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THE UROTHELIUM IN FIC (FELINE INTERSTITIAL CYSTITIS) CAT BLADDERS EXHIBITS ALTERED RESPONSES TO UTP—ELUCIDATED USING OPTICAL IMAGING

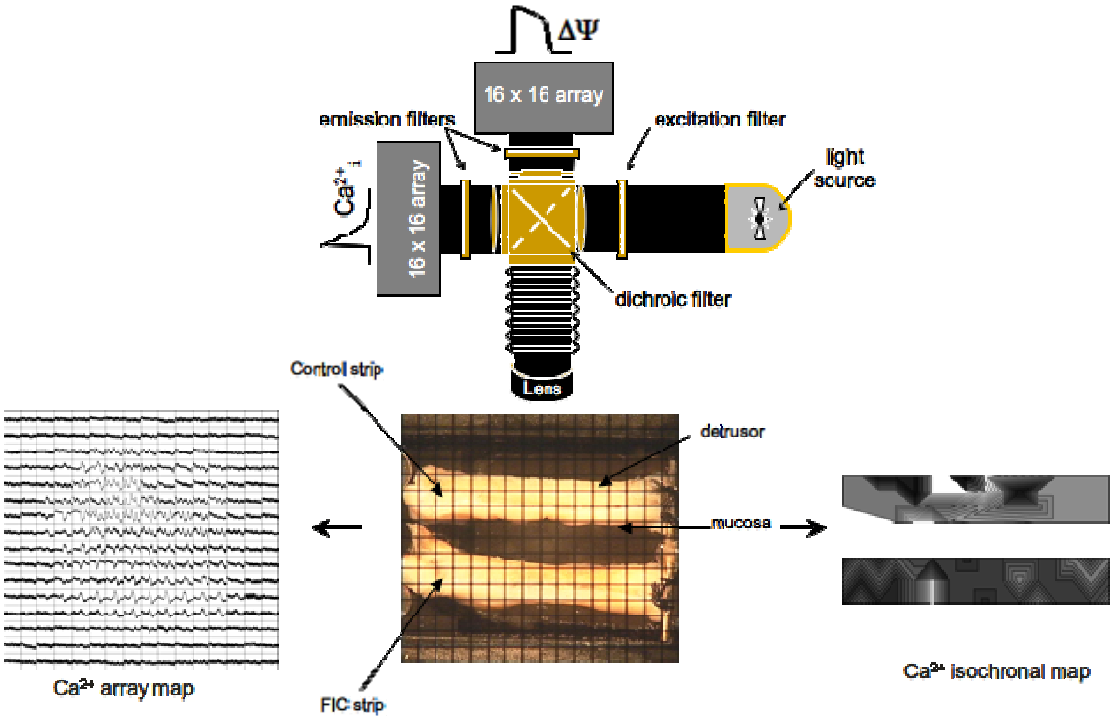
Hypothesis / aims of study

Interstitial cystitis is a condition that causes chronic pelvic pain, specifically attributed to the urinary bladder. The cause and mechanism for the sensitization of the bladder is still not fully understood. However, there is evidence that the urothelial cells in feline interstitial cystitis (FIC) are sensitized and have altered responses to mechanical stimulation. Furthermore, FIC cells were shown to release ATP *via* IP_3 pathway activation, a characterisic not found in control cells [1]. Hence, we investigated the role of ATP and the functional differences between FIC and control cat bladders using optical imaging.

Study design, materials and methods

Bladder samples from healthy age and sex matched control and FIC cats were used for this study. Single full thickness strips, approximately 5 mm in width, were dissected dome to base from control and FIC bladders. The strips were stained with Ca^{2+} (10 uM Rhod-2AM) and voltage sensitive (10 uM Di-4-ANEPPS) dyes. Both control and FIC strips where mounted at one end to a fixed platform with the cut edge facing up, to allow crossectional imaging from the different layers of the bladder. The other ends of the muscle strips were connected to a tension transducer to monitor contractile activity. The bladder strips were placed into an organ bath with Tyrode's solution (95% O_2 and 5% CO_2, pH7.35) at 37°C and stretched to 1 g of tension. 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.

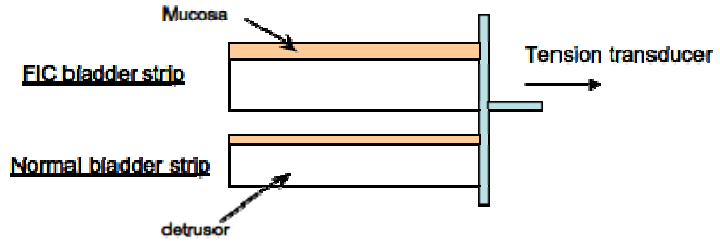
Figure 1. Schematic of the optical imaging setup with FIC and control cat bladder strips

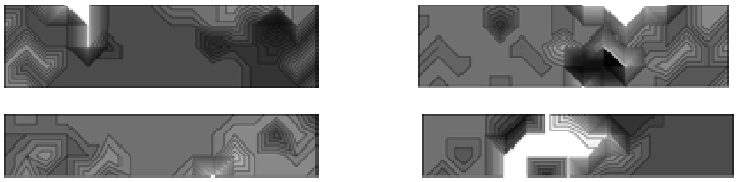


Results

In the bladders of FIC cats, optical imaging demonstrated that Ca^{2+} and voltage activity was initiated in the urothelial/suburothelial region in response to stretch, low-dose (50 nM) carbachol, and 10 uM ATP or UTP (n=2). This response was not seen in the normal bladder strip, where optical activity originated from the detrusor region. Application of the non selective P2 receptor antagonist, PPADS (10 uM), caused a marked reduction in optical activity in FIC strips. When the mucosa was dissected away there was a significant decrease in optical and contractile activity (not shown).

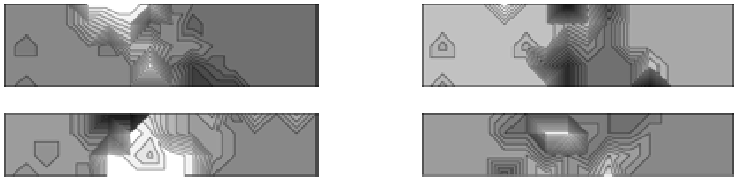
Figure 2. Ca^{2+} isochronal maps of normal cat and FIC bladder strips with various interventions





Resting conditions

50nM carbachol



10μM UTP

10μM PPADS

Interpretation of results

FIC bladders showed a significant amount of optical activity in the urothelial/suburothelial region. This activity was absent in control strips, where Ca^{2+} transients were limited to the detrusor layer. Optical activity in FIC strips was increased with UTP and reduced in the presence of PPADS, suggesting the involvement of P2Y-receptors.

Concluding message

The urothelium in FIC cat bladders has been shown to have enhanced sensitivity which may be due to the increased release of ATP. This may account for the increased optical activity observed in the FIC preprations. This study supports histological evidence from previous studies [2], where the functional changes seen can be attributed to the increased expression of P2Y-receptors.

References

1. AJP Renal Physiol (2003) 285; F423-29
2. AJP Renal Physiol (2004) 287; F1084-91

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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 Commity