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Ikeda Y[1], Fry C[2], Roppolo J[1], Zabbarova I[1], McCarthy C[1], Hagdorn-Smith N[1], Birder L[1], DeGroat W[1], Kanai A[1]

1. University of Pittsburgh, 2. Imperial College London

INCREASED GAP JUNCTION CONNECTIVITY IN THE SUBUROTHELIAL REGION ENHANCES OVERACTIVITY IN PATHOLOGICAL BLADDERS—MEASURED USING OPTICAL IMAGING

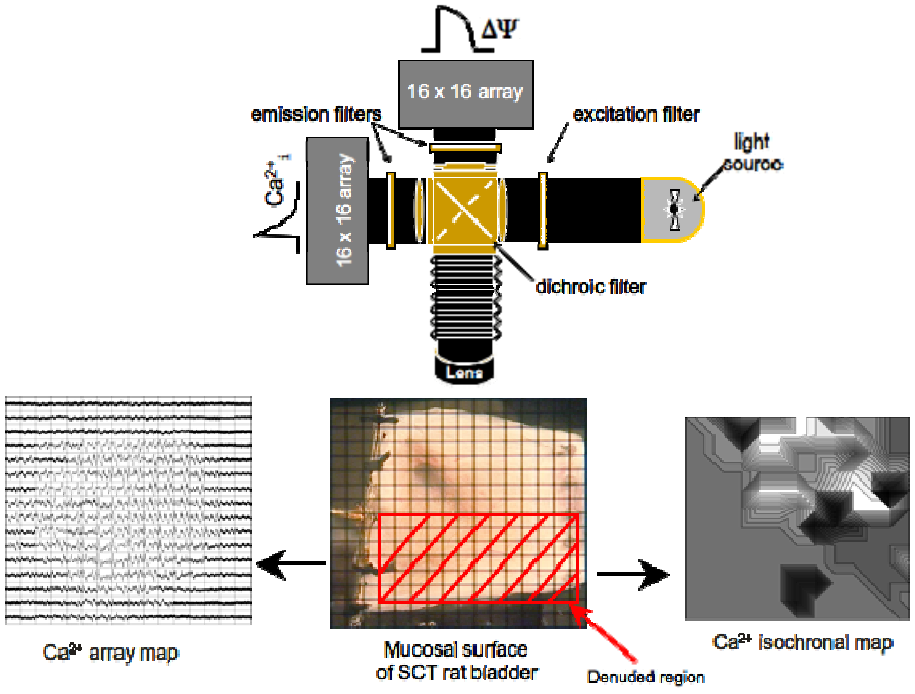
Hypothesis / aims of study

In the bladders of spinal cord transected (SCT) rats, we have histological evidence for increased gap junction expression in the urothelium (Connexin 26) and suburothelial myofibroblasts (Cx43) while expression in the detrusor (Cx 45) remained unchanged. In addtion, there was an increase in the magnitude and regularity of spontaneous bladder contractions. Gap junction blockade abolished these spontaneous contractions. Accordingly, our aim was to determine if enhanced gap junction connectivity correlates with increases in spontaneous activity. We hypothesize that increased communication within the urothelium and/or between lamian propria myofibroblasts plays a role in detrusor overactivity.

Study design, materials and methods

Bladders from normal adult (3 months old) and spinal cord transected (T_8-T_{10}, 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 intact or partially denuded of the mucosal surface. The bladders were then stained using Ca^{2+}- (10 uM Rhod-2-AM) and voltage- (10 uM 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% O_2 and 5% CO_2, 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 for up to 256 optical action potentials and intracellular Ca^{2+} 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 the optical imaging setup with a SCT bladder sheet



Results

SCT rat bladder sheets demonstrated high amplitude, low frequency spontaneous contractions with a regular periodicity. In comparison, normal adult bladders showed irregular low amplitude, high frequency contractions. Application of the gap junction blocker, 18-beta-glycyrrhetinic acid (10 uM), caused a significant reduction in contractile and optical activity in SCT rat bladders. However this was not shown to be the case in normal adult bladder preparations, where the gap junction blocker had no significant effect on contractile or optical activity (Figure 2). Optical imaging of partially denuded SCT bladders showed that spontaneous activity originated from the mucosal layer and could be abolished by gap junction blockade (Figure 3).

Figure 2. Effect of 18-beta-glycyrrhetinic acid on the optical and spontaneous contractile activity in normal adult and SCT rat bladders

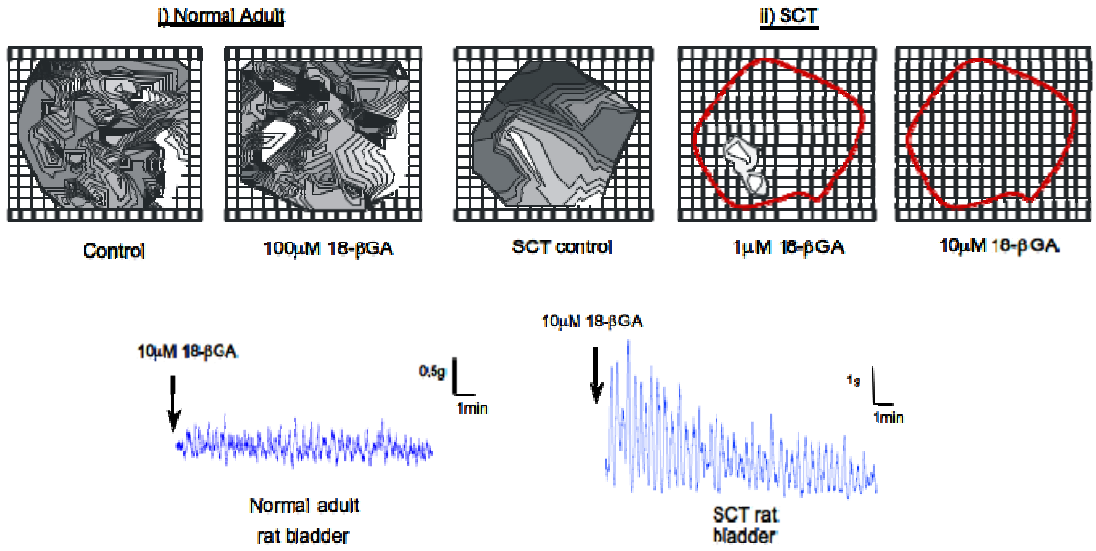
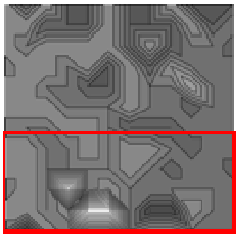


Figure 3. Effect of 18-beta-glycyrrhetinic acid on mucosal activity in partially denuded SCT bladder
(Red boxed area denotes mucosal denuded region)



Ca^{2+} isochronal map

Interpretation of results

This study supports histological evidence from previous studies, where the functional changes can be attributed to increased gap junction expression in the urothelial/suburothelial region of SCT rat bladders. Spontaneous optical and contractile activity was markedly reduced by gap junction blockade. This suggests that there is a signaling mechanism that requires electrical coupling between cells in the urothelium/subruothelium to drive spontaneous contractions.

Concluding message

Factors released from the urothelium may drive spontaneous contractions by activating suburothelial myofibroblasts. We hypothesize that these myofibroblasts form networks *via* gap junctions to transmit signals to the detrusor. Furthermore, that increases in connexin expression results in enhanced propagation which drive spontanous contractions.

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ANIMAL SUBJECTS: This study followed the guidelines for care and use of laboratory animals and was approved by University of Pittsburgh Institutional Animal Care and Use Committee