Acute effects of parainfluenza virus on epithelial electrolyte transport

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Running Head: Effects of Sendai virus on ion transport

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Summary

Parainfluenza viruses are important causes of respiratory disease in both children and adults. In particular, they are the major cause of the serious childhood illness croup (laryngo-tracheo-bronchitis). The infections produced by them are associated with the accumulation of ions and fluid in the respiratory tract. It is not known, however, whether this accumulation is due to a direct effect of the viruses on ion and fluid transport by the respiratory epithelium. Here we show that a model parainfluenza virus (Sendai virus), in concentrations observed during respiratory infections, activates Cl⁻ secretion and inhibits Na⁺ absorption across the tracheal epithelium. It does so by binding to a neuraminidase-insensitive glycolipid, possibly asialoGM1, triggering the release of ATP which then acts in an autocrine fashion on apical P2Y receptors to produce the observed changes in ion transport. These findings indicate that fluid accumulation in the respiratory tract associated with parainfluenza virus infection is attributable, at least in part, to direct effects of the virus on ion transport by the respiratory epithelium.
The volume of fluid in the respiratory tract is determined by the balance between the rate of fluid secretion and the rate of fluid absorption by the respiratory epithelium. The secretion of fluid is due to the movement of \( Cl^- \) into the lumen through CFTR \( Cl^- \) channels and \( Ca^{2+} \)-activated \( Cl^- \) channels in the apical membranes of the epithelial cells and is driven by the \( Na^+-K^+-2Cl^- \) cotransporter in their basolateral membranes (1). The absorption of fluid is due to the movement of \( Na^+ \) across the apical membrane through epithelial \( Na^+ \) channels (1-3). The \( Na^+ \) is then pumped out of the cytosol across the basolateral membrane by the \( Na^+,K^+-ATPase \).

Given the importance of ion transport across the respiratory epithelium in determining the volume of the lung surface fluid, it is not surprising that disturbances in it lead to pathological changes in the volume of lung fluid. Hence excessive activity of epithelial \( Na^+ \) channels, such as occurs in cystic fibrosis (4), leads to dehydration of the respiratory surfaces, whereas reduced activity of epithelial \( Na^+ \) channels, such as occurs in pseudohypoaldosteronism type I, is associated with an increase in lung surface fluid (3). Similarly, reduced activity of the epithelial \( Na^+ \) channels in the respiratory epithelium has been implicated in the development of high altitude pulmonary oedema (5), neonatal respiratory distress syndrome (6), cardiogenic pulmonary oedema (7) and serous otitis media (8).

Parainfluenza viruses are a major cause of respiratory disease (9), producing laryngo-tracheo-bronchitis (croup) in children (10) as well as bronchiolitis and pneumonia in children (11,12) and in adults (13). They are disseminated by large-droplet spread and are highly contagious, over 75% of children being infected by parainfluenza viruses at least once during their first 5 years of life (9). The infections caused by them are
associated with fluid accumulation in the respiratory tract, which ranges in severity from rhinitis (9) and serous otitis media (14) to a life-threatening adult respiratory disease syndrome (15). The question thus arises whether parainfluenza viruses could be directly affecting ion transport by the respiratory epithelium. One common respiratory virus, influenza virus, has been shown to directly inhibit Na⁺ absorption by the respiratory epithelium (16). This inhibition is due to the binding of the hemagglutinin in the viral coat to a neuraminidase-sensitive receptor in the apical membrane of the epithelium and is mediated by activation of phospholipase Cβ and protein kinase C (16). Despite the similarity of their names, which reflects the similarity of the clinical features they produce, influenza viruses and parainfluenza viruses are unrelated (9): influenza viruses have a segmented genome and replicate in the nucleus, whereas the parainfluenza viruses have a non-segmented genome and replicate in the cytoplasm (9). Thus it is not possible to assume that parainfluenza viruses affect epithelial ion transport in the same way as influenza viruses.

In the present study we performed experiments to determine whether parainfluenza viruses rapidly alter ion transport by respiratory epithelia. We found that not only do they inhibit absorption of the Na⁺ by these epithelia, they also activate epithelial secretion of Cl⁻. Furthermore, we found that the mechanism by which parainfluenza viruses alter epithelial ion transport differs markedly from that used by influenza viruses.
Experimental procedures

**Viruses.** Sendai virus, the gift of Dr A. Mühlbacher (John Curtin School of Medical Research, Canberra ACT), was grown for 2 days in the allantoic cavity of 10-day old embryonated hen’s eggs. Aliquots of allantoic fluid containing the virus were stored at 80°C. The viral stock solution was titred on monolayers of MDCK cells (17).

**Cell culture.** M-1 mouse cortical collecting duct cells, provided by Dr C. Korbmacher (University of Erlangen, Germany), were grown to confluence for three days on permeable supports (Transwell-Coll, Costar, Cambridge MA, USA) in DMEM/F12 media containing: 10% fetal calf serum, glutamine (2 mM), penicillin (100,000 U/l), streptomycin (100,000 U/l) and dexamethasone (0.1 µM).

**Ussing chamber experiments.** QS mice were killed by cervical dislocation. The trachea was then removed, freed of connective tissue, opened longitudinally and divided into small pieces which were stored in a chilled solution containing (mM): NaCl 145, KCl 3.8, D-glucose 5, MgCl₂ 1, HEPES 5, Ca-gluconate 1.3, pH 7.4. Each tracheal piece was mounted in an Ussing chamber (18) having a circular aperture of 0.95 mm². The apical and basolateral surfaces of the epithelium were perfused continuously with aerated solutions at a rate of 10-20 ml/min (chamber volume 1 ml) at 37°C. The bath solution contained (mM): NaCl 145, KH₂PO₄ 0.4, K₂HPO₄ 1.6, D-glucose 5, MgCl₂ 1, Ca-gluconate 1.3, pH 7.4. All experiments were carried out under open-circuit conditions. The transepithelial potential difference (V_{te}) was recorded relative to the serosal side and current was defined as positive when conventional current flowed in the apical to serosal direction. The transepithelial resistance (R_{te}) was determined and the equivalent short circuit current calculated as previously described (16).
Compounds: 3-isobutyl-1-methylxanthine (IBMX), forskolin, amiloride, bumetanide, uridine 5′-triphosphate (UTP), pertussis toxin holoenzyme, pertussis toxin B oligomer, carbachol, BAPTA-AM, bisindolylmaleimide I (BIM), hexokinase and suramin were all obtained from Sigma (Castle Hill, NSW). The phospholipase C inhibitors, U-73122 and edelfosine, the inactive analogue of U-73122, U-73343, the MAP kinase inhibitors, SB-203580 and U-0126, and the purinergic receptor antagonist, pyridoxalphosphate-6-azophenyl-2′,4′-disulfonic acid (PPADS) were from Calbiochem (Alexandria, NSW). 1-phenyl-2-hexadecanoylamino-3-morpholino-1-propanol (PPMP) was from Adelab Scientific (Norwood, SA). Neuraminidase was from Boehringer (Mannheim, Germany). The anti-ASGM1 antibody was from Wako (Japan).

Statistics: Results are presented as means ± SEM (n = number of tissues tested). Statistical significance was assessed using unpaired Student’s t-tests at a probability level of P < 0.05. Asterisks indicate statistically significant differences from control.
Results

The baseline electrical properties of mouse tracheal epithelium: The rate at which the tracheal epithelium secretes Cl\(^-\) ions and absorbs Na\(^+\) ions can be measured by determining the current flow generated by the movement of these ions across the epithelium. We can do this by measuring the electrical potential difference across the epithelium (the transepithelial potential, \(V_{te}\)) and the change in the transepithelial potential produced by the passage of a standard test pulse of current. We can then use Ohm’s Law to estimate the resistance of the epithelium to the flow of the test pulse of current (the transepithelial resistance, \(R_{te}\)), as well as the current that is required to generate the observed transepithelial potential (the so-called short circuit current, \(I_{sc} = V_{te} / R_{te}\)).

To make these measurements, we placed a small piece of trachea in an Ussing chamber, an organ bath that permits the luminal and interstitial surfaces of the tissue to be superfused by separate solutions and the continuous measurement of transepithelial potential and resistance. Under control conditions, we found the potential difference across the tracheal epithelium to be \(-7.3 \pm 0.6\) mV (\(n = 23\)), with the lumen being negative to the interstitium and the electrical resistance of the epithelium to be \(79.4 \pm 5.2\) \(\Omega\)cm\(^2\) (\(n = 23\)). From these measurements we calculated the short circuit current across the epithelium to be \(98.3 \pm 7.8\) \(\mu\)Acm\(^{-2}\) (\(n = 23\)). This current flow was largely due to transport of Na\(^+\) through amiloride-sensitive Na\(^+\) channels, as the application of amiloride (10 \(\mu\)M), a selective inhibitor of these channels, to the apical surface of the epithelium reduced the short circuit current by \(90.2 \pm 6.9\) \(\mu\)Acm\(^{-2}\) (\(n = 23\)), a 92% reduction.

The effects of Sendai virus: Exposure of the apical membrane of mouse tracheal epithelium to Sendai virus (10\(^6\) pfu/ml) caused the transepithelial
potential and the short circuit current to become transiently more negative
($\Delta V_{te} = -1.2 \pm 0.2 \text{ mV}, n = 8; \Delta I_{sc} = -43.1 \pm 9.0 \mu\text{Acm}^{-2}$; Fig. 1A). This
transient occurred within approximately 1 minute of adding the virus to the
epithelium and lasted approximately 5 minutes. It could have been due
either to an increase in the rate of Cl$^-$ secretion or to an increase in the
rate of Na$^+$ absorption. We found that it could not be prevented by
inhibiting Na$^+$ absorption by the addition of 10 $\mu$M amiloride to the apical
membrane (data not shown), hence could not be attributed to an increase in
the rate of Na$^+$ absorption. It could be inhibited, however, by blocking
the secretion of Cl$^-$ by the addition to the basolateral membrane of 100 $\mu$M
bumetanide, an inhibitor of the Na$^+$-K$^+$-2Cl$^-$ cotransporter (data not shown).
Hence the initial transient stimulation of the short circuit current was
due to an increase in the rate of Cl$^-$ secretion. We further found that this
transient stimulation of short circuit current required an increase in the
intracellular concentration of Ca$^{2+}$, as it could be prevented by manoeuvres
that clamp cytosolic Ca$^{2+}$ at a low level, such as the removal of
extracellular Ca$^{2+}$ or loading the cytosol with the Ca$^{2+}$ chelator, BAPTA
(Fig. 4). Hence the initial increase in short circuit current produced by
Sendai virus was due to activation of Ca$^{2+}$-activated Cl$^-$ channels in the
apical membrane of the epithelium.

After the epithelium had been exposed to the virus for 1 hour (Fig. 1A),
the transepithelial potential had fallen from $-5.6 \pm 0.6 \text{ mV} (n = 8)$ to $-3.3$
$\pm 0.2 \text{ mV} (n = 8)$ and the transepithelial resistance had increased from $49.7$
$\pm 5.0 \Omega\text{cm}^2 (n = 8)$ to $67.5 \pm 8.4 \Omega\text{cm}^2 (n = 8)$. From these measurements we
calculated that the short circuit current across the epithelium had
decreased from $-117.7 \pm 17.1 \mu\text{Acm}^{-2} (n = 8)$ to $-52.7 \pm 6.6 \mu\text{Acm}^{-2} (n = 8)$.
This decrease in current flow during prolonged exposure to parainfluenza
virus was almost entirely due to a reduction in the rate of amiloride-
sensitive Na$^+$ transport (Fig. 1B). In paired control experiments, the ion
transport activity of the epithelium did not change over this period (Fig. 1B). The addition of allantoic fluid also did not affect the ion transport activity of the epithelium (data not shown). We further found that, as we had previously observed for influenza virus (16), the action of Sendai virus on the epithelium was not prevented by UV-inactivation of the virus. UV-inactivated virus produced an initial transient increase in short circuit current of 23.4 ± 6.0 µAcm⁻² (n = 5), and, after an hour of exposure, reduced the rate of amiloride-sensitive Na⁺ absorption from -89.4 ± 9.3 µAcm⁻² (n = 5) to -50.1 ± 4.2 µAcm⁻² (n = 5).

To check whether Sendai virus exerted a non-specific toxic effect, we examined other parameters of the function of the tracheal epithelium. In particular, we examined the effect of the virus on: (i) the rate of Cl⁻ secretion by the epithelium in response to an increase in intracellular cyclic AMP produced by exposure to the activator of adenylate cyclase, forskolin (18); (ii) the rate of Cl⁻ secretion in response to an increase in intracellular Ca²⁺ produced by muscarinic agonist carbachol (18,19); and (iii) the rate of electrogenic cotransport of Na⁺ and glucose across the epithelium (16,20). We found (Fig. 1C) that although exposure to Sendai virus (10⁶ pfu/ml) for 1 hour reduced the response to 100 µM forskolin, it did not affect the response to 100 µM carbachol or the rate of electrogenic glucose transport. The virus thus appears not to have exerted a non-specific toxic effect.

Finally, we examined the dependency of the ion transport effects of Sendai virus on the concentration of virus bathing the apical membrane. We found that both the initial transient stimulation of the short circuit current (Fig. 1D) and the longer term inhibition of the amiloride-sensitive Na⁺ absorption (Fig. 1E) were dependent on the viral concentration over the range 10⁴ to 10⁶ pfu/ml. By comparison, levels of Sendai virus in the lungs of infected mice reach 7 × 10⁶ pfu/g wet weight of lung tissue or higher.
The apical membrane target for Sendai virus: We have previously reported that the effects of influenza virus on ion transport in the mouse trachea can be inhibited by treatment of the apical membrane with neuraminidase (16). In the present study, however, we found that pre-incubation with neuraminidase (0.1 and 1 U/ml) for 30 min had no impact on the actions of Sendai virus (data not shown). Since glycolipids have been reported to act as membrane receptors for paramyxoviruses, including Sendai (24), we also examined the possibility that the effects of Sendai virus might be mediated by a glycolipid. We first examined the effect of blocking glycolipid synthesis using PPMP, an inhibitor of the enzyme GlcCer synthase that requires 24 hours or more to be effective (25). Because we found that incubation of the excised tracheal epithelium for 24 hours led to an irreversible deterioration of its ion transporting function, we performed these studies using monolayers of the M1 mouse collecting duct cell line, a cell line which, like the tracheal epithelium, absorbs Na\(^+\) through amiloride-sensitive Na\(^+\) channels (26,27). Furthermore, M1 cells respond to Sendai virus in a similar manner to tracheal epithelium, showing an initial transient stimulation in short circuit current followed by a longer term inhibition in the amiloride-sensitive current (Figs 2E and 2F). We found that pre-incubation of M1 mouse collecting duct cells in PPMP (20-40 µM) for 24 hours almost completely inhibited both the initial transient stimulation of short circuit current (Fig. 2F) and the reduction of the amiloride-sensitive current (Fig. 2E) that follows the addition of 10\(^6\) pfu/ml Sendai to the apical bathing solution. In contrast, the inhibition of the amiloride-sensitive current produced by influenza virus in M1 cells was not affected by pre-incubation in PPMP (data not shown).

We further explored the role of glycolipids in mediating the effects of Sendai virus by testing the effect of an antibody directed against the
ganglioside asialoGM₁. This ganglioside has been previously identified as the apical receptor by which many bacterial pathogens evoke mucus and cytokine production by epithelia (28,29). We found that pre-incubation for 20 minutes in an anti-asialoGM₁ antibody in a dilution of 1 in 100 completely inhibited the effects of Sendai virus (Figs 2A, 2B and 2D). Interestingly, as reported in other systems (28,29), when added to tracheal epithelium at the lower dilution of 1 in 20, the antibody acted as an agonist, evoking a transient stimulation of short circuit current followed by a long-term depression of the amiloride-sensitive Na⁺ absorption (Fig. 2C).

**Sendai virus acts by triggering ATP release:** We next investigated whether Sendai virus acted by triggering the release of ATP, as has been reported following the exposure of human respiratory cells to *Pseudomonas aeruginosa* (28). We found that enzymatic destruction of ATP secreted from the epithelium by the inclusion in the apical solution of hexokinase plus glucose (30) completely inhibited the effects of Sendai virus (Figs 3A, 3B and 3C). Furthermore, the effects of the virus were inhibited by the purinergic receptor antagonists, suramin and PPADS (Figs 3C, 3D and 3E).

Given that binding of asialoGM₁ had been reported to increase intracellular Ca²⁺ (28,29), we investigated whether the actions of Sendai virus are mediated by changes in intracellular Ca²⁺. We did this by incubating the epithelium in a low Ca²⁺ (1 µM) solution buffered with 1 mM EGTA (Figs 4A and 4B) or by pre-incubating the epithelium with the membrane permeant Ca²⁺ chelator BAPTA-AM (10 µM) for 30 minutes (Figs 4C and 4D). We found that both treatments inhibited the initial transient stimulation of short circuit current produced by Sendai virus (Figs 4B and 4D), but did not affect the longer term inhibition of amiloride-sensitive Na⁺ absorption (Figs 4A and 4C).
The findings that the effects of Sendai virus are mediated by ATP acting on purinergic receptors and that they are mediated in part by increasing intracellular Ca$^{2+}$ suggested roles also for phospholipase Cβ and protein kinase C. Consistent with this we found that U-73122 (10 µM; Figs 5D and 5F) and edelfosine (10 µM; data not shown), which are blockers of phospholipase C, inhibited the effects of the virus, whereas the inactive isomer of U-73122, U-73343 (20 µM), was without effect (data not shown). We also found that an inhibitor of protein kinase C, BIM I (100 nM), partially inhibited the transient response to Sendai virus (Fig. 5F), although it did not prevent the inhibition of amiloride-sensitive Na$^+$ absorption (Fig 5E).

The increased intracellular Ca$^{2+}$ concentration that accompanies binding of asialoGM1 has been reported to activate p38 and p42/44 MAP kinases (29,31). We found, however, that neither inhibition of p38 MAP kinase with 25 µM SB-203580 nor inhibition of p42/44 MAP kinase with 25 µM U-0126 interfered with the actions of Sendai virus on ion transport (Figs 5A, 5B and 5F).

Finally, we examined whether a pertussis toxin-sensitive G protein mediates the effects of Sendai virus. We performed these studies in the presence of BIM I, an inhibitor of protein kinase C, because we have previously found that the B-oligomer of the toxin, which is enzymatically inactive acts as a hemagglutinin, inhibits amiloride-sensitive Na$^+$ absorption in mouse tracheal epithelium as a result of activating protein kinase C (32). When we added Sendai virus to tracheal epithelium that had been pre-treated with pertussis toxin in the presence of BIM I (100 nM), we found that the magnitude of the transient stimulation of short circuit current was not different from that observed in the presence of BIM I alone (Fig. 5F). This treatment, however, abolished completely the effects of Sendai virus on the amiloride-sensitive Na$^+$ absorption (Fig. 5C). Exposure of the epithelium to the isolated B-oligomer of the toxin in the presence of BIM I...
was without effect (data not shown). Thus the action of Sendai virus on the amiloride-sensitive Na\(^+\) absorption would appear to be mediated by a pertussis toxin-sensitive G protein.

**The actions of UTP on the tracheal epithelium have a similar pharmacology to those of Sendai virus:** As reported above, the effects of Sendai virus on epithelial ion transport are mediated by the autocrine action of ATP on apical P2Y receptors. Direct activation of these receptors by exogenous nucleotides should therefore reproduce the effects of the virus. We have previously shown that apical UTP (100 \(\mu M\)) causes a rapid stimulation of Ca\(^{2+}\)-activated Cl\(^-\) secretion followed by inhibition of amiloride-sensitive Na\(^+\) absorption (32) (c.f. Figs 6A and 6B) in the tracheal epithelium. Furthermore, the P2Y antagonist, suramin, inhibits both the activation of Cl\(^-\) secretion (data not shown) and the inhibition of the amiloride-sensitive Na\(^+\) absorption (Fig. 6B) produced by UTP. Both actions of UTP were also blocked by the inhibitor of phospholipase C, U-73122 (32) (Fig. 6B). Finally, UTP shows a similar divergence in the mechanisms by which it controls Cl\(^-\) secretion and Na\(^+\) absorption to that we had observed with Sendai virus: treatment of the epithelium with the protein kinase C inhibitor BIM I inhibits the effect of UTP on Cl\(^-\) secretion, but leaves the inhibitory effect of UTP on the amiloride-sensitive Na\(^+\) absorption unchanged (32) (Fig. 6B).

**Discussion**

We have found that mouse parainfluenza virus I (Sendai virus) produces rapid changes in ion transport across mouse tracheal epithelium. We observed these effects at concentrations comparable to those observed in the nasal mucosa and lungs of animals during experimentally induced infections, which reach \(7 \times 10^6\) pfu/g wet weight of lung tissue and higher within 3 days of inoculation with the virus (21,23,33). In addition, the
nature of the changes in ion transport observed, viz. an increase in Cl$^-$ secretion together with an inhibition of Na$^+$ absorption, suggests that they may play a significant role in the fluid accumulation in the respiratory tract that accompanies parainfluenza infections (9,23). Since these effects of Sendai virus were also observed in the M1 collecting duct cell line, it would seem that they are not mediated by immune cells in the tracheal mucosa.

The mechanism by which Sendai produces its effects is summarised in Figure 7. It first binds to a glycolipid, which may be asialoGM$_1$, although our findings with the anti-sialoGM1 antibody are also consistent with the weak agonist activity of this antibody having desensitized the glycolipid target for Sendai virus. The binding of the virus then triggers ATP release leading to autocrine activation of apical P2Y receptors and activation of phospholipase C$\beta$. The pathways that activate Cl$^-$ secretion and inhibit amiloride-sensitive Na$^+$ absorption then diverge. The activation of Cl$^-$ secretion is dependent on an increase in intracellular Ca$^{2+}$ as well as on the activation of protein kinase C, whereas the inhibition of the amiloride-sensitive Na$^+$ absorption is independent of increases in intracellular Ca$^{2+}$ and the activity of protein kinase C. It is noteworthy that this divergence in the regulation of these two ion transport processes is also observed when apical P2Y receptors are directly stimulated by UTP or ATP (present results, (32,34)).

The effects of Sendai virus on epithelial ion transport differ markedly from those of influenza viruses: (i) Sendai virus acts via a neuraminidase-insensitive glycolipid, whereas the influenza receptor is neuraminidase-sensitive (16); (ii) Sendai virus produces an initial transient stimulation of Cl$^-$ secretion, whereas influenza does not (16); and (iii) although both viruses inhibit the amiloride-sensitive Na$^+$ absorption, Sendai virus does so via a pertussis toxin-sensitive G protein, whereas influenza does so via
a pertussis toxin-insensitive G protein and protein kinase C (16). The ability of Sendai virus to evoke Cl− secretion is reminiscent, however, of the ability of rotaviruses to trigger Cl− secretion by the intestinal epithelium. This action of rotaviruses is apparently due to a viral non-structural protein, NSP4, activating phospholipase Cβ which then increases intracellular Ca2+ and triggers Cl− secretion by intestinal villus cells (35-37). It is not known whether ATP release mediates these actions of NSP4, although the present findings suggest that this possibility should be considered.

The present findings also need to be considered in the light of the recent report that respiratory syncytial virus (RSV) infections inhibit the rate of fluid clearance from the airways (38). In their studies, Davis and colleagues showed that three days after infection of mice with RSV there was a marked inhibition in the rate of clearance of a standardised volume of fluid instilled into the lungs through the trachea. This inhibition was accompanied by a decline in the amiloride-sensitive component of the fluid clearance, and was apparently mediated by the secretion of UTP from the respiratory mucosa. From these studies, it was not possible to determine whether these effects were the direct result of the contact of the virus with the epithelium, or the consequence of an immune response to the infection. The findings in the present study, however, suggest that they are likely to be due to a direct interaction between the viral particles and the epithelium.

The phenomenon that most closely resembles the action of Sendai virus on epithelial transport is the stimulation of epithelial cytokine and mucus production by exposure to Pseudomonas aeruginosa and Staphylococcus aureus. These bacteria trigger mucus and interleukin production by binding apical asialoglycolipids, leading to release of ATP, autocrine activation of purinergic receptors and increased intracellular Ca2+ (28,29). Taking these
reports together with our present finding that Sendai virus modulates epithelial ion transport we propose that altered epithelial ion transport and the production of mucus and cytokines are all part of a stereotypic response of airway epithelia to contact with pathogens. In this response, an increase in the volume of fluid bathing the surface of the epithelium hydrates the increased amounts of mucus being secreted and leads to an increase in the rate of mucus clearance so as to facilitate transport of the pathogens out of the lung (39). The possibility that the acute changes in electrolyte transport we have observed form part of a stereotyped epithelial response to pathogens is supported by reports that both *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* inhibit Na⁺ transport by the respiratory epithelium (40-43). A further implication of our findings is that the release of ATP from epithelia, which has been considered to be an epithelial response to mechanical stimuli (44), may also play a critical role in co-ordinating epithelial responses to pathogens.

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Figure Legends

**Figure 1**: Effects of Sendai virus on ion transport by mouse trachea. Panel A. Original recording of the transepithelial voltages (V<sub>te</sub>) and the effects of amiloride (A; 10 µM) prior to and following one hour incubation with Sendai virus (10<sup>6</sup> pfu/ml) or with control buffer. The initial transient increase in transepithelial potential that follows addition of the virus is marked by †. The transepithelial potential (V<sub>te</sub>) in the absence of the test current pulse is marked with an arrow. Panel B. Effects of 1 h incubation in Sendai virus or control buffer on amiloride-sensitive short circuit currents (I<sub>sc-Amil</sub>). Panel C. Effects of 1 h incubation in Sendai virus on the short circuit current responses (∆I<sub>sc</sub>) induced by carbachol (CCH, 100 µM), by a mixture of IBMX (100 µM) and forskolin (2 µM) (cAMP), or by luminal glucose (5 mM). Panel D. Concentration-response relation for the initial transient increase in short circuit current (Initial ∆I<sub>sc</sub>) produced by Sendai virus. Initial ∆I<sub>sc</sub> was measured as the difference between the peak I<sub>sc</sub> observed following the addition of Sendai and the I<sub>sc</sub> observed immediately prior to its addition. Panel E. Concentration-response relation for the inhibition of I<sub>sc-Amil</sub> produced by incubation in Sendai virus for 1 hour. Statistically significant effects are marked with an asterisk.

**Figure 2**: Investigation of the membrane target for Sendai virus. Panel A. Original recording of the transepithelial voltages (V<sub>te</sub>) in mouse trachea and effects of amiloride (Amil; 10 µM) prior to and following one hour incubation with Sendai virus (10<sup>6</sup> pfu/ml) in the absence or presence of an ASGM1 antibody (Anti-ASGM1; 1:100 dilution). Panel B. Effects on the amiloride-sensitive short circuit current (I<sub>sc-Amil</sub>) of 1 hour incubation in Sendai virus (10<sup>6</sup> pfu/ml) in the absence or presence of Anti-ASGM1 antibody (1:100). Panel C. Effect of 1:100 and 1:20 dilutions of Anti-ASGM1 antibody on I<sub>sc-Amil</sub> measured 10-15 minutes after adding the antibody. Panel D. Impact on the initial transient increase in short circuit current (Initial ∆I<sub>sc</sub>) produced by Sendai virus (10<sup>6</sup> pfu/ml) of exposure to a 1:100
dilution of Anti-ASGM1 antibody. Panel E. The impact of 24 h incubation of M1 cells with PPMP (20 μM) or with control solution on the effect on $I_{sc-Amil}$ of 1 h exposure to Sendai virus (10^6 pfu/ml). Panel F. The impact of 24 h incubation of M1 cells with PPMP (20 μM) or with control solution on Initial $ΔI_{sc}$ produced by Sendai virus (10^6 pfu/ml). Initial $ΔI_{sc}$ was measured as the difference between the peak $I_{sc}$ observed following the addition of Sendai and the $I_{sc}$ observed immediately prior to its addition. Statistically significant effects are marked with an asterisk.

**Figure 3** The actions of Sendai virus are mediated by release of ATP. Panel A. Original recording of the transepithelial voltage ($V_{te}$) in mouse trachea and of the effects of amiloride (Am; 10 μM) prior to and following incubation for 1 hour with Sendai virus (10^6 pfu/ml) in the presence of hexokinase (5 U/ml) plus glucose (15 mM). Panels B-E. Effect on the initial transient stimulation of short circuit current (Initial $ΔI_{sc}$) and on the inhibition of the amiloride-sensitive short circuit current ($I_{sc-Amil}$) produced by Sendai virus under control conditions or in the presence of hexokinase plus glucose (Panels B and C), suramin (100 μM; Panels B and D) and PPADS (100 μM; Panels B and E), respectively. $I_{sc-Amil}$ was measured 1 h after the addition of Sendai virus (10^6 pfu/ml). Initial $ΔI_{sc}$ was measured as the difference between the peak $I_{sc}$ observed following the addition of Sendai and the $I_{sc}$ observed immediately prior to its addition.

**Figure 4**: Role of Ca^{2+} in mediating the effects of Sendai virus (10^6 pfu/ml). Panels A and B show the effects of incubation in low (1 μM) extracellular Ca^{2+} solution on the initial transient increase in short circuit current (Initial $ΔI_{sc}$) seen following the addition of Sendai virus and on the amiloride-sensitive short circuit current ($I_{sc-Amil}$) measured 1 h after the addition of Sendai virus. Panels C and D show the effects of treatment with BAPTA-AM on Initial $ΔI_{sc}$ following the addition of Sendai virus and on the amiloride-sensitive short circuit current ($I_{sc-Amil}$) measured 1 h after the addition of Sendai virus. Initial $ΔI_{sc}$ was measured as the difference between the peak $I_{sc}$ observed following the addition of
Sendai and the $I_{sc}$ observed immediately prior to its addition. Statistically significant effects are marked with an asterisk.

**Figure 5:** Second messengers that mediate the actions of Sendai virus. Effects of incubation in Sendai virus ($10^6$ pfu/ml) for 1 hour on theamiloride-sensitive short circuit current ($I_{sc-Amil}$) in the absence or presence of U-0126 (25 $\mu$M; Panel A), SB-203580 (25 $\mu$M, Panel B), PTX (250 ng/ml) together with BIM (0.1 $\mu$M; Panel C), U-73122 (10 $\mu$M; Panel D) and BIM (0.1 $\mu$M; Panel E). Panel F. Summarizes the effects of these agents on the initial transient increase in short circuit current (Initial $\Delta I_{sc}$) observed following the addition of Sendai virus ($10^6$ pfu/ml). Initial $\Delta I_{sc}$ was measured as the difference between the peak $I_{sc}$ observed following the addition of Sendai and the $I_{sc}$ observed immediately prior to its addition. Statistically significant effects are marked with an asterisk.

**Figure 6:** Effects of UTP (100 $\mu$M) on ion transport in mouse trachea. Panel A. Original recording of $V_{te}$ and the effects of amiloride (A; 10 $\mu$M) before and after UTP. Panel B. The effects of UTP on the amiloride-sensitive short circuit current ($I_{sc-Amil}$) under control conditions or in the presence of suramin (100 $\mu$M), U-73122 (10 $\mu$M) and PTX (250 ng/ml) plus BIM (0.1 $\mu$M), respectively. Statistically significant differences from the control are marked with an asterisk. Statistically significant differences from UTP are marked with a hash.

**Figure 7:** Cell model for the mechanisms by which Sendai virus rapidly regulates ion transport in mouse tracheal epithelium.
Fig. 1

A

Sendai / Sendai 1h

Control / Control 1h

B

C

D

E

Isc-Amil (µA/cm²)

Isc-Amil (µA/cm²)

CCH    cAMP    Glucose

Abc (µA/cm²)

Isc-Amil (µA/cm²)

Sendai (log pfu/ml)

Sendai (log pfu/ml)

(4)

(4)

(6)

(6)
Fig. 2
Fig. 3
Fig. 4

A

B

C

D

Isc-Aml (μA/cm²)

Initial Alsc (μA/cm²)

Isc-Aml (μA/cm²)

Initial Alsc (μA/cm²)

Low Ca²⁺

normal Ca²⁺

BAPTA-AM

control

* Sendai

(3)

(5)

(6)

(3)

(5)
Fig. 5

A

Isoc-Amil (µA/cm²)

80

60

40

20

0

U-0126

SB-203580

B

Initial Asc (µA/cm²)

80

60

40

20

0

SB-203580

U-0126

C

Isoc-Amil (µA/cm²)

80

60

40

20

0

PTX/BIM

D

Isoc-Amil (µA/cm²)

200

160

120

80

40

0

U-73122

E

Isoc-Amil (µA/cm²)

80

60

40

20

0

BIM

F

Initial Asc (µA/cm²)

50

40

30

20

10

0

Control

U-0126

SB-203580

PTX/BIM

U-73122

BIM
Fig. 7
Acute effects of parainfluenza virus on epithelial electrolyte transport
Karl Kunzelmann, Jens Koenig, Jane Sun, Nicholas J. King, Guna Karupiah, John A.
Young and David I. Cook

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