solution and plated, along with urothelium cells, onto sterilized 4 x 12mm polyacrylic coverslips and cultured until confluent. The presence of smooth muscle, IC and urothelial cells was confirmed by positive staining for alpha-smooth muscle actin (detrusor), vimentin + c-kit (IC) and pan-cytokeratin (urothelial cells). **Ca^{2+} mapping of cultured cells.** Cells were stained with a Ca^{2+} -sensitive dye (Rhod2-AM, 1 microM) and mapped using methods described previously (2). Up to 3 coverslips of cells were placed into the recording chamber in different combinations and continuously perfused with modified Tyrode's solution (95% O_2 / 5% CO_2 , maintained at 35 Celsius). The recording chamber was then sealed over with a glass coverslip to minimize motion artifact. Drugs were taken from 10 mM stock and added into the perfusion solution from an injection port located before the recording chamber.

Results

Ca^{2+} trace maps from cultured detrusor smooth muscle cells are depicted in figure A-D and a photo image of the recording chamber with cell coverslips is shown in figure F. In these experiments, three coverslips of smooth muscle were recorded and the regions enclosed in red denote separate coverslips on the trace map. Detrusor cells were not spontaneously active under control conditions (figure A). Ca^{2+} transients were elicited in response to 100 microM ATP, ADP and the muscarinic agonist arecaidine (10 μM), but not to 100 μM UTP (figure A-D). Suburothelial interstitial cells in contrast showed spontaneous Ca^{2+} transients under control conditions (figure E).



Interpretation of results

These results demonstrate an *in vitro* method to study the direct interactions of urothelial, interstitial and detrusor smooth muscle cells. The ability of the urothelium or interstitial cells to modulate smooth muscle activity can be studied by using the smooth muscle culture as a "sensor" to detect released signalling factors. Smooth muscle cells did not respond to UTP, however it has been demonstrated that urothelial and possibly IC can respond to this purine (3). Therefore in future experiments UTP will be utilized as a way to selectively stimulate the release of urothelial and IC factors, and measure their response on

smooth muscle activity. Appropriate antagonists will be used to identify these factors. This approach is not possible in in vivo or in tissue preparations.

Concluding message

Demonstration of direct communication between urothelium, IC and detrusor cells has been difficult to achieve. We believe the cultured cell method presented here will allow for such studies. Under pathological conditions there is a proliferation of IC throughout the bladder and increased release of transmitters from the urothelium. It is possible that the combination of these events contribute to increased urge, urgency and potentially detrusor overactivity. Pharmacological modulation of the IC network in the bladder may represent a new avenue for the treatment of overactive bladder symptoms and thus further study of these interactions, particularly from pathological bladders, is necessary.

References

1. Autonomic Neurosci, 153 (1-2); 33-40, 2010.
2. Am J Physiol Renal Physiol, 295(2); F454-61, 2008.
3. Am J Physiol Renal Physiol, 294(4); F821-9, 2008.

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<i>Is this a clinical trial?</i>	No
<i>What were the subjects in the study?</i>	ANIMAL
<i>Were guidelines for care and use of laboratory animals followed or ethical committee approval obtained?</i>	Yes
<i>Name of ethics committee</i>	University of Pittsburgh Institutional Animal Care and Use Committee