Cell to Cell Communication in Response to Mechanical Stress via Bilateral Release of ATP and UTP in Polarized Epithelia

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Abstract. Airway epithelia are positioned at the interface between the body and the environment, and generate complex signaling responses to inhaled toxins and other stresses. Luminal mechanical stimulation of airway epithelial cells produces a propagating wave of elevated intracellular Ca\(^{2+}\) that coordinates components of the integrated epithelial stress response. In polarized airway epithelia, this response has been attributed to IP\(_3\) permeation through gap junctions. Using a combination of approaches, including enzymes that destroy extracellular nucleotides, purinergic receptor desensitization, and airway cells deficient in purinoceptors, we demonstrated that Ca\(^{2+}\) waves induced by luminal mechanical stimulation in polarized airway epithelia were initiated by the release of the 5' nucleotides, ATP and UTP, across both apical and basolateral membranes. The nucleotides released into the extracellular compartment interacted with purinoceptors at both membranes to trigger Ca\(^{2+}\) mobilization. Physiologically, apical membrane nucleotide-release coordinates airway mucociliary clearance responses (mucin and salt, water secretion, increased ciliary beat frequency), whereas basolateral release constitutes a paracrine mechanism by which mechanical stresses signal adjacent cells not only within the epithelium, but other cell types (nerves, inflammatory cells) in the submucosa. Nucleotide-release ipsilateral and contralateral to the surface stimulated constitutes a unique mechanism by which epithelia coordinate local and distant airway defense responses to mechanical stimuli.

Key words: intercellular Ca\(^{2+}\) wave • nucleotide-release • airway epithelium • mechanical stimulus • P\(_2\)-receptors

Introduction

Airway epithelia in vivo are exposed to a spectrum of chemical and mechanical stresses. As a part of normal airways defense, epithelia must mount coordinated responses to prevent damage/toxicity. Because the airways epithelia are not innervated, it has not been clear how airway epithelial cells respond in a coordinated fashion to perform these defense-related activities.

Because many of the epithelial defense functions are regulated by changes in intracellular Ca\(^{2+}\) levels, it has been attractive to focus on regulation of Ca\(^{2+}\) signaling in cells exposed to stress. Indeed, mechanically induced intercellular Ca\(^{2+}\) waves have been described in several cell types, including epithelial cells studied under nonpolarized conditions (e.g., hepatocytes, mammary cells) (Enomoto et al., 1994; Schlosser et al., 1996; Frame and de Feijter, 1997), as well as a variety of nonepithelial cells (e.g., basophilic leukemia cells, glial cells, and insulin-secreting cells; Osipchuk and Cahalan, 1992; Cao et al., 1997; Newman and Zahs, 1997). It has been proposed that the propagation of Ca\(^{2+}\) waves in these cells is mediated by extracellular nucleotides. In contrast, a distinct mechanism mediating intercellular Ca\(^{2+}\) waves has been proposed for polarized airway epithelial cells (Boitano et al., 1992; Hansen et al., 1993; Sneyd et al., 1995; Sanderson, 1996; Dirksen 1998; Felix et al., 1998). In this model, IP\(_3\) generated in the mechanically stimulated cell permeates to adjacent cells via gap junctions, resulting in release of Ca\(^{2+}\) from internal stores and propagation of a Ca\(^{2+}\) wave. A number of observations have raised the possibility that extracellular nucleotide signaling may also be pertinent to polarized airway epithelia. For example, polarized airway epithelial cells functionally express P2Y-purinoceptors that are activated by both adenine and uridine nucleotides (Brown et al., 1991; Mason et al., 1991; Paradiso et al., 1995; Hwang et al., 1996; Lazarowski et al., 1997a; Cressman et al., 1998, 1999; Homolya et al., 1999). Fur-
thermore, mechanical stimulation induces release of cellular ATP from airway epithelial cells (Felix et al., 1996; Grygorczyk and Hanrahan, 1997; Watt et al., 1998). More recently, we reported that mechanical stress also elicits UTP-release from airway epithelial cells (Lazarowski et al., 1997b).

Therefore, we tested the hypothesis that extracellular ATP and UTP mediate mechanically induced intracellular Ca\(^{2+}\) waves in airway epithelia. We generated unique airway epithelial model systems, including nasal epithelial cell lines from wild-type and P2Y\(_{2}\)-way epithelial model systems, including nasal epithelial immortalized (x\(^{-}\)).

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Incubated with 5 mM ATP and UTP mediate mechanically induced intercellular Ca\(^{2+}\) waves in airway epithelia. We generated unique airway epithelial model systems, including nasal epithelial cell lines from wild-type and P2Y\(_{2}\)-way epithelial model systems, including nasal epithelial immortalized (x\(^{-}\)).

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Immortalized nasal epithelial cells obtained from normal and P2Y\(_{2}\)-receptor deficient mice) and P2Y\(_{2}\)-way (x\(^{-}\)) cells reconstituted with a P2Y-receptor specific for UTP (human P2Y\(_{2}\); Community et al., 1995; Nguyen et al., 1995; Lazarowski et al., 1997b), to investigate this hypothesis. Importantly, most cultures were grown on polarized supports that allowed access to apical or basolateral surfaces for selective addition of reagents.

**Materials and Methods**

**Airway Epithelial Cell Cultures**

Immortalized nasal epithelial cell lines obtained from normal and P2Y\(_{2}\)-receptor (x\(^{-}\)) mice (Homola et al., 1999) were maintained on 24-mm Transwell Col filters (pore diameter 0.45 μm; Costar) in Ham's F12-based medium containing 10 μg/ml insulin, 5 μg/ml transferrin, 1 μM hydrocortisone, 30 mM triiodothyronine, 25 ng/ml epidermal growth factor, 3.75 μg/ml endothelial cell growth substance, 0.8 mM Ca\(^{2+}\) (total), and an equal amount of 3T3 fibroblast-conditioned DME containing 2% FBS. For imaging studies, the cells were seeded at 5 × 10^4 cells/cm\(^2\) density on Transwell Col filters previously coated with 0.03 mg/ml Vitrogen. Studies were carried out with confluent mouse cultures 5–7 d after seeding. Confluence was assessed visually and by measuring transepithelial electrical resistance (R\(_{t}\)) with an EVOM (WPI). Mean R\(_{t}\) were 344 ± 31 Ω cm\(^2\); n = 122.

For studies with nonpolarized cultures, the cells were seeded on glass coverslips previously coated with 0.3 mg/ml Vitrogen, and cultured for 6–8 h.

To generate a P2Y\(_{2}\)-R (x\(^{-}\)) cell line expressing the human P2Y\(_{2}\)-receptor, the plasmid harboring the DNA sequence of the human P2Y\(_{2}\)-receptor was obtained from Drs. T.K. Harden and R.A. Nicholas (The University of North Carolina, Chapel Hill, NC). A retroviral expression vector was generated as described previously (Comstock et al., 1997). For infection of the P2Y\(_{2}\)-R (x\(^{-}\)) mouse epithelial cell line, the cells were seeded at 3 × 10^4 cells/cm\(^2\)-density, cultured overnight, incubated with the retrovirus and polybrene (8 μg/ml) for 2 h, and then were washed. For selection, the culture was maintained in medium containing 100 μg/ml hygromycin for 24 d. A time/concentration sufficient to kill all cells in mock-infected cultures. For control experiments, a cell line was generated by transducing the P2Y\(_{2}\)-R (x\(^{-}\)) mouse epithelial cell line with a retroviral vector expressing Hygro (alone (Comstock et al., 1997).

For primary human airway cultures, bronchial epithelial cells from lung transplant donors were processed according to protocols as described elsewhere (Matsumi et al., 1998a). For imaging studies, disaggregated airway epithelial cells were seeded at 10^4 cells/cm\(^2\)-density on Transwell Col filters in Ham's F12-based medium supplemented with 5 ng/ml insulin, 500 ng/ml hydrocortisone, 10 ng/ml epidermal growth factor, 3.75 μg/ml endothelial cell growth substance, and 1 mM Ca\(^{2+}\) (total). The human cultures were studied 10–12 d after seeding when significant R\(_{t}\) were established (x = 66 ± 20 Ω cm\(^2\); n = 11).

\[\text{Ca}^{2+}\] Measurements

The cell cultures were washed with hormone free Ham's F12 medium and incubated with 5 μM Fura-2 AM for 30 min at 37°C in the presence of vec-

**Abbreviations used in this paper:** [Ca\(^{2+}\)], intracellular calcium concentration; CFTR, cystic fibrosis transmembrane conductance regulator; P2Y\(_{2}\)-R (x\(^{-}\)), P2Y\(_{2}\)-receptor deficient mice; PAPS, adenosine 3'-phosphate 5'-phosphosulfate; ROI, region of interest.
tor agonist carbachol (see Fig. 1, a and c), and the absence of Fura-2 leakage.

To quantitate wave propagation, \([\text{Ca}^{2+}]\) was measured in a circular region of interest (ROI; 27.5-μm diameter) at the point of stimulation (ROI_0), and at distances 70- and 140-μm (≈4 and ≈8 cells) from the stimulated cell (ROI_70 and ROI_140, respectively; see bright field image in Fig. 1 c, left). The magnitude of the \(\text{Ca}^{2+}\) response was greatest in the stimulated cell and declined as a function of time and distance from this cell (middle). The \(\text{Ca}^{2+}\) level in ROI_0 typically reached its peak eight seconds after the stimulus, whereas peak responses were recorded at 12 and 16 s for ROI_70 and ROI_140, respectively (n = 20). The \([\text{Ca}^{2+}]\) at times of peak responses were used for calculating and plotting mean maximum \(\text{Ca}^{2+}\) responses (right).

**\(\text{Ca}^{2+}\) Wave Propagation Across Discontinuous Airway Epithelia: Sensitivity to Apyrase**

We used two approaches to test whether the intercellular \(\text{Ca}^{2+}\) waves required cell–cell contact. First, we generated short-term cultures (overnight) in which the nonpolarized epithelial cells were not confluent, i.e., the cells grew in separated islands (Fig. 2 a). Mechanical stimulation of a single cell induced a \(\text{Ca}^{2+}\) wave (top row) that was transmitted to cells not in physical contact with the stimulated cell. Apyrase (10 U/ml, grade V), an enzyme that rapidly hydrolyses 5'-nucleotide-triphosphates to monophosphates, prevented the propagation of the \(\text{Ca}^{2+}\) signal to the adjacent cells (bottom row), suggesting that the mediator of the \(\text{Ca}^{2+}\) wave was a 5'-nucleotide. The carbachol-induced \(\text{Ca}^{2+}\) response was not affected by apyrase (data not shown).

The second approach tested whether the concept of extracellular nucleotide release was also pertinent after the airway epithelial cells had fully polarized (Fig. 2 b). A small number of cells were physically removed from a region of confluent polarized culture to produce a linear gap and a single cell was stimulated on one side of this discontinuity. The propagating \(\text{Ca}^{2+}\) wave skipped over the gap (Fig. 2 b, top row), indicating involvement of a released,
diffusible substance(s). Note that the magnitude of the responses was not different at equal distances from the stimulated cell (ROI_70 and 140), irrespective of the presence of a gap. Apyrase abolished the transmission of the Ca$^{2+}$ spread (Fig. 2 b, bottom row).

**Evidence that Apyrase Sensitive Mediators (Nucleotide) Are Released from both Apical and Basolateral Surfaces in Response to Mechanical Stimulation**

In polarized airway epithelial cell cultures, apyrase added selectively to the apical bath alone attenuated, but did not block the mechanically induced intercellular Ca$^{2+}$ wave (Fig. 3, top row, compare to Fig. 1). However, addition of apyrase to both the apical and basolateral baths completely abolished wave propagation as defined by the absent Ca$^{2+}$ responses at ROI_70 and 140, without affecting carbachol-induced responses (Fig. 3, lower row). The Ca$^{2+}$ responses in the stimulated cell were markedly (~70%) reduced, but not completely prevented with bilateral apyrase. Thus, we conclude that apyrase-sensitive nucleotides released from polarized epithelial cells into both extracellular compartments participated in the Ca$^{2+}$ wave propagation.
Evidence for Nucleotide (P2) Receptors in Mediating Ca2+ Waves

We next investigated whether the released nucleotides interacted with P2-receptors to generate intercellular Ca2+ waves. To test for expression of P2-receptors in mouse airway epithelia, we characterized nucleotide-induced Ca2+ responses in our preparations (Fig. 4 a). ATP and UTP were effective on both apical and basolateral surfaces, whereas ADP was more effective on the basolateral surface. The equipotency of ATP and UTP suggested P2Y2-receptor expression on the apical membrane (Nicholas et al., 1996). The effectiveness of ADP in addition to ATP/UTP at the basolateral surface suggested expression of both P2Y1 and P2Y2-receptors at this barrier (see below).

To determine whether P2-receptor activation was required for generation of intercellular Ca2+ waves, homologous desensitization protocols were performed. Selective pretreatment of the apical surface with ATP (300 μM) attenuated, but did not prevent, the propagation of Ca2+ signals as compared with control: Δ[Ca2+]i in ROI_70 12 s after mechanical stimulation after apical ATP pretreatment was 35 ± 20 nM (n = 3) versus 171 ± 16 nM (n = 20, control). Similarly, selective pretreatment of the basolateral surface with ATP (300 μM) reduced, but did not abolish, Ca2+ wave propagation as compared with control: Δ[Ca2+]i in ROI_70 were 101 ± 10 nM, and 171 ± 16 nM; n = 3 and 20, respectively. In contrast, simultaneous exposure of P2-receptors on both epithelial surfaces to ATP abolished the propagation of Ca2+ waves (Δ[Ca2+]i in ROI_70 = 0.7 ± 2.8 nM; Fig. 4, b and c). As a test for the selectivity of ATP pretreatment, the carbachol-induced Ca2+ responses were tested at the end of each protocol and were not affected by this maneuver (Fig. 4 c).

To test whether the inhibitory effect of ATP pretreatment on Ca2+ waves occurred at the level of P2-receptor desensitization or downstream in the signaling pathway (e.g., due to desensitization of IP3 receptors), the spread of mechanically induced Ca2+ waves was measured after carbachol pretreatment. No significant differences were found in mechanically induced Ca2+ waves after carbachol pretreatment as compared with control (Δ[Ca2+]i in ROI_70 were 140 ± 24 nM and 171 ± 16 nM, respectively; n = 5 and 20).

These results strongly suggest that extracellular nucleotides released into both apical and basolateral baths upon mechanical stimulation interact with P2-receptors to generate intercellular Ca2+ waves. Again, the Ca2+ response in the mechanically stimulated cell itself was substantially reduced by maneuvers that modulated extracellular nucleotide levels/P2-receptor sensitivity, indicating that the elevation in [Ca2+]i in that cell was dominated by nucleotide release, but also involved a second mechanism.

Adenine Nucleotides Released Across the Basolateral Barrier in Response to Mechanical Stimulation Interact with a P2-receptor

As a second test for the requirement for P2-receptors in Ca2+ wave propagation, and as a reagent to identify which released nucleotides participated in the Ca2+ wave propagation, we studied intercellular Ca2+ waves in nasal epithelial cells from P2Y2-R (−/−) mice (Homolya et al., 1999). Consistent with the absence of P2Y2-R expression, these cells exhibited no significant Ca2+ responses to nucleotides added to the apical bath, and the responses to UTP on the basolateral surface were abolished (Fig. 5 a). The equipotency and equiefficiency of ADP and ATP after basolateral administration suggested that the residual P2Y-receptor on the basolateral membrane of P2Y2-R (−/−) cells was the adenine-selective P2Y1-receptor. The observation that these responses were blocked by the specific P2Y1-
Mechanical stimulation of a single P2Y2-R (−−) cell within a polarized culture induced Ca2+ waves (Fig. 5 b) that were significantly smaller in magnitude than in wild-type [P2Y2-R (+/+) cultures (Δ[Ca2+]i, in ROI_70 were 106 ± 9 nM and 171 ± 16 nM, respectively; n = 11 and 20, P < 0.01). This reduction in magnitude likely reflected the contribution of P2Y2-R to this response.

In contrast to wild-type cells, the propagation of Ca2+ signals was abolished in P2Y2-R (−−) cells by selective basolateral pretreatment with ATP or by basolateral administration of PAPS (Fig. 5 c). The carbachol-induced Ca2+ responses again were not significantly different after ATP pretreatment (201 ± 48 nM) or in the presence of PAPS (127 ± 8 nM) from those in control experiments (187 ± 47 nM), indicating that the inhibitory effect of ATP or PAPS was specific to basolateral P2Y1-receptors. These data are consistent with the notion that apical mechanical stimulation of P2Y2-R (−−) cells induced Ca2+ waves by the basolateral release of adenine nucleotides interacting with basolateral P2Y1-receptors.

Uridine Nucleotides Are also Released from Murine Airway Epithelia

Next, we studied whether UTP also contributed to the propagation of mechanically induced Ca2+ waves. Our approach was to test whether the expression of a UTP-specific receptor in an airway epithelial system that did not exhibit Ca2+ waves would reconstitute this response. P2Y2-R (−−) cells deficient in native UTP-sensitive purinoceptors in the presence of basolateral PAPS to block P2Y1-receptors do not exhibit Ca2+ waves in response to mechanical stimulation (see Fig 5 c, right). We infected this cell line with a retrovirus containing the cDNA of the human P2Y4-receptor, which is highly selective for UTP.
over ATP or UDP (Communi et al., 1995; Nguyen et al., 1995; Lazarowski et al., 1997b). The functional expression and characterization of the P2Y4-receptor was verified by the nucleotide-induced Ca\(^{2+}\) responses (Fig. 6a). The cells transduced with the Hygro\(^{-}\)-only vector (Control) showed no significant Ca\(^{2+}\) responses to nucleotides, but exhibited carbachol responses (left). In contrast, UTP, but not other triphosphate nucleotides nor UDP, stimulated substantial elevations in cytosolic Ca\(^{2+}\) level in P2Y\(_4\)-R transduced cells (right). The UTP-induced Ca\(^{2+}\) response was not affected by the P2Y\(_1\)-receptor antagonist (PAPS): \(\Delta[Ca^{2+}]_i\) were 130 ± 38 nM and 124 ± 28 nM in response to 1 \(\mu\)M UTP in the presence and absence of 100 \(\mu\)M PAPS (n = 3 and 4), respectively.

Mechanical stimulation of a single cell did not induce Ca\(^{2+}\) wave in the P2Y\(_2\) (-/-) cells transduced with the Hygro\(^{-}\)-only vector, when 100 \(\mu\)M PAPS was present in the basolateral bath (Fig. 6, b and c, left), whereas large Ca\(^{2+}\) waves were observed in P2Y\(_2\)-R transduced cells (right). Desensitization of the P2Y\(_4\)-receptors with bilateral UTP pretreatment abolished the Ca\(^{2+}\) wave propagation in the P2Y\(_4\)-receptor expressing cells: \(\Delta[Ca^{2+}]_i\) in ROI_70 after UTP pretreatment was 1.27 ± 0.14 nM, n = 3. These results strongly suggest that focal mechanical stimulation induced extracellular UTP release, which contributed to the generation of Ca\(^{2+}\) waves.

**Nucleotide-release and Ca\(^{2+}\) Waves in Human Airway Epithelia**

To assess the relevance of our findings in mice to other species, we studied human bronchial epithelial cells in well-differentiated, polarized cultures. These cells also exhibited mechanically induced intercellular Ca\(^{2+}\) waves (Fig. 7a). Wave spread was attenuated by apical addition of apyrase and completely abolished by bilateral treatment with apyrase (Fig. 7b), indicating that the phenomenon of bilateral 5’ nucleotide-release observed in the mouse model systems was relevant to human airway cells.

**Discussion**

Unlike previous studies (Boitano et al., 1992; Hansen et
We demonstrated initially that for nonpolarized airway epithelial cells grown in discontinuous culture, i.e., islands (Matsui et al., 1997), cell–cell contact was not required for mechanically induced intercellular Ca\(^{2+}\) waves in airway epithelial cells, consistent with a role for a diffusible substance as had been reported by others (Fig. 2). We extended this concept to studies that asked whether Ca\(^{2+}\) waves were transmitted across physical gaps created in polarized, confluent airway epithelia. Ca\(^{2+}\) waves jumped across these gaps, indicating a role again for a diffusible substance when airway epithelia are polarized. A series of experiments (Figs. 2, 3, and 7) revealed that the diffusible substance(s) were sensitive to apyrase, an enzyme that cleaves 5'-purine and pyrimidine nucleotides into monophosphate nucleotides that are not recognized by P2Y-receptors. These data thus indicate a role for 5'-triphosphate nucleotides in this response. Further investi-
gation revealed that both adenine and uridine 5' nucleotides were released in response to mechanical stimulation and that the release occurred both ipsilateral and contralateral to the mechanical stimulus. The contribution of adenine nucleotides to Ca\(^{2+}\) wave propagation was demonstrated by the experiments with the P2Y\(_2\)-R (-/-) cells, in which a residual adenine nucleotide-selective receptor (P2Y\(_1\)) expressed on the basolateral membrane was activated by adenine nucleotides released across that barrier (Fig. 5). The participation of uridine nucleotides (UTP) in this response was demonstrated in studies with P2Y\(_2\)-R (-/-) cells reconstituted with the UTP-specific hP2Y\(_4\)-R (Fig. 6). The observation that apyrase was required in both baths to block mechanically induced Ca\(^{2+}\) waves in wild-type cells (Fig. 3) and P2Y\(_2\)-R (-/-) cells expressing hP2Y\(_4\) (Fig. 6) demonstrated that both adenine and uridine nucleotides were released across both barriers.

The involvement of P2-receptors in sensing released nucleotides and triggering Ca\(^{2+}\) release was likewise demonstrated in a series of studies. Pretreatment with ATP to induce homologous desensitization abolished Ca\(^{2+}\) waves, consistent with a role for P2Y-receptors (Fig. 4). The contribution of the P2Y\(_2\)-receptor to the epithelial response was studied using P2Y\(_2\)-R deficient nasal cells and revealed a 40–50% reduction in responses in cells without P2Y\(_2\)-R as compared with wild-type cells (Figs. 5 c, left and 1 c, right). The contribution of the P2Y\(_1\)-receptor on the basolateral membrane of murine airway epithelia was demonstrated by the virtual abolition of Ca\(^{2+}\) waves in P2Y\(_2\)-R deficient cells when the basolateral P2Y\(_1\)-R was blocked with the P2Y1-R antagonist PAPS (Fig. 5 c, right) or desensitized by ATP pretreatment. Since only bilateral maneuvers resulted in complete inhibition of mechanically induced Ca\(^{2+}\) waves in wild-type cells (Figs. 3, 4, b–c, and 7 b), we conclude that P2-receptors on both epithelial surfaces contribute to Ca\(^{2+}\) wave formation.

Despite previous reports that blockers of gap junctional communication inhibited intercellular Ca\(^{2+}\) waves in airway epithelia (Sanderson et al., 1990; Boitano et al., 1992, 1998) and other cell types (Tordjmann et al., 1997; Toyofuku et al., 1998), our data provide little evidence for a role for IP\(_3\) permeation through gap junctions in mediating Ca\(^{2+}\) waves in polarized airway epithelia. Further, our polarized epithelial preparations exhibited little gap junctional communication as evidenced by Lucifer yellow and calcine dye transfer measurements (data not shown), which mimics the paucity of gap junctions reported in mature airway epithelia in vivo (Carson et al., 1989). Further, a requirement for IP\(_3\) permeation through gap junctions in Ca\(^{2+}\) wave propagation was ruled out on the basis of our experiments with discontinuous cultures that demonstrated wave transmission was mediated by a diffusible,
hibited a rise in \( \text{Ca}^{2+} \) (P2Y1 and P2Y2). Our studies leave the question open and in a paracrine fashion adjacent cells via P2Y-receptors source. The model (Fig. 8) accordingly describes the nucleotide-induced \( \text{Ca}^{2+} \) release of 5\( \text{P} \). The observation that the kinetics of wild-type mouse nasal cells and could detect no release the regenerating signal hypothesis, we used luciferin/ nucleotide-induced nucleotide release) mechanism. As a test of the hydrolytic products of released triphosphates. diphosphates are directly released from cells or appear as wave propagation, it being possible that nucleotide-induced \( \Delta \text{Ca}^{2+} \) (Figs. 4 a and 5 c) to the corresponding dose–effect relationships for nucleotide-induced \( \Delta \text{Ca}^{2+} \) (Larsen et al., 1992). These calculations indicated that 6–9 fmol ATP (~20% of the total cellular ATP content of a single cell) would be required to produce the magnitude or pattern of responses observed.

In conclusion, these studies demonstrated that a response of airway epithelia to local mechanical stress is the production of intercellular \( \text{Ca}^{2+} \) waves mediated by release of nucleotides into the extracellular space that interact with P2Y-receptors. The \( \text{Ca}^{2+} \) wave propagation in polarized airway epithelia does not require intercellular IP3 permeation. Thus, polarized airway epithelia behave, in general, similarly to other cell types by producing intercellular signaling through extracellular nucleotide-release. From the organ-level physiologic perspective, the generation of \( \text{Ca}^{2+} \) waves within airway epithelia coordinates intraepithelial defense mechanisms on airway surfaces (e.g., salt and water transport, ciliary beat frequency, and mucin secretion; Mason et al., 1991; Lethem et al., 1993; Geary et al., 1995) in response to mechanical stress. Our studies extend the spectrum of extracellular nucleotide signaling (Burnstock, 1997; Ferguson et al., 1997) by demonstrating that local mechanical stress induces release of both adenine and uridine nucleotides, which suggests that cells expressing uridine receptors (e.g., P2Y2, P2Y4, and/or P2Y6-R) can respond to the epithelial-derived signals. Importantly, we demonstrate that epithelia can transduce the information of luminal (apical) mechanical stress into a signal (5\( \text{P} \) nucleotides) released into the contralateral compartment. We speculate that basolateral release of nucleotides may be useful not only for coordination of local interepithelial cell responses to mechanical stresses, but also serves as a paracrine signal coordinating the responses of cells (e.g., nerves and inflammatory cells) in the submucosal space of the airway wall.

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