Feline interstitial cystitis results in mechanical hypersensitivity and altered ATP release from bladder urothelium

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Birder, L. A., S. R. Barrick, J. R. Roppolo, A. J. Kanai, W. C. de Groat, S. Kiss, and C. A. Buffington. Feline interstitial cystitis results in mechanical hypersensitivity and altered ATP release from bladder urothelium. Am J Physiol Renal Physiol 285: F423–F429, 2003—ATP can be released from a variety of cell types by mechanical stimulation; however, the mechanism for this release and the influence of pathology are not well understood. The present study examined intracellular signaling mechanisms involved in swelling-evoked (exposure to a hypotonic solution) release of ATP in urothelial cells from normal cats and cats diagnosed with interstitial cystitis (feline interstitial cystitis; FIC). Using the luciferin-luciferase bioluminescent assay, we demonstrate that swelling-evoked ATP release is significantly elevated in FIC cells. In both normal and FIC cells, ATP release was significantly decreased (mean 70% decrease) by application of blockers of stretch-activated channels (amiloride or gadolinium), as well as brefeldin A and monensin (mean 90% decrease), suggesting that ATP release occurs when ATP-containing vesicles fuse with the plasma membrane. Swelling-evoked release was reduced after removal of external calcium (65%), and release was blocked by incubation with BAPTA-AM or agents that interfere with internal calcium stores (caffeine, ryanodine, heparin, or 2-aminoethoxydiphenyl borate). In addition, agents known to act through inositol 1,4,5-triphosphate (IP3) receptors (thapsigargin, acetylcholine) release significantly more ATP in FIC compared with normal urothelium. Taken together, these results suggest that FIC results in a novel hypersensitivity to mechanical stimuli that may involve alterations in IP3-sensitive pathways.

EPITHELIAL CELLS ARE OFTEN subjected to mechanical disturbances, such as shear force, changes in cell volume due to alterations in osmolarity, and mechanical stretch or distension (14, 27). For example, epithelial cells that form the lining of the urinary bladder (uroepithelial cells) are consistently subjected to mechanical stretch as well as changes in osmolarity during the course of a normal micturition cycle (28). These changes may lead to a coordinated endocytosis and exocytosis at the cell membrane, changes in epithelial morphology, and release of bioactive substances such as nitric oxide or ATP (4, 28).

A number of studies have lent support for the idea that mechanical stimuli can evoke the release of ATP from epithelial cells lining “tubes” or “sacs” such as the urinary bladder (10). In addition, extracellular ATP, most likely of urothelial origin, has been implicated in the distension-evoked activation of bladder afferents (14). Once released from epithelial cells after bladder stretch, ATP is thought to activate purinergic receptors on submucosal afferent fibers and thus may play a role in sensory functions such as nociception (10). In fact, mice lacking the P2X3 purinergic receptor subunit (normally expressed by a subset of bladder afferents) exhibit normal distension-evoked urothelial ATP release but diminished reflex bladder contractions and voiding behavior and a reduction in the behavioral (pain) response to injection of ATP (30). Thus mechanically evoked ATP release from epithelial cells may play a major role in both volume- and pain-mediated reflexes.

Despite the growing interest in the sensory role of ATP, the mechanisms responsible for mediating mechanically evoked ATP release are not well understood. Several lines of evidence suggest that ATP release may involve mechanosensitive ATP channels, ATP transporters, or fusion of ATP-containing vesicles with the plasma membrane (i.e., exocytosis) (6). It has been suggested that the latter may be in part a Ca2+-dependent process, whereby increased intracellular Ca2+ can trigger secretion of ATP and other bioactive mediators.

The release of algogenic agents such as ATP has been demonstrated after tissue injury or inflammation (13). Here, altered release of ATP from either injured or sensitized cells may play a direct role in sensitizing nociceptors and thereby contribute to the initiation of pain and inflammatory responses. In models of rheumatoid arthritis, increased ATP levels are thought to contribute to activation of nociceptors because antagonists of ATP reduce pain behavior (12).

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Although altered release of bioactive mediators such as ATP has been demonstrated in pathological bladder conditions such as interstitial cystitis (IC) (26), the mechanism for this altered release is unknown. IC is a chronic pelvic pain syndrome of unknown cause and no generally accepted treatment (24). Symptoms include pain referable to the urinary bladder and increased frequency and urgency of urination. IC may affect more than 700,000 American women and a significant proportion of men with prostatitis or prostatodynia. A comparable disorder has been found in domestic cats, and this syndrome, which exhibits many of the hallmarks of IC in patients, is termed feline interstitial cystitis (FIC) (8). The aims of the present study were to evaluate whether FIC alters swelling-induced ATP release from bladder urothelium. In addition, a number of agents were also evaluated to examine the mechanisms for ATP release and for FIC-induced urothelial hypersensitivity to mechanical stimulation.

MATERIALS AND METHODS

All procedures were conducted in accordance with Institutional Animal Care and Use Committee policies.

Animals

Healthy and FIC adult cats were used for this study. All cats with FIC were obtained as donations from clients due to a history of chronic recurrent stranguria, hematuria, pollakiuria, and/or urination in inappropriate locations and were evaluated at The Ohio State University (OSU) Veterinary Teaching Hospital. Evaluation consisted of a complete physical examination (including body weight), complete blood count, serum biochemical analysis, urinalysis, urine bacteriological culture, and cystoscopy. Cystoscopy was performed using a 9-F rigid pediatric cystoscope (Karl Storz, Endoscopy America, Culver City, CA) in female cats and a 3-F flexible fiber optic cystoscope (Five Star Medical, Sun Jose, CA) in male cats. The diagnosis of FIC was based on compatible history and consideration of standard National Institutes of Health inclusion and exclusion criteria after the results of the above laboratory tests were obtained, including the presence of submucosal petechial hemmorhages (glomerulations) at cystoscopy (8). Healthy, age-matched cats obtained from commercial vendors and determined to be free of disease and signs referable to the lower urinary tract according to the same diagnostic criteria as cats with FIC were used as controls. All cats were housed in stainless steel cages in the OSU animal facilities and allowed to acclimate to their environment for at least 3 mo before the study.

Urothelial Cell Culture

Preparation and characterization of urothelial cultures have been described in previous reports (2). Briefly, bladders were excised from deeply anesthetized (α-chloralose, 60–70 mg/kg; 2% halothane) cats (of either sex), cut open, and gently stretched (urothelial side up). Anesthesia was determined to be adequate for surgery by periodic testing for the absence of a withdrawal reflex to a strong pinch of a hind paw and absence of an eyelid reflex to tactile stimulation of the cornea. After tissue removal, all animals were killed via an overdose of anesthetic. The tissue was incubated overnight in minimal essential medium (Cellgro, Mediatech, Herndon, VA), penicillin/streptomycin/fungizone, and 2.5 mg/ml disodium ATP (Invitrogen, Rockville, MD). The urothelium was gently scraped from underlying tissue, treated with 0.25% trypsin, and resuspended in keratinocyte medium (Invitrogen). The dissociated cell suspension (0.1 ml, 50,000–150,000 cells/ml) was plated on the surface of collagen-coated dishes and maintained in culture for 1–3 days. Because long-term maintenance of cells in culture could significantly change the properties of some types of cells (1), the cells in this study were examined after a short time in culture. In general, all cells were used within the first 3 days after plating. All cells in culture were cytokeratin positive (DAKO, Carpinteria, CA) and, therefore, were presumably of epithelial origin.

Measurement of ATP Release

In this study, we used exposure to a hypotonic solution as an in vitro method for evoking mechanical stress. It has been established that hypotonic stress or swelling shares a number of common characteristics with mechanical stretch or distension (15). In fact, our previous studies have demonstrated a similar release of ATP from urothelium using both hypotonicity- and stretch-evoked paradigms (4). The extracellular ATP concentration was measured by using luciferin-luciferase bioluminescence (5). Cells were seeded on collagen-coated culture plates, and each plate contained an average of 10^5 cells before the experiment. The data are presented as femtomoles ATP released in all cases, and values for each experiment were normalized per milligram protein. Because a mechanical disturbance has been demonstrated to alter release of ATP, all cells were carefully washed with oxygenated physiological solution containing (in mM) 4.8 KCl, 120 NaCl, 1.1 MgCl2, 2.0 CaCl2, 11 glucose, and 10 HEPES (pH 7.4; 25°C; 1 ml/min flow rate) until a stable baseline was achieved. The media was switched from isotonic to a hypotonic (244–260 mosM) Ringer solution, and 100–1,000 μl samples were collected using a Retriever II automated fraction collector (Isco, Lincoln, NE). The luciferin-luciferase reagent (100 μl; Adenosine Triphosphate Assay Kit, Sigma) was added to each sample, and bioluminescence was read using a luminometer (TD-20/20, Sunnyvale, CA). The detection limit was ~10 fmol ATP/sample. The concentration of all agents tested was chosen based on effectiveness in other cell types (epithelial, endothelial, or smooth muscle). All antagonists were incubated for a minimum of 10 min before sample collection. Because phosphates and bicarbonates found in most physiological media aggressively chelate Gd^{3+}, disabling its stretch-activated channel (SAC)-blocking ability and often leading to false-negative results (11), Gd^{3+} studies were performed with bicarbonate- and phosphate-free HEPES-buffered solutions in all experimental and control groups. Unless otherwise noted, all chemicals were obtained from Sigma and were of reagent grade or better.

Data Analysis

Pooled data results are given as means ± SE, and statistical significance was determined using Student’s unpaired t-test. Each figure represents data collected from a minimum of six independent experiments from a minimum of six different cats. P ≤ 0.05 was regarded as significant.

RESULTS

FIC Alters Swelling-Evoked, But Not Basal, ATP Release From Urothelial Cells

It has been well established that fluid movement during perfusion can elicit release of ATP due to effects

AJP-Renal Physiol • VOL 285 • SEPTEMBER 2003 • www.ajprenal.org
of shear force. In either preparation, slow perfusion elicited a small but not significant ($P > 0.05$) increase in the amount of ATP over nonperfused baseline. Basal release of ATP was similar for both normal (20 ± 3 fmol ATP) and FIC (35 ± 5 fmol ATP) urothelial cells. In both normal and FIC urothelial cells, constant perfusion of a hypotonic solution (20% decrease in osmolarity; 244–260 mosM) elicited a substantial release of ATP compared with isotonic perfusion at the same flow rate. In contrast to normal urothelial cells, FIC urothelium exposed to the same treatment released a significantly larger amount of ATP (285 ± 45 (FIC) vs. 140 ± 22 fmol ATP (normal)) (Fig. 1, Table 1). No significant differences in hypotonicity-evoked ATP release were detected between cells cultured from 1–3 days in either preparation (data not shown). Multiple applications of hypotonic stimuli (applied to the same cells) elicited similar increases in ATP (range 125–165 fmol ATP in normal; 260–340 fmol ATP in FIC). To test for cell viability and the possible contribution of cell lysis to ATP release after hypotonic tests, two dyes, one of which (0.23 μM ethidium homodimer-1) enters through the damaged membrane of dead cells and binds to nucleic acids (produces a bright red fluorescence in dead cells), and 0.12 μM calcein-AM, which is retained in live cells (producing a green fluorescence in live cells), were added to cultures before hypotonic swelling (Live/Dead viability/cytotoxicity assay kit, Molecular Probes, Eugene, OR). Both calcein and ethidium homodimer-1 can be viewed simultaneously using a conventional fluorescein long-pass filter. Afterward, live and dead cells were counted in three random locations in each well (6 control and 6 after hypotonic stimuli) in every experimental condition. Each experiment typically yielded one to two damaged cells/culture plate, demonstrating that cell lysis after hypotonic swelling is not a contributing factor in ATP release (Fig. 1).

**FIC-Mediated Alterations in Swelling-Evoked ATP Release Are Not Altered by Cell Passage**

To evaluate whether the alterations in ATP release in FIC urothelium may be an inherent defect within the urothelium or possibly a transient in vivo insult to the urothelium that disappears in culture, ATP release with the application of hypotonic swelling was compared after passage and replating of cells (up to 3 passages were tested). In each passage, hypotonic swelling evoked an increase in ATP concentration similar to that in the first culture (Fig. 1). Passaged FIC cells compared with normal cells also displayed a significantly greater release of ATP after swelling. All experiments were performed in similar numbers of cells plated and used within 1–3 days after initial plating.

**Modulation of ATP Release**

**Inhibition of SACs.** Amiloride or Gd$^{3+}$, both of which block certain types of SACs, was tested on hypotonicity-evoked release of ATP. Application of either Gd$^{3+}$ (10 μM; 20-min incubation) or amiloride (10 μM; 20-min incubation) significantly decreased ATP released by a hypotonic stimulus (60–75% maximal decrease; Fig. 2, Table 1) in both normal and FIC urothelium. Although there was no significant difference in the effect of Gd$^{3+}$ on FIC compared with normal urothelium, Gd$^{3+}$ was more effective than amiloride in both preparations in decreasing hypotonicity-evoked ATP release.

**Inhibition of vesicular exocytosis or trafficking.** Stretchlor activated release of ATP may also be a result of vesicular exocytosis. We evaluated both an inhibitor of vesicle formation, monensin, as well as brefeldin A (BFA), which disrupts the Golgi complex and vesicle trafficking to the cell surface, thereby blocking protein secretion (19). Both monensin (10 μM) as well as BFA (10 μM) blocked hypotonicity-evoked release of ATP from both normal and FIC urothelial cells (Fig. 2, Table 1).

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**Fig. 1.** Swelling-evoked ATP release in normal and feline interstitial cystitis (FIC) urothelial cells. Graph depicts amount of swelling-evoked ATP release (fmol ATP release/mg protein) from isolated normal or FIC urinary bladder urothelial cells (A) as well as after passage and replating of cells (2 passages are depicted (B and C)). Values are means ± SE from recordings in a minimum of 6 independent experiments. Photomicrograph (B) depicts a group of urothelial cells after exposure to hypotonic stimuli. Arrow indicates a cell with damaged membrane, which exhibited a bright red ethidium homodimer-1-induced fluorescence. In remaining cells, calcein dye is retained and produces a green fluorescence in live cells. Ethidium homodimer-1 is excluded by the intact membrane of live cells. *$P < 0.05$.**
Role of Ca$^{2+}$ in ATP Release

The inhibitory effect of monensin and BFA suggests that swelling-mediated release of ATP is mediated in part by vesicular exocytosis and raises the possibility that it is a Ca$^{2+}$-dependent process. This was evaluated by reducing external Ca$^{2+}$ (0 Ca$^{2+}$/EGTA buffer; 1-h perfusion). Hypotonicity-evoked ATP release in both normal and FIC urothelium was significantly reduced (mean reduction, 65%) in 0 Ca$^{2+}$. In contrast, reducing external Ca$^{2+}$ did not alter basal release in either normal or FIC urothelium. Longer perfusion times in Ca$^{2+}$-free extracellular media did not further decrease ATP release in either preparation. However, incubation with the Ca$^{2+}$ chelator BAPTA-AM (2 μM; 30 min; 0 Ca$^{2+}$) resulted in a complete block of swelling-evoked ATP release (Table 1, Fig. 3A). The effect of elevating intracellular Ca$^{2+}$ by incubation with the ionophore A-23187 (10 μM; 20 min) was also tested (3). In the presence of Ca$^{2+}$ but in the absence of stretch or other stimuli, the ionophore alone released a significant amount of ATP (230 ± 49 fmol ATP) from both normal and FIC urothelial cells, with no significant difference between the two preparations.

The reduction of hypotonicity-evoked ATP release by elimination of external Ca$^{2+}$ and complete blockade with BAPTA suggests an involvement of internal Ca$^{2+}$ stores. The role of intracellular Ca$^{2+}$ concentration was further evaluated by treating urothelial cells with caffeine (10 mM), which depletes intracellular Ca$^{2+}$ stores (22). Prolonged exposure to caffeine (10 mM; in the absence of external Ca$^{2+}$) diminished the stretch-evoked ATP concentration in both normal (79% decrease) and FIC (88% decrease) urothelial cells. Similar results were obtained after incubation with ryanodine (10 μM; 30 min) (Table 1, Fig. 3B), a selective blocker of the ryanodine receptor Ca$^{2+}$ release channel (16). Both agents released a low concentration of ATP (20–40 fmol ATP) in the absence of hypotonic stimulation.

The effects of two agents, heparin and 2-aminoethoxydiphenyl borate (2-APB), known to interfere with inositol 1,4,5-triphosphate (IP$_3$) receptor (IP$_3$R) channels, were also tested. While either agent alone released a low amount of ATP (10–35 ± 7 fmol ATP), incubation with either heparin (10 μM; 30 min) or 2-APB (20 μM, 20 min) significantly decreased hypotonicity-evoked ATP release (Table 1, Fig. 3C). The residual release [likely generated through release via ryanodine receptors (RyRs)] was blocked after incubation with ryanodine (in the presence of 2-APB). These experiments suggest hypotonicity-evoked ATP release involves two intracellular Ca$^{2+}$ components, release through both IP$_3$ and RyRs.

Enhanced ATP Release in FIC Urothelium May Be Due to Altered IP$_3$ Sensitivity

A brief incubation with the Ca$^{2+}$ ionophore A-23187 (10-s exposure; in the absence of Ca$^{2+}$), which did not release ATP alone, potentiated hypotonicity-evoked ATP release in normal urothelium (mean 300%) but did not alter release in FIC urothelium (Fig. 4). To further test whether intracellular Ca$^{2+}$ or Ca$^{2+}$ sensitivity is altered in FIC, agents that stimulate IP$_3$ receptors were tested. Administration of the Ca$^{2+}$ pump inhibitor thapsigargin (10 μM) in the absence of external Ca$^{2+}$ or mechanical stimulation elicited a significantly larger ATP release in FIC (240 ± 25 fmol ATP) compared with normal (110 ± 18 fmol ATP) urothelium (Table 1, Fig. 4). Another agent, acetylcholine (10 μM), known to stimulate the IP$_3$ pathway, also elicited ATP release (Table 1, Fig. 4). This release was significantly elevated in FIC (190 ± 22 fmol ATP) compared with normal urothelium (85 ± 16 fmol ATP) (Fig. 4), and this release was significantly decreased [78% decrease; 42 ± 6 (FIC) vs. 18 ± 6 fmol ATP]

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**Table 1. ATP release in normal or FIC urothelium**

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<thead>
<tr>
<th></th>
<th>Normal</th>
<th>FIC</th>
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<tbody>
<tr>
<td>Basal ATP release (0 Ca$^{2+}$)</td>
<td>20 ± 3</td>
<td>35 ± 5</td>
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<tr>
<td>Basal ATP release (Ca$^{2+}$)</td>
<td>17 ± 2</td>
<td>31 ± 4</td>
</tr>
<tr>
<td>Hypotonicity alone</td>
<td>140 ± 22</td>
<td>285 ± 45</td>
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<tr>
<td>Acetylcholine (0 Ca$^{2+}$)</td>
<td>85 ± 16</td>
<td>190 ± 22</td>
</tr>
<tr>
<td>Thapsigargin (0 Ca$^{2+}$)</td>
<td>110 ± 18</td>
<td>240 ± 25</td>
</tr>
<tr>
<td>Hypotonicity + Amiloride</td>
<td>56 ± 17</td>
<td>114 ± 95</td>
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<tr>
<td>Gadolinium</td>
<td>35 ± 8</td>
<td>72 ± 11</td>
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<tr>
<td>Brefeldin A</td>
<td>12 ± 4</td>
<td>18 ± 3</td>
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<tr>
<td>Monensin</td>
<td>8 ± 2</td>
<td>12 ± 3</td>
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<tr>
<td>0 Ca$^{2+}$</td>
<td>50 ± 11</td>
<td>100 ± 14</td>
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<tr>
<td>A-23187 (0 Ca$^{2+}$)</td>
<td>197 ± 28</td>
<td>189 ± 58</td>
</tr>
<tr>
<td>BAPTA (0 Ca$^{2+}$)</td>
<td>5 ± 2</td>
<td>4 ± 4</td>
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<tr>
<td>Caffeine (0 Ca$^{2+}$)</td>
<td>11 ± 2</td>
<td>12 ± 2</td>
</tr>
<tr>
<td>2-APB (0 Ca$^{2+}$)</td>
<td>27 ± 5</td>
<td>18 ± 5</td>
</tr>
<tr>
<td>Heparin (0 Ca$^{2+}$)</td>
<td>22 ± 3</td>
<td>20 ± 6</td>
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<tr>
<td>Ryanodine (0 Ca$^{2+}$)</td>
<td>5 ± 0.9</td>
<td>17 ± 4</td>
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Values are means ± SE expressed as fmol ATP released. FIC, feline interstitial cystitis; 2-APB, 2-aminoethoxydiphenyl borate.
DISCUSSION

This study established that urothelial cells from cat urinary bladder release ATP in response to hypotonic swelling and that this release is upregulated in cells from cats with FIC. An augmentation in ATP release evoked by mechanical stretching has been reported (26). The similarity in these observations lends further support for the proposal that FIC is a naturally occurring model of human IC. Although the mechanism(s) by which mechanical stimuli can evoke the release of ATP from urothelial or other epithelial cell types is unclear, our findings demonstrate that ATP release occurs in part by a Ca\(^{2+}\)-dependent vesicular exocytotic process that involves an IP\(_3\) signaling pathway which may be altered in FIC.

Our results are in agreement with previous reports that hypotonic swelling, a widely used model of mechanical stimulation, releases ATP from various cell types, including epithelial cells (7). Although mechanical stimulation produced by hypotonic swelling and direct stretch may not be entirely equivalent, our characterization of ATP release in urothelial cells has suggested an involvement of SACs. The effectiveness of the SAC antagonist Gd\(^{3+}\) or amiloride to diminish swelling-evoked ATP release in both normal and FIC urothelium supports this view. Lanthanides such as Gd\(^{3+}\) have been demonstrated to inhibit stretch-evoked changes in cell volume and can also inhibit SACs (31), which have been implicated in ATP release from other types of epithelial cells. Although amiloride has been used to block epithelial sodium channels (ENaCs) and Gd\(^{3+}\) has been used to block SACs, both agents in high concentrations have also been demonstrated to exert a nonselective block of voltage-gated and mechanogated ENaCs, Ca\(^{2+}\) channels, and numerous nonselective cation channels (15). Therefore, the interpretation of the blocking effects of these agents in the present experiments might be complex. The effectiveness of both blockers to decrease ATP release provides support for a multistep process linking mechano-transduction and exocytosis in ATP release from bladder urothelium.

A possible role of vesicular exocytosis in ATP release was examined by evaluating the effects of agents that disrupt the Golgi complex and thereby block mem-

Fig. 3. Role of Ca\(^{2+}\) in ATP release. A: swelling-evoked ATP release in normal or FIC urothelium is diminished in the absence of extracellular Ca\(^{2+}\) [extracellular Ca\(^{2+}\) concentration ([Ca\(^{2+}\)]\(_o\); 100 \(\mu\)M EGTA] and ablated by 2 \(\mu\)M BAPTA-AM. B: swelling-evoked ATP release in normal or FIC urothelium is significantly decreased after incubation with ryanodine (Ryan; 10 \(\mu\)M) or caffeine (Caf; 10 mM) in the absence of extracellular Ca\(^{2+}\). C: swelling-evoked ATP release in normal or FIC urothelium is significantly decreased after incubation with 2-aminoethoxydiphenyl borate (2-APB; 20 \(\mu\)M) or heparin (Hep; 10 \(\mu\)M) in the absence of extracellular Ca\(^{2+}\). Values are means ± SE from recordings in a minimum of 6 independent experiments. \(*P \leq 0.05.\)

Fig. 4. Enhanced ATP release may be due to altered inositol 1,4,5-trisphosphate sensitivity. A: brief addition of a Ca\(^{2+}\) ionophore (A-23187; 2 \(\mu\)M; 2 min) potentiates swelling-evoked ATP release in normal but not in FIC urothelium. B: effect of either thapsigargin (Thap; 10 \(\mu\)M) or acetylcholine (Ach; 10 \(\mu\)M) alone, in the absence of swelling or extracellular Ca\(^{2+}\), released significantly greater ATP in FIC compared with normal urothelium. Values are means ± SE from recordings in a minimum of 6 independent experiments. \(*P \leq 0.05.\)
brane/protein trafficking. BFA blocks protein secretion from cells by suppressing vesicular transport between the Golgi complex and the endoplasmic reticulum (17). BFA treatment blocked hypotonicity-evoked ATP release from urothelial cells. Monensin, which is a sodium ionophore that inhibits vesicle formation and can also prevent receptor recycling (6), had a similar effect.

Our studies also revealed that removal of extracellular Ca\(^{2+}\) suppressed ATP release, whereas chelation of free intracellular Ca\(^{2+}\) with BAPTA-AM abolished it. In addition, elevation of intracellular Ca\(^{2+}\) with the ionophore A-23187 (10 \(\mu\)M) evoked ATP release as noted in other cell types (25), including endothelium and epithelium. These results suggest that swelling-mediated ATP release is due to both Ca\(^{2+}\) influx and release from intracellular stores.

At least two types of Ca\(^{2+}\) release channels, including those that are ryanodine and IP\(_3\) sensitive, seem to be involved in ATP release. Hypotonicity-evoked ATP release was attenuated but not completely blocked by incubation with ryanodine (a selective blocker of RyRs) or caffeine, an agent that activates ryanodine-sensitive Ca\(^{2+}\) release channels and depletes Ca\(^{2+}\) stores. Hypotonicity-evoked ATP release was also reduced but not completely blocked by heparin, a potent blocker of IP\(_3\)Rs, as well as by the IP\(_3\) antagonist 2-APB. The latter was more effective in FIC urothelial cells compared with normal urothelial cells, although a residual response (~20% in FIC) still remained. The residual (IP\(_3\) insensitive) component was completely blocked by ryanodine and thus corresponds to ATP release via RyRs. In some cell types, heparin activates RyRs, raising the possibility that other pathways in addition to those sensitive to IP\(_3\) are involved in ATP release.

These findings suggest that the increased ATP release in FIC may involve activation of the IP\(_3\) signaling pathway. This was tested by applying thapsigargin, a sarco-endoplasmic reticulum Ca\(^{2+}\)-ATPase inhibitor, which causes depletion of intracellular Ca\(^{2+}\) stores. In the absence of external Ca\(^{2+}\) and mechanical stimuli, thapsigargin elicited a robust release of ATP, which was significantly greater in FIC urothelial cells compared with normal cells. Furthermore, the IP\(_3\)-linked muscarinic agonist acetylcholine produced a significantly greater ATP release in FIC compared with normal urothelium, and this release was inhibited by the IP\(_3\) antagonist 2-APB.

A change in the sensitivity of ATP release to intracellular Ca\(^{2+}\) was also evident in FIC cells when the effects of the Ca\(^{2+}\) ionophore A-23187 were examined. Administration of a high concentration of the ionophore alone evoked ATP release to a similar extent in both FIC and normal urothelial cells, suggesting that the sensitivity to Ca\(^{2+}\) is not changed. In contrast, a brief application of the ionophore in the presence of hypotonic swelling potentiated an evoked release of ATP in normal but not in FIC urothelium. These data, taken together with the enhancement of ATP release by thapsigargin or acetylcholine, suggest that FIC may involve alterations in IP\(_3\)-sensitive pathways. It is possible that this change may occur as a result of environmental conditions or an inherent defect within the urothelial cells. For example, inflammation has been shown to alter the expression of a number of Ca\(^{2+}\)-sensor proteins, involved in regulating transmitter exocytosis (18, 29). In the present experiments, the urothelial hypersensitivity does not diminish after cell passage, suggesting that this abnormality may be inherent within the urothelium and not due to an effect of inflammation or injury. While uncovering the mechanism for the alterations in swelling-mediated ATP release requires further experimentation, these results together suggest that FIC results in abnormalities in a Ca\(^{2+}\)-dependent exocytotic pathway.

What is the significance of urothelial hypersensitivity leading to altered ATP release? It has been shown that distension or changes in pressure can release ATP from epithelial cells lining various organs such as the urinary bladder and that this has the potential to activate purinergic (P2X\(_3\) and/or P2X\(_{2/3}\)) receptors on submucosal afferents in close proximity to the epithelium. Distension-evoked activation of bladder afferents can be reduced by purinergic antagonists including suramin or pyridoxal phosphate-6-azophenyl-2′,4′-disulfonic acid (20, 30). In addition, recent studies have demonstrated that ATP administered intravesically induces urinary bladder hyperactivity, most likely by affecting submucosal C fibers (21). These and other data lend support for the idea that bladder afferents, which lie in close proximity to epithelial cells, may play a role in “neural-epithelial” function. Taken together, the results support the idea that increased levels of ATP released from sensitized urothelial cells after distension may activate submucosal purinergic afferents, leading to bladder pain in IC.

Augmented ATP release from urothelial cells could also trigger changes in cell-cell signaling within the urothelium. In a number of cell types, mechanical stimulation induces an immediate and transient elevation of Ca\(^{2+}\) (mediated by P2 purinergic receptors) that quickly spreads to neighboring cells (17). Support for the idea that ATP release may play an autocrine role in urothelial function comes from evidence that urothelial cells also exhibit purinergic (P2) receptors (10). Thus propagation of intercellular Ca\(^{2+}\) “waves” could occur due to release of a diffusible mediator, such as ATP. In fact, triggering of Ca\(^{2+}\) waves within epithelial cells is thought to be a key element in cell signaling, excitability, proliferation, and even cell death (23).

While it has been established that release of Ca\(^{2+}\) from intracellular stores in small discrete regions may have little effect on global cytoplasmic Ca\(^{2+}\) concentrations, it may lead to abnormal local changes in Ca\(^{2+}\) concentration. In some types of cells, this local release of Ca\(^{2+}\), which has been termed Ca\(^{2+}\) “sparks” or “puffs,” is known to activate channels/processes in close proximity to the release site. Thus augmented distension-evoked secretion of ATP from urothelial cells in IC may lead to an autocrine activation of urothelial purinergic receptors and the initiation of Ca\(^{2+}\) waves. Further studies are needed to evaluate the role of urothelial ATP release in both neural-epi-
theal as well as interepithelial signaling within the urinary bladder.

In conclusion, our findings show that IC in cats results in an altered swelling-evoked release of ATP from urothelium. These changes in urothelial hypersensitivity may lead to sensory and urothelial deficits and may play a role in bladder pathologies such as IC.

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DISCLOSURES

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