INTERSTITIAL CELLS DRIVE OVERACTIVITY IN PATHOLOGICAL BLADDERS—DETERMINED USING OPTICAL IMAGING

Hypothesis / aims of study
Interstital cells have been shown to play a pacemaker role in a variety of smooth muscles, for example, in the intestine and the ureters. Interstitial-like cells have also been identified to be present throughout the bladder, characterized morphologically via a number of markers including the tyrosine kinase receptor, c-kit [1]. The activity of these cells have been shown to be modulated by ATP, acting through P2Y6 receptors [2]. Under normal circumstances, these interstital-like cells do not appear to function as pacemakers. However, following spinal cord transection (SCT), the bladders developed large amplitude contractions with a regular periodicity. Accordingly, our aim was to determine if myofibroblasts are responsible for pacemaker activity in pathological bladders.

Study design, materials and methods
Bladders from normal adult (3 months old) and spinal cord transected (T8-T10, 2 weeks postoperative) rats were harvested and cut from outlet to dome along the dorsal aspect to form a sheet. The sheet preparations were either left intact or partially denuded of the mucosal surface. The bladders were then stained using Ca2+- (10 μM Rhod-2-AM) and voltage- (10 μM Di-4-ANEPPS) sensitive dyes. After staining, the bladders were transferred to a recording chamber, where the base of the bladder was secured to a fixed platform with pins, while the dome was connected to a tension transducer. The bladder sheets were perfused with Tyrode’s solution (95% O2 and 5% CO2, pH 7.35) at 37 °C, stretched to 1 g of tension and imaged from the mucosal surface. Isochronal maps were generated from the local activation time-points from up to 256 optical action potentials and intracellular Ca2+ transients using cross-correlation analysis. The schematic for the optical imaging set-up is shown in Figure 1. Drugs were added to the bath from stock solutions for final working concentrations.

Figure 1. Schematic of optical imaging set-up with a SCT rat bladder sheet

Results
Spontaneous detrusor contractions were significantly higher in SCT animals compared to controls. These contractions were enhanced by stretch and low-dose carbachol to a greater extent in the pathological bladders. Furthermore, the c-kit inhibitor Gleevec (30 μM) and the P2Y6 receptor antagonist MRS2578 (10 μM) reduced the amplitude of contractions by a 55±8.9% and 54±18%, respectively (n=4; Figure 2). There was no significant response to Gleevec or MRS2578 in normal adult bladders. The partially denuded preparations of pathological bladders showed that enhanced Ca2+ and voltage activity was initiated only in the mucosal/suburothelial region when exposed to low dose carbachol. Similar responses were achieved using 10 μM UTP (Figure 3).

Figure 2. Effects of MRS2578 and Gleevec on spontaneous contractions in SCT rat bladders
Figure 3. Alterations in Ca^{2+} transients in partially denuded SCT bladder sheets in response to carbachol, UTP and Gleevec (Red boxed areas denote mucosa denuded regions)

Interpretation of results
These results suggest a role for suburothelial myofibroblasts in modulating detrusor activity through signaling factors released from the urothelium. This activity can be inhibited by using blockers for c-kit and P2Y_6 receptors, which have been shown to be localized to suburothelial myofibroblasts.

Concluding message
The urothelium has been shown to release multiple factors, including ATP [3], in response to various stimuli. The targets for these chemical factors have not been clearly elucidated, but may include lamina propria myofibroblasts whose stimulation may enhance detrusor activity. This enhancement was inhibited in pathological bladders by blocking c-kit and P2Y_6 receptors, suggesting that alterations in urothelial and/or myofibroblast function may be contributing factors to detrusor overactivity.

References
1. BJU Int. 2006. 97(3): p. 612-6
2. BJU Int. 2006. 97(6): p. 1327-31

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