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Mucosal Muscarinic Receptors Enhance Bladder Activity in Cats With Feline Interstitial Cystitis

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Abstract

Purpose—Interstitial cystitis is a chronic pelvic pain syndrome of which the origin and mechanisms involved remain unclear. In this study Ca^{2+} transients in the bladder wall of domestic cats diagnosed with naturally occurring feline interstitial cystitis were examined.

Materials and Methods—Cross-sections of full-thickness bladder strips from normal cats and cats with feline interstitial cystitis were examined by optically mapping Ca^{2+} transients and recording tension. Responses of Ca^{2+} activity and detrusor contractions to pharmacological interventions were compared. In addition, pharmacological responses were compared in mucosa denuded preparations.

Results—Optical mapping showed that feline interstitial cystitis bladders had significantly more spontaneous Ca^{2+} transients in the mucosal layer than control bladders. Optical mapping also demonstrated that feline interstitial cystitis bladders were hypersensitive to a low dose (50 nM) of the muscarinic receptor agonist arecaidine when the mucosal layer was intact. This hypersensitivity was markedly decreased in mucosa denuded bladder strips.

Conclusions—In feline interstitial cystitis cat bladders there is increased Ca^{2+} activity and sensitivity of muscarinic receptors in the mucosal layer, which can enhance smooth muscle spontaneous contractions.

Keywords

urinary bladder; cystitis, interstitial; cats; cat diseases; receptors, muscarinic

INTERSTITIAL cystitis is a chronic pelvic pain syndrome with symptoms similar to those of bacterial cystitis (urinary frequency, urgency, nocturia and pain) with no indication of bacterial infection on conventional urine culture or cytology. Research into the causes and mechanisms underlying IC have been hampered by the limited number of animal models that mimic the idiopathic nature of the disease. However, domestic cats have been shown to have a syndrome called FIC, which shares many characteristics seen in humans with IC.¹

Numerous studies based on the FIC model have shown evidence that bladder urothelium dysfunction is a factor contributing to IC symptomology.² The urothelium is a highly impermeable barrier that prevents toxic components in urine from crossing to muscle. It also

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acts as a sensory organ that responds to mechanical and chemical stimulation by releasing neurotransmitters, such as ACh,³ ATP⁴ and nitric oxide.⁵ It is hypothesized that these factors can act on suburothelial afferent nerves and interstitial cells to modulate bladder activity during filling.

In FIC cases the urothelium has been shown to have decreased transepithelial resistance, and increased water and urea permeability compared to controls in response to hydrodistention.² This indicates that barrier function is compromised, which could lead to the sensitization of sensory nerves by irritants from urine crossing into the muscle layer. Alterations in the expression of purinergic receptors,⁶ stretch induced release of ATP⁷ and nitric oxide release⁸ from FIC urothelial cells have also been identified. Results of these studies suggest that ATP release may also be modulated by muscarinic receptors on the urothelium.⁷ When ACh is released from urothelial cells by mechanical stimulation, it may act in autocrine fashion to evoke ATP release. Alterations in ACh release or signaling pathways may contribute to urothelial dysfunction in FIC cases.

We examined the role of muscarinic receptor activation in full-thickness cross-sections of FIC and normal adult cat bladder strips with or without mucosa. Optical mapping of Ca^{2+} transients was done to measure the changes in fluorescence intensity induced by calcium entering the cell, mainly through voltagegated calcium channels. Tension measurements were made to record spontaneous smooth muscle contractions.

MATERIALS AND METHODS

All procedures were conducted in accordance with Ohio State University and University of Pittsburgh institutional animal care and use committee policies.

Animals

Six healthy cats and 5 with FIC were used in this study. All cats with FIC were obtained as donations from clients and FIC was diagnosed at Ohio State University Veterinary Teaching Hospital using established criteria.⁹ Healthy, age matched control cats were obtained from commercial vendors and determined to be free of disease and signs referable to the lower urinary tract symptoms according to the same diagnostic criteria as cats with FIC. All cats were housed in stainless steel cages at the Ohio State University animal facility and allowed to acclimate to their environment for at least 3 months before being studied.

Bladder Excision

The bladder was excised from deeply anesthetized (98% O_2 /2% isoflurane) cats. Anesthesia was determined to be adequate for surgery by periodically testing for absence of the withdrawal reflex to a strong pinch of the hind paw and absence of an eye blink reflex to tactile stimulation of the cornea. After removing the tissue the animal was sacrificed by an overdose of sodium pentobarbital (70 to 80 mg/kg intravenously).

Tissue Preparation for Optical Imaging and Tension Recording

Strips approximately 5 mm wide were cut dome to base from control and FIC cat bladders. Each strip was stained with the Ca^{2+} sensitive dye rhod-2-AM (1 μM in Ca^{2+} -free Tyrode's solution) (Molecular Probes®) and incubated at 37°C for 30 minutes. The dye was removed and the tissue was washed 3 times with Tyrode's solution.

After staining, the outlet end of a normal and FIC bladder strip was pinned to a fixed platform in a temperature controlled recording chamber. The strips were placed with the cut surface facing up to allow imaging of the different layers of the bladder wall. The dome end

was tied with 5-zero silk suture to a bar made of hypodermic tubing, which was then connected to a tension transducer. The tissue strips were perfused and allowed to equilibrate in Tyrode's solution with 95% O₂/5% CO₂ at 37°C. Contractile activity was measured by a tension transducer attached to a hydraulic micro-manipulator (Narishige) controlled by a stepper motor to enable precise stretch protocols. In mucosa denuded strips the mucosa was dissected by cutting through the lamina propria region of the bladder strip with care taken not to damage the detrusor layer.

Optical Mapping Setup

Intracellular Ca²⁺ transients were recorded from full-thickness bladder cross-sections, as described previously.^{10,11}

Signal Analysis

Conduction delays of the Ca²⁺ transients were determined from the cross-correlation analysis of their waveforms. A custom-built photodiode array imaging system (fig. 1) was used to record calcium transients from the cross-sections of 2 bladder walls (fig. 2, *A* and *B*). Cross-correlation analysis was used to determine the conduction delays between calcium transients to construct isochronal maps (fig. 2, *D*), as previously described.¹² Individual traces in a windowed region were overlaid using custom software to determine the earliest transient to occur on each wall in the windowed region. These transients served as the reference traces for correlation analysis (fig. 2, *C*). For this analysis we calculated the correlation coefficient between a reference trace and each calcium transient at shifting time points, that is the maximal correlation occurring when the 2 signals were optimally overlaid. The maximal correlation coefficients and corresponding time delays were calculated for the windowed calcium transient in every pixel that recorded a signal.

Ca²⁺ transients were visually represented as propagating waves by generating isochrones linking points on the bladder with similar conduction delays. Lighter and darker regions of the grayscale isochronal maps indicated the earliest and latest regions, respectively, to undergo activation.

Solutions and Chemicals

For optical imaging studies bladder strips were bathed in Tyrode's solution containing 118 mM NaCl, 25 mM NaHCO₃, 4.7 mM KCl, 1.2 mM MgCl₂·0.6H₂O, 1.8 mM CaCl₂, 6.0 mM glucose and 5.0 mM Na pyruvate gassed with 95% O₂/5% CO₂ (pH 7.4). For Ca²⁺ sensitive dye staining modified Ca²⁺ free Tyrode's solution was used, composed of 118 mM NaCl, 4.0 mM KCl, 6.0 mM glucose, 5.0 mM Na pyruvate, 0.4 mM NaH₂PO₄ and 10 mM HEPES. Test chemicals were added to the physiological solution as aliquots from aqueous or dimethyl sulfoxide stock solutions.

Statistics

The occurrence of spontaneous and arecaidine mediated Ca²⁺ activity in FIC and normal cat bladder mucosa was compared using Fisher's exact test with significance considered at $p < 0.05$.

RESULTS

Optical Maps of Spontaneous and Muscarinic Evoked Ca²⁺ Transients in FIC and Normal Bladder Strips

Optical mapping of spontaneous intracellular Ca²⁺ transients, expressed as isochronal maps to show where initiation began, revealed that initiation started at different sites in FIC vs

normal adult cat bladder wall cross-sections. Spontaneous Ca^{2+} transients in FIC bladders began in the mucosa and subsequently involved the detrusor, whereas all those in normal cat bladders began in the detrusor (fig. 3, *A* and *B*). This enhanced spontaneous activity in the mucosa was observed in all 5 FIC preparations examined and in 2 of 6 normal cat bladder strips, indicating significantly more spontaneous activity in FIC mucosa than in normal bladders (Fisher's exact test right $p = 0.045$).

Histology of normal and FIC cat bladders showed significant changes in the structure, particularly in the lamina propria (fig. 3, *C* and *D*). In FIC bladders there was a suburothelial gap, edema in the submucosa and increased visibility of submucosal blood vessels in the absence of inflammatory infiltrate. These findings, which are characteristic of neurogenic inflammation, may indicate the potential cause of the change in spontaneous activity seen on optical imaging.

Optically measured spontaneous Ca^{2+} transients initiated small bladder contractions, as measured using a tension transducer in FIC and normal bladder strips. The addition of low dose (50 nM) arecaidine, a muscarinic agonist, enhanced the amplitude and frequency of these contractions in the 2 types of strip. Figure 4 shows the Ca^{2+} isochronal maps of FIC and control cat bladder strips recorded simultaneously in the presence of 50 nM arecaidine. In FIC strips arecaidine mediated Ca^{2+} transients were initiated from the urothelium and the detrusor layer, whereas in normal bladder strips they only began and spread out from the detrusor. Although the resulting activity recorded was the sum of the contractions of FIC and normal bladder strips, figure 4 shows that the pattern of tension recording resulted predominately from Ca^{2+} transients generated in the mucosa of the FIC strip. Activation in the mucosa in response to 50 nM arecaidine was observed in all 5 FIC preparations and in 2 of 6 normal cat bladder strips. This demonstrates that the urothelium in cats with FIC was more sensitive to muscarinic agonists than the urothelium in control animals (Fisher's exact test right $p = 0.045$). In contrast, adding 10 μM of the α -adrenergic agonist phenylephrine had no effect on either preparation in altering contractile or optical activity.

Effect of Mucosa Removal on Arecaidine Evoked Optical and Contractile Activity

The effects of muscarinic and adrenergic agonists were also tested in mucosa denuded bladder preparations. Without the mucosa spontaneous Ca^{2+} transients and contractions were initiated in the detrusors of FIC and control cat bladder strips, and could be enhanced by low dose (50 nM) arecaidine but not by phenylephrine (fig. 5, *A* and *B*). Figure 5, *C* shows the Ca^{2+} isochronal maps of intact and mucosa denuded FIC bladder strips. In the intact FIC strip spontaneous activity was initiated in the mucosa, whereas in the denuded preparation activity was initiated in the detrusor, similar to that seen in normal cat detrusor. These findings suggest that spontaneous activity still occurs in the detrusor of FIC bladders, although activity in the mucosa precedes that in muscle.

One of the 5 FIC strips tested showed significant enhancement of spontaneous detrusor contractions in response to 50 nM arecaidine, which was abolished when the mucosa was removed from the FIC strip (fig. 6).

DISCUSSION

Although the cause(s) and mechanism(s) responsible for IC remain unclear, there is increasing evidence that urothelium has a significant role in the development and/or progression of the condition. In this study we observed differences in spontaneous Ca^{2+} transient activity in FIC and normal cat bladders. Urothelium appeared to modulate contractile and signaling activity in the bladder strips from cats with FIC but not in strips from healthy cats. The optical mapping approach allowed us to determine the initiation sites

of Ca^{2+} transients that evoked muscle contractions spontaneously and in response to stimuli. We found that the initiation of spontaneous activity in cats with FIC appeared to occur from the mucosal surface, whereas normal cat bladders showed random patterns of activity originating from the detrusor muscle layer, most likely corresponding to activity intrinsic to smooth muscle.¹³ In FIC strips signals appeared to propagate from the urothelium/suburothelium into the detrusor layer, which may reflect a mechanism for increased sensitivity associated with IC. These results suggest the propagation of signals from the mucosal layer in cats with FIC that does not occur in normal cat bladders. This might be due to augmentation of the release of various factors, such as ACh or ATP, from urothelium. These may in turn act on suburothelial structures such as sensory nerve endings, leading to afferent activation and possibly to sensitization.

The mechanism of the propagation of signals from urothelium to detrusor has not been fully elucidated. Studies of the bladder of other species indicate that suburothelial myofibroblasts/interstitial cells may be involved in the response of urothelial signaling factors, for example ATP and nitric oxide. These released factors are thought to stimulate interstitial cell activity, which could then modulate intrinsic smooth muscle activity by communicating to other interstitial cells in the detrusor layer via gap junctions or directly to smooth muscle cells.¹⁰ Structural changes in the FIC urothelium/suburothelium also suggest that there might be changes in the interstitial cell network as a result of neurogenic inflammation.¹⁴

We did not find any significant difference in the effect of muscarinic stimulation by arecaidine on contractile activity in FIC vs normal bladders except in 1 bladder in a cat with FIC. This may have been due to variability in symptoms, which has been shown to occur in IC cases. It is possible that there is a range of muscarinic receptor sensitivity, in which in some cases mucosal sensitivity is high enough to induce detrusor overactivity, as we observed. We have previously reported that the stimulated release of ACh from the detrusor in cats with FIC was not different from that in healthy cats.¹⁵ However, in the current study Ca^{2+} isochronal maps revealed different patterns of activation between the groups. The spread of Ca^{2+} activity from the mucosa to the muscle layer was found in FIC bladders spontaneously or in response to arecaidine, implying the activation of muscarinic receptors in urothelium. This result was further supported by removing the mucosa, which decreased the effect of arecaidine on spontaneous activity. This suggests that there is an increase in urothelial ACh release and/or mucosal muscarinic receptor sensitivity in FIC bladders. Changes in nonneuronal ACh signaling has also been noted in the bladder after spinal cord injury¹⁶ and as a consequence of aging.¹⁷

As previously stated, urothelial cells are capable of synthesizing and releasing ACh in response to bladder distention.³ Previous studies in cultured urothelial cells indicate that ATP release is potentiated by urothelial muscarinic receptor activation.⁷ Increased ACh release from the mucosa could theoretically lead to increased ATP release. This could be a major contributing factor to IC symptoms because ATP has been implicated in bladder afferent sensitization, possibly through $\text{P2X}_{3/2}$ receptors located on sensory terminals in the mucosal layer.¹⁸

Increased plasma concentrations of NE¹⁹ as well as increased content and release from the bladder¹⁵ have been found in cats with FIC. This is thought to be a result of alterations in central sympathetic activity, which in turn may have consequences on afferent activity. In this study there appeared to be no significant change in optical or contractile activity caused by the α_1 -adrenoceptor agonist phenylephrine in FIC or control bladders. This suggests that there is little change in contractile activity caused by NE in the bladder by α_1 -adrenoceptors. However, NE may still have a significant role regarding sensory function, which was not evaluated in this study.

We have previously noted significant changes in urothelial barrier function, stretch mediated release of factors and purinergic receptor expression in bladders from cats with FIC.⁶ Our current study extends these findings by demonstrating increased Ca^{2+} activity in the mucosal layer of FIC bladders. We hypothesize that urothelium or suburothelial nerves/myofibroblasts contribute to this enhanced activity. Briefly, we have identified a shift in the locus of the propagation of excitatory signals from detrusor to urothelium and an alteration in the role of muscarinic receptors in excitatory signaling from the urothelium in cats with FIC. Further characterization of the specific receptors involved as well as how mucosal muscarinic receptors augment the detrusor response in FIC cases is currently under investigation.

CONCLUSIONS

Increased spontaneous Ca^{2+} activity is present in the mucosal layer of the bladder of cats with FIC compared to that in normal adult controls. There was also a differential response to muscarinic activation between these bladders that depended on the presence of the mucosa. Changes in the expression or sensitivity of mucosal muscarinic receptors may have a contributing role in IC symptoms.

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Abbreviations and Acronyms

ACh	acetylcholine
ATP	adenosine triphosphate
FIC	feline IC
IC	interstitial cystitis
NE	noradrenaline

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Figure 1.
Dual photodiode array optical imaging system used to record Ca^{2+} transients.

Figure 2.

Generation of grayscale isochronal maps from Ca²⁺ transient array maps of FIC and control cat bladder strips (A). Ca²⁺ transient maps (B) were analyzed using cross-correlation analysis by manually selecting reference channel with earliest transient, to which all other channels were correlated. Note windowed Ca²⁺ transient, reference trace and maximal correlation (C). Grayscale isochronal maps were generated to represent pattern of Ca²⁺ spread and conduction delays for each bladder strip (D).

Figure 3.

Pattern of spontaneous Ca^{2+} activity in isochronal maps from FIC (*A*) and normal (*B*) cat bladder strips. FIC cat bladder strip shows that Ca^{2+} activity originated in mucosa, while in normal bladder strip activity originates in detrusor. Isochronal delay was 0.12 milliseconds for each map. White areas indicate earliest Ca^{2+} transients. Darker areas indicate later time points. Histological micrographs reveal normal (*C*) and FIC (*D*) cat bladder wall. Black bars indicate urothelial, lamina propria and muscle layers, respectively (*C*). Reduced from $\times 40$.

Figure 4.

Optical mapping response of FIC and normal cat bladder strip to 50 nM arecaidine. Isochronal maps (A) were generated from Ca²⁺ transients (B) recorded simultaneously in FIC and control cat bladder strips after treatment with 50 nM arecaidine with isochronal delay of 0.15 milliseconds each. Ca²⁺ transients showed that activity was initiated in FIC strip mucosa and in control strip detrusor. Overlay (C) of Ca²⁺ transients from 1 pixel at earliest initiation site (black trace) with tension measurements from 2 strips (red trace) shows that mucosal transients precede and drive contractions in FIC strip, indicating that enhanced spontaneous activity originated from mucosal layer of FIC strip and not of normal bladder strip.

Figure 5.

Pattern of spontaneous Ca^{2+} activity in isochronal map of mucosa denuded FIC cat bladder strip. Photo images show mucosa intact (*A*) and denuded (*B*) cat bladder strips overlain with reticule defining photodiode array alignment. Ca^{2+} isochronal maps from same FIC strip before (*C*) and after (*D*) mucosa removal demonstrate that denuding FIC strip altered intrinsic Ca^{2+} activity pattern, showing that it originated from detrusor layer and likely represents smooth muscle intrinsic activity. Isochronal delay was 0.12 milliseconds.

Figure 6.

Effects of 50 nM arecaidine on tension recordings in full-thickness and mucosa denuded FIC cat bladder strips. Adding 50 nM arecaidine induced phasic contractile activity (*A*) and dissecting mucosa caused significant decrease in arecaidine mediated contractile response (*B*). *g*, gm.