

Acute Effects of Parainfluenza Virus on Epithelial Electrolyte Transport*

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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 (laryngotracheobronchitis). The infections produced by parainfluenza viruses are associated with the accumulation of ions and fluid in the respiratory tract. It is not known, however, whether this accumulation is because of a direct effect of the viruses on ion and fluid transport by the respiratory epithelium. Here we show that a model parainfluenza virus (the 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 asialo-GM1, 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 cystic fibrosis transmembrane conductance regulator protein Cl^- channels and Ca^{2+} -activated Cl^- channels in the apical membranes of the epithelial cells and is driven by the $\text{Na}^+/\text{K}^+/\text{2Cl}^-$ cotransporter in their basolateral membranes (1). The absorption of fluid is because of 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 $\text{Na}^+/\text{K}^+/\text{ATPase}$.

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 edema (5), neonatal respiratory distress syndrome (6), cardiogenic pulmonary edema (7), and serous otitis media (8).

Parainfluenza viruses are a major cause of respiratory disease (9), producing laryngotracheobronchitis (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 are 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, the influenza virus, has been shown to directly inhibit Na^+ absorption by the respiratory epithelium (16). This inhibition is because of 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 $\text{C}\beta$ 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 the 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 eggs from hens. Aliquots of allantoic fluid containing the virus were stored at 80 °C. The viral stock solution was titrated on monolayers of Madin-Darby canine kidney cells (17).

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Cell Culture—M-1 mouse cortical-collecting duct cells, provided by Dr. C. Korbacher (University of Erlangen, Germany), were grown to confluence for 3 days on permeable supports (Transwell-Coll, Costar, Cambridge, MA) in Dulbecco's modified Eagle's medium/F12 medium containing 10% fetal calf serum, glutamine (2 mM), penicillin (100,000 units/liter), streptomycin (100,000 units/liter) and dexamethasone (0.1 μ M).

Ussing Chamber Experiments—Quackenbush-Swiss mice were killed by cervical dislocation. The trachea were then removed, freed of connective tissue, opened longitudinally, and divided into small pieces that were stored in a chilled solution containing 145 mM NaCl, 3.8 mM KCl, 5 mM D-glucose, 1 mM MgCl₂, 5 mM HEPES, 1.3 mM calcium gluconate, 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 145 mM NaCl, 0.4 mM KH₂PO₄, 1.6 mM K₂HPO₄, 5 mM D-glucose, 1 mM MgCl₂, 1.3 mM calcium gluconate, 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 was calculated as described previously (16).

Compounds—3-Isobutyl-1-methylxanthine, forskolin, amiloride, bumetanide, UTP, pertussis toxin holoenzyme, pertussis toxin B oligomer, carbachol, BAPTA-AM,¹ bisindolylmaleimide I (BIM), hexokinase, and suramin were all obtained from Sigma. 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, pyridoxal phosphate-6-azophenyl-2',4'-disulfonic acid (PPADS) were all from Calbiochem. 1-Phenyl-2-hexadecanoylamino-3-morpholino-1-propanol (PPMP) was from Adela Scientific (Norwood, SA). Neuraminidase was from Roche Applied Science. The anti-ASGM1 antibody was from Wako (Japan).

Statistics—Results are presented as the means \pm S.E. (n = number of tissues tested). Statistical significance was assessed using unpaired Student's *t* tests at a probability level of $p < 0.05$. Asterisks in figures 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 ± 0.6 mV ($n = 23$), with the lumen being negative to the interstitium and the electrical resistance of the epithelium to be $79.4 \pm 5.2 \Omega \text{ cm}^2$ ($n = 23$). From these measurements we calculated the short circuit current across the epithelium to be $98.3 \pm 7.8 \mu\text{A cm}^{-2}$ ($n = 23$). This current flow was largely because of the transport of Na⁺ through amiloride-

sensitive Na⁺ channels, as the application of amiloride (10 μ M), a selective inhibitor of these channels, to the apical surface of the epithelium reduced the short circuit current by $90.2 \pm 6.9 \mu\text{A cm}^{-2}$ ($n = 23$), a 92% reduction.

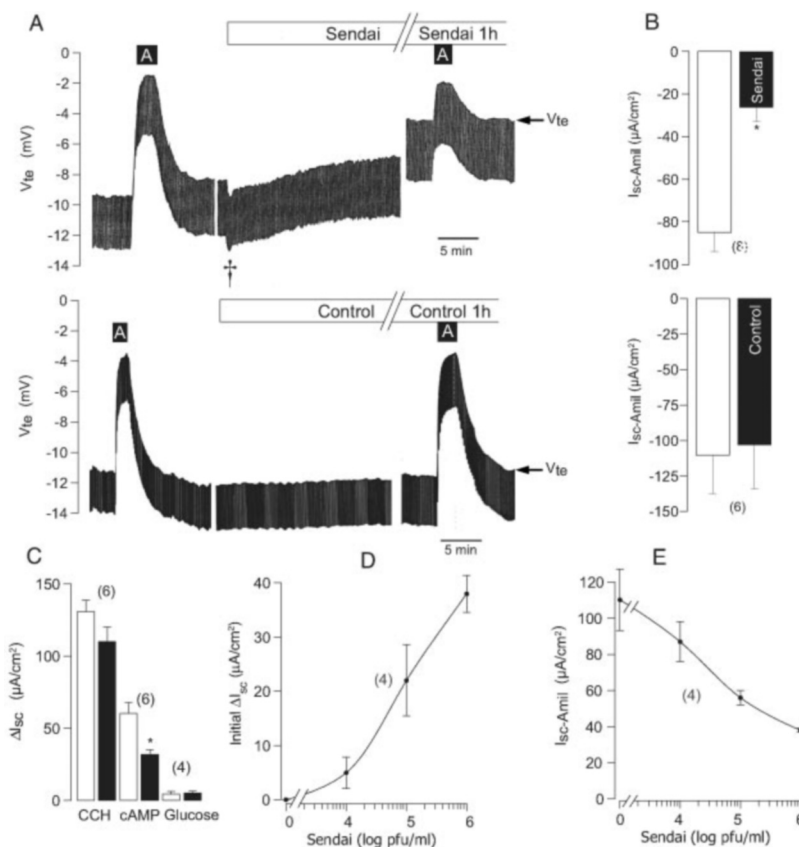
The Effects of Sendai Virus—Exposure of the apical membrane of mouse tracheal epithelium to the Sendai virus (10⁶ pfu/ml) caused the transepithelial potential and the short circuit current to become transiently more negative (Fig. 1A, $\Delta V_{te} = -1.2 \pm 0.2$ mV, $n = 8$; $\Delta I_{sc} = -43.1 \pm 9.0 \mu\text{A cm}^{-2}$). This transient occurred within ~ 1 min of adding the virus to the epithelium and lasted ~ 5 min. 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 μ M amiloride to the apical membrane (data not shown), and 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 μ M bumetanide, an inhibitor of the Na⁺-K⁺-2Cl[−] cotransporter (data not shown). Hence the initial transient stimulation of the short circuit current was because of 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 Ca²⁺, as it could be prevented by maneuvers that clamp cytosolic Ca²⁺ at a low level, such as the removal of extracellular Ca²⁺ or loading the cytosol with the Ca²⁺ chelator, BAPTA (see Fig. 4). Hence the initial increase in short circuit current produced by Sendai virus was because of the activation of Ca²⁺-activated Cl[−] channels in the apical membrane of the epithelium.

After the epithelium had been exposed to the virus for 1 h (Fig. 1A), the transepithelial potential had fallen from -5.6 ± 0.6 mV ($n = 8$) to -3.3 ± 0.2 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 declined from $-117.7 \pm 17.1 \mu\text{A cm}^{-2}$ ($n = 8$) to $-52.7 \pm 6.6 \mu\text{A cm}^{-2}$ ($n = 8$). This decrease in current flow during prolonged exposure to parainfluenza virus was almost entirely because of 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 acid 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 the Sendai virus on the epithelium was not prevented by an UV inactivation of the virus. A UV light-inactivated virus produced an initial transient increase in the short circuit current of $23.4 \pm 6.0 \mu\text{A cm}^{-2}$ ($n = 5$) and, after 1 h of exposure, reduced the rate of amiloride-sensitive Na⁺ absorption from $-89.4 \pm 9.3 \mu\text{A cm}^{-2}$ ($n = 5$) to $-50.1 \pm 4.2 \mu\text{A cm}^{-2}$ ($n = 5$).

To check whether the Sendai virus exerted a nonspecific toxic effect, we examined the 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 the adenylate cyclase forskolin (18), (ii) the rate of Cl[−] secretion in response to an increase in intracellular Ca²⁺ produced by the 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 the Sendai virus (10⁶ pfu/ml) for 1 h reduced the response to 100 μ M forskolin, it did not affect the response to 100 μ M carbachol or the rate of electrogenic glucose transport. The virus thus appears not to

¹ The abbreviations used are: BAPTA-AM, 1,2-bis(2-aminophenoxy)ethane-*N,N,N',N'*-tetraacetic acid tetrakis(acetoxymethyl ester); BIM, bisindolylmaleimide I; MAP, mitogen-activated protein; PPADS, pyridoxal phosphate-6-azophenyl-2',4'-disulfonic acid; PPMP, 1-phenyl-2-hexadecanoylamino-3-morpholino-1-propanol; pfu, plaque-forming unit; Ω , ohm; GM1, Gal β 1-3GalNAc β 1-4(GlcNAc2-3)Gal β 1-4Glc β 1-1Cer.

FIG. 1. Effects of Sendai virus on ion transport by mouse trachea. *A*, original recording of the transepithelial voltages (V_{te}) and the effects of amiloride (*A*; 10 μ M) prior to and following a 1-h incubation with the Sendai virus (10^6 pfu/ml) or with control buffer. The initial transient increase in transepithelial potential that follows the addition of the virus is marked by a dagger. The transepithelial potential (V_{te}) in the absence of the test current pulse is marked with an arrow. *B*, effects of a 1-h incubation in Sendai virus or control buffer on amiloride-sensitive short circuit currents ($I_{sc-Amil}$). *C*, effects of a 1-h incubation in Sendai virus on the short circuit current responses (ΔI_{sc}) induced by carbachol (CCH, 100 μ M), by a mixture of isobutylmethylxanthine (100 μ M) and forskolin (2 μ M) (cAMP), or by luminal glucose (5 mM). *D*, concentration-response relation for the initial transient increase in short circuit current (Initial ΔI_{sc}) produced by the 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. *E*, concentration-response relation for the inhibition of $I_{sc-Amil}$ produced by incubation in Sendai virus for 1 h. Statistically significant effects are marked with an asterisk.



have exerted a nonspecific toxic effect.

Finally, we examined the dependence of the ion transport effects of the 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 10^4 – 10^6 pfu/ml. By comparison, the levels of the Sendai virus in the lungs of infected mice reach 7×10^6 pfu/g of wet weight of lung tissue or higher (21–23).

The Apical Membrane Target for Sendai Virus—We have previously reported that the effects of the influenza virus on ion transport in the mouse trachea can be inhibited by the treatment of the apical membrane with neuraminidase (16). In the present study, however, we found that pre-incubation with neuraminidase (0.1 and 1 units/ml) for 30 min had no impact on the actions of the Sendai virus (data not shown). Because 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 glucosylceramide synthase that requires 24 h or more to be effective (25). Because we found that the incubation of the excised tracheal epithelium for 24 h 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 that, 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 (Fig. 2, E and F). We found that pre-incubation of M1 mouse collecting duct cells in PPMP (20–40 μ M) for 24 h almost completely inhibited both the initial tran-

sient 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 the 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 the 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 min in an anti-asialoGM1 antibody in a dilution of 1 in 100 completely inhibited the effects of the Sendai virus (Fig. 2, A, B, and D). 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 the 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 the Sendai virus (Fig. 3, A–C). Furthermore, the effects of the virus were inhibited by the purinergic receptor antagonists, suramin, and PPADS (Fig. 3, C–E).

Given that binding of asialoGM1 had been reported to increase intracellular Ca^{2+} (28, 29), we investigated whether the actions of the Sendai virus are mediated by changes in intracellular Ca^{2+} . We did this by incubating the epithelium in a low Ca^{2+} (1 μ M) solution buffered with 1 mM EGTA (Fig. 4, A and B) or by pre-incubating the epithelium with the membrane-per-

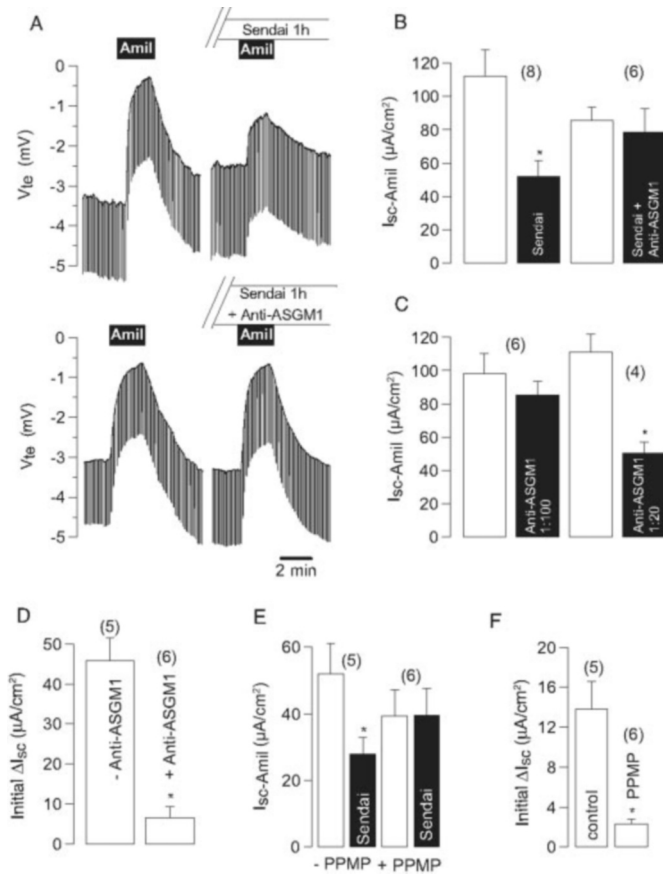


FIG. 2. Investigation of the membrane target for the Sendai virus. A, original recording of the transepithelial voltages (V_{te}) in mouse trachea and effects of amiloride ($Amil$; $10 \mu M$) prior to and following a 1-h incubation with the Sendai virus (10^6 pfu/ml) in the absence or presence of an ASGM1 antibody ($Anti-ASGM1$; 1:100 dilution). B, effects on the amiloride-sensitive short circuit current ($I_{sc-Amil}$) of a 1-h incubation in Sendai virus (10^6 pfu/ml) in the absence or presence of anti-ASGM1 antibody (1:100). C, effect of 1:100 and 1:20 dilutions of anti-ASGM1 antibody on $I_{sc-Amil}$ measured 10–15 min after adding the antibody. D, impact on the initial transient increase in short circuit current ($Initial \Delta I_{sc}$) produced by the Sendai virus (10^6 pfu/ml) of exposure to a 1:100 dilution of Anti-ASGM1 antibody. Panel E. The impact of a 24-h incubation of M1 cells with PPMP ($20 \mu M$) or with control solution on the effect on $I_{sc-Amil}$ of a 1-h exposure to Sendai virus (10^6 pfu/ml). F, the impact of a 24-h incubation of M1 cells with PPMP ($20 \mu M$) 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.

meant Ca^{2+} chelator BAPTA-AM ($10 \mu M$) for 30 min (Fig. 4, C and D). We found that both treatments inhibited the initial transient stimulation of short circuit current produced by the Sendai virus (Fig. 4, B and D) but did not affect the longer term inhibition of amiloride-sensitive Na^+ absorption (Fig. 4, A and C).

The findings that the effects of the 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 $C\beta$ and protein kinase C. Consistent with this we found that U-73122 ($10 \mu M$; Fig. 5, D and F) and edelfosine ($10 \mu M$; data not shown), which are blockers of phospholipase C, inhibited the effects of the virus, whereas the inactive isomer of U-73122, U-73343 ($20 \mu M$), 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 the Sendai virus (Fig. 5F), although it did not

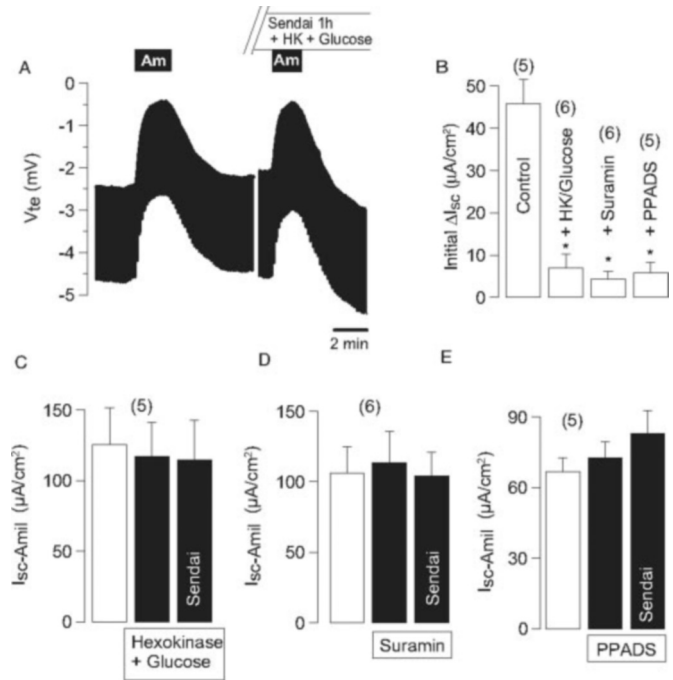


FIG. 3. The actions of the Sendai virus are mediated by release of ATP. A, original recording of the transepithelial voltage (V_{te}) in mouse trachea and of the effects of amiloride (Am ; $10 \mu M$) prior to and following incubation for 1 h with Sendai virus (10^6 pfu/ml) in the presence of hexokinase (5 units/ml) plus glucose (15 mM). B–E, effect on the initial transient stimulation of short circuit current ($Initial \Delta I_{sc}$) and on the inhibition of the amiloride-sensitive short circuit current ($I_{sc-Amil}$) produced by the Sendai virus under control conditions or in the presence of hexokinase plus glucose (B and C), suramin (B and D, $100 \mu M$) and PPADS (B and E, $100 \mu M$), 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.

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 $25 \mu M$ SB-203580 nor inhibition of p42/44 MAP kinase with $25 \mu M$ U-0126 interfered with the actions of Sendai virus on ion transport (Fig. 5, A, B, and F).

Finally, we examined whether a pertussis toxin-sensitive G protein mediates the effects of the 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 and inhibits amiloride-sensitive Na^+ absorption in mouse tracheal epithelium as a result of activating protein kinase C (32). When we added Sendai virus to the 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 the 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

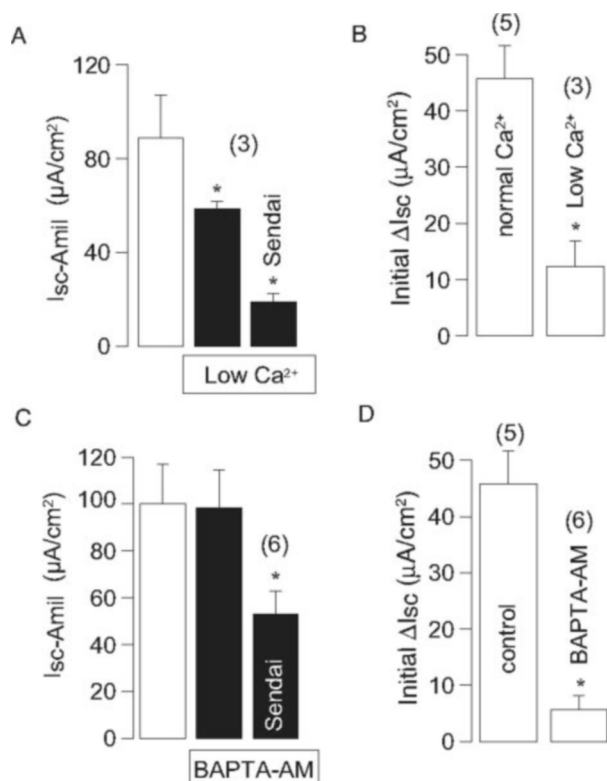


FIG. 4. Role of Ca²⁺ in mediating the effects of the Sendai virus (10⁶ pfu/ml). *A* and *B* show the effects of incubation in low (1 μM) extracellular Ca²⁺ solution on the initial transient increase in short circuit current (*Initial* ΔI_{sc}) seen following the addition of the Sendai virus and on the amiloride-sensitive short circuit current (*I*_{sc-Amil}) measured 1 h after the addition of Sendai virus. *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.

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 μM) causes a rapid stimulation of Ca²⁺-activated Cl⁻ secretion followed by inhibition of amiloride-sensitive Na⁺ absorption (32) (Fig. 6, compare *A* with *B*) 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. 6*B*) produced by UTP. Both actions of UTP were also blocked by the inhibitor of phospholipase C, U-73122 (32) (Fig. 6*B*). Finally, UTP shows a similar divergence in the mechanisms by which it controls Cl⁻ secretion and Na⁺ absorption to what 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. 6*B*).

DISCUSSION

We have found that the mouse parainfluenza virus I (Sendai virus) produces rapid changes in ion transport across mouse tracheal epithelium. We observed these effects at concentrations comparable with those observed in the nasal mucosa and lungs of animals during experimentally induced infections, which reach 7 × 10⁶ pfu/g of wet weight of lung tissue and higher within 3 days of inoculation with the virus (21, 23, 33).

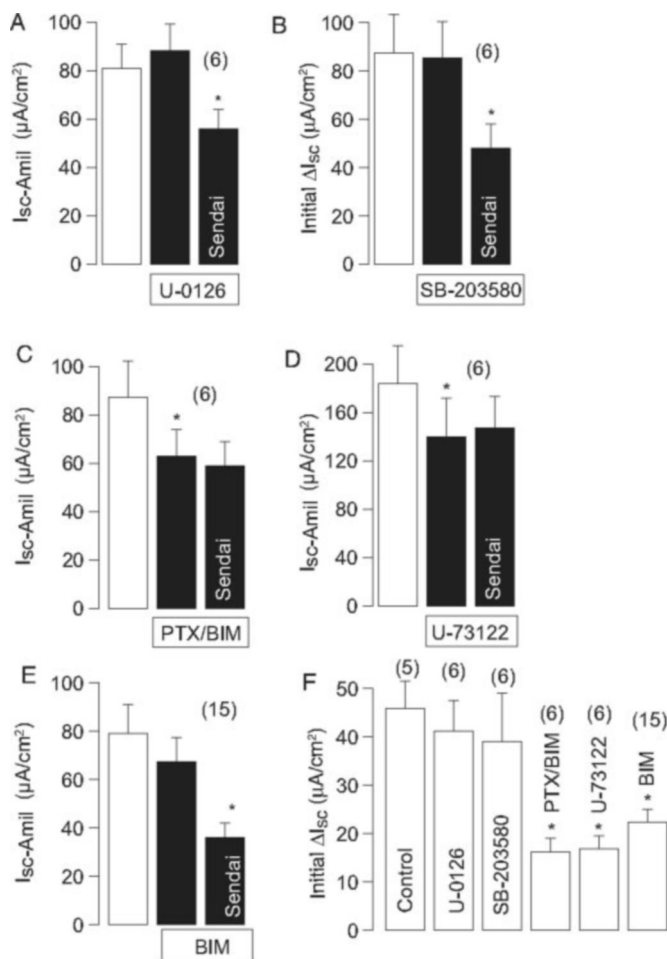


FIG. 5. Second messengers that mediate the actions of the Sendai virus. Effects of incubation in Sendai virus (10⁶ pfu/ml) for 1 h on the amiloride-sensitive short circuit current (*I*_{sc-Amil}) in the absence or presence of U-0126 (*A*, 25 μM), SB-203580 (*B*, 25 μM), PTX (250 ng/ml) together with BIM 1 (*C*, 0.1 μM), U-73122 (*D*, 10 μM) and BIM (*E*, 0.1 μM). *F*, summarizes the effects of these agents on the initial transient increase in short circuit current (*Initial* ΔI_{sc}) observed following the addition of Sendai virus (10⁶ pfu/ml). Initial ΔI_{sc} was measured as the difference between the peak *I*_{sc} observed following the addition of the Sendai and the *I*_{sc} observed immediately prior to its addition. Statistically significant effects are marked with an asterisk.

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). Because 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 summarized in Fig. 7. It first binds to a glycolipid, which may be asialoGM1, 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 an ATP release leading to the autocrine activation of apical P2Y receptors and activation of phospholipase Cβ. The pathways that activate Cl⁻ secretion inhibit amiloride-sensitive Na⁺ absorption then diverge. The activation of Cl⁻ secretion is dependent on an increase in intracellular Ca²⁺ 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 Ca²⁺ and the activity of protein kinase C. It is noteworthy

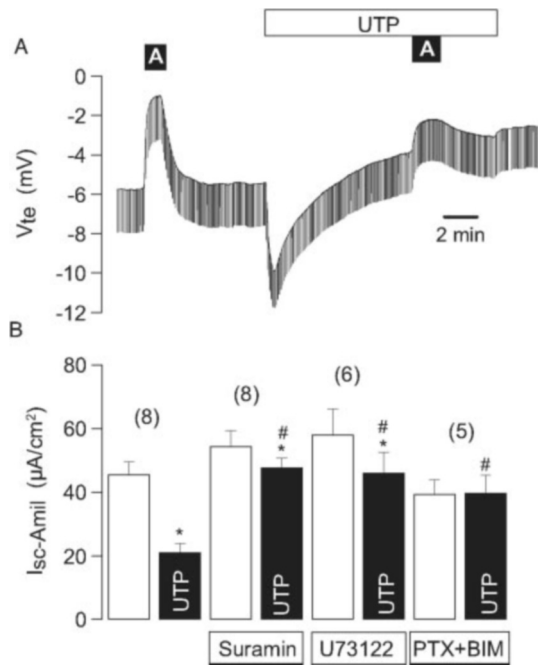


FIG. 6. Effects of UTP (100 μ M) on ion transport in mouse trachea. A, original recording of V_{te} and the effects of amiloride (A, 10 μ M) before and after UTP. B, the effects of UTP on the amiloride-sensitive short circuit current ($I_{sc-Amil}$) under control conditions or in the presence of suramin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

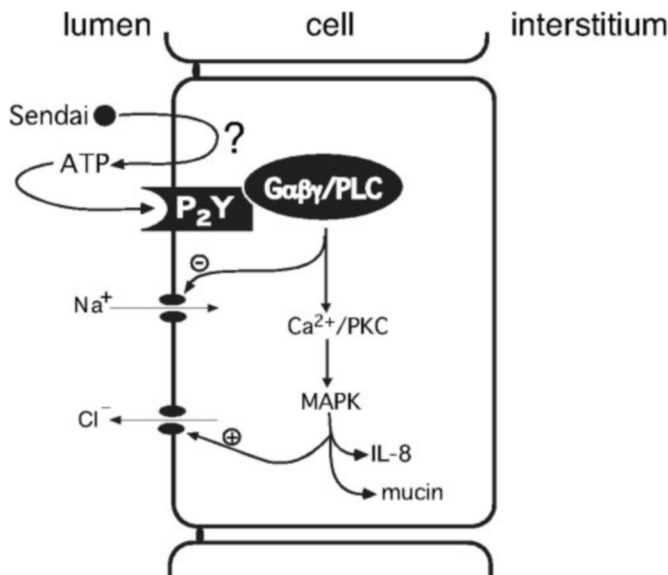


FIG. 7. Cell model for the mechanisms by which the Sendai virus rapidly regulates ion transport in mouse tracheal epithelium.

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 (this work and Refs. 32 and 34).

The effects of the 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 the 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 because of a viral non-structural protein, NSP4, activating phospholipase $C\beta$, which then increases intracellular Ca^{2+} 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 infections inhibit the rate of fluid clearance from the airways (38). In their studies, Davis and colleagues (38) showed that 3 days after the infection of mice with respiratory syncytial virus there was a marked inhibition in the rate of clearance of a standardized 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 because of a direct interaction between the viral particles and the epithelium.

The phenomenon that most closely resembles the action of the Sendai virus on epithelial transport is the stimulation of epithelial cytokine and mucus production by exposure to *P. aeruginosa* and *Staphylococcus aureus*. These bacteria trigger mucus and interleukin production by binding apical asialoglycolipids, leading to the release of ATP, autocrine activation of purinergic receptors, and increased intracellular Ca^{2+} (28, 29). Taking these reports together with our present finding that the 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 *P. 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 coordinating epithelial responses to pathogens.

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