Basolateral chloride current in human airway epithelia

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1Howard Hughes Medical Institute, Departments of 2Internal Medicine, 3Pediatrics, and 4Physiology and Biophysics, Roy J. and Lucille A. Carver College of Medicine, University of Iowa, Iowa City, Iowa; and 5Center for Oral Biology, University of Rochester Medical Center, Rochester, New York

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Itani OA, Lamb FS, Melvin JE, Welsh MJ. Basolateral chloride current in human airway epithelia. Am J Physiol Lung Cell Mol Physiol 293: L991–L999, 2007. First published July 27, 2007; doi:10.1152/ajplung.00077.2007.—Electrolyte transport by airway epithelia regulates the quantity and composition of liquid covering the airways. Previous data indicate that airway epithelia can absorb NaCl. At the apical membrane, cystic fibrosis transmembrane conductance regulator (CFTR) provides a pathway for Cl− absorption. However, the pathways for basolateral Cl− exit are not well understood. Earlier studies, predominantly in cell lines, have reported that the basolateral membrane contains a Cl− conductance. However, the properties have varied substantially in different epithelia. To better understand the basolateral Cl− conductance in airway epithelia, we studied primary cultures of well-differentiated human airway epithelium. The basolateral membrane contained a Cl− current that was inhibited by 4,4′-disothiocyanostilbene-2,2′-disulfonic acid (DIDS). The current-voltage relationship was nearly linear, and the halide selectivity was Cl− > Br− > I−. Several signaling pathways increased the current, including elevation of cellular levels of cAMP, activation of protein kinase C (PKG), and reduction of pH. In contrast, increasing cell Ca2+ and inducing cell swelling had no effect. The basolateral Cl− current was present in both cystic fibrosis (CF) and non-CF airway epithelia. Likewise, airway epithia from wild-type mice and mice with disrupted genes for ClC-2 or ClC-3 all showed similar Cl− currents. These data suggest that the basolateral membrane of airway epithelia possesses a Cl− conductance that is not due to CFTR, ClC-2, or ClC-3. Its regulation by signaling pathways and coordinated regulation of Cl− conductance in both apical and basolateral membranes may be important in controlling transepithelial Cl− movement.

chloride channel; absorption; adenosine 3′,5′-cyclic monophosphate; cystic fibrosis

A THIN LAYER OF SURFACE LIQUID (ASL) covers the apical side of airway epithelia. The ASL contributes to pulmonary host defense by facilitating mucociliary clearance and by providing an environment for the elimination of inhaled bacteria and other microorganisms (5, 13, 30, 40). The quantity and composition of ASL are tightly regulated by ion transport across airway epithelia. These epithelia can either secrete or absorb liquid depending on environmental conditions and the signals they receive. Both of these processes depend on the cystic fibrosis transmembrane conductance regulator (CFTR) anion channel located in the apical membrane of airway epithelia. Loss of CFTR function in cystic fibrosis (CF) renders the epithelia defective at both liquid secretion and absorption (9, 45).

Previous studies of airway epithelia have provided insight into the mechanisms of ion and liquid transport (5, 30, 40). For secretion, Cl− is transported across the basolateral membrane by the Na+/K+/2Cl− cotransporter (NKCC) and across the apical membrane by CFTR. Na+ follows through the paracellular pathway. The energy needed for Cl− secretion is provided by the basolateral Na+/K+/ATPase. K+ channels recycle K+ across the basolateral membrane. The secretory process also accommodates HCO3−, with HCO3− entering the cell through Na+/HCO3− cotransporters and anion exchangers and exiting through apical CFTR anion channels (23, 35). Electrolyte secretion by airway epithelia generates a driving force for liquid secretion.

For absorption by airway epithelia, Na+ enters cells across their apical membrane through epithelial Na+ channels (ENaC) (5, 30, 34, 40). Cl− could follow Na+ either through the cell or through the paracellular pathway. The paracellular pathway is cation selective (7), suggesting that some Cl− absorption may occur through the cell. More direct evidence for transcellular Cl− absorption comes from the study of CF epithelia; the loss of CFTR, and hence apical Cl− entry, reduces Cl− and Na+ absorption (9, 45). Reduced salt absorption is also consistent with the well-characterized reduction in NaCl absorption in the CF sweat gland duct (32). These observations raised the question of how Cl− exits across the basolateral membrane of airway epithelia. Note, however, that the rate of Cl− and Na+ absorption by CF airway epithelia is a subject of controversy, and some data suggest that loss of CFTR increases Cl− and Na+ absorption (4, 21).

Although a basolateral Cl− conductance in airway epithelia has been described in several studies, its properties and regulation remain uncertain. Moreover, there are substantial differences in the literature that may depend on the model system used. In addition, there are no reports of the properties and regulation of the basolateral Cl− conductance in well-differentiated human airway epithelia. Willumsen et al. (41) reported a small basolateral Cl− conductance in human airway epithelia. Other studies reported the presence of an outwardly rectifying basolateral Cl− channel in the submucosal gland cell line Calu-3 (17, 37, 42), where the channel was regulated by cAMP agonists. In bovine airway epithelia, basolateral Cl− conductance was attributed to an inwardly rectifying Cl− channel that was regulated by agents that elevate cAMP levels (39). In cultured airway epithelia from the rat, an outwardly rectifying basolateral Cl− conductance was reported to influence cAMP-stimulated Cl− secretion (16). Studies using whole cell patch clamp of human airway epithelia revealed a swelling-induced,
hyperpolarization-activated, outwardly rectifying Cl⁻ current and Ca²⁺-activated, depolarization-activated Cl⁻ current (12). Similar studies in mouse airway epithelia revealed a linear, voltage-independent Cl⁻ current, an inwardly rectifying Cl⁻ current, and a Cl⁻ current activated by hyperpolarization and depolarization (38).

Basolateral Cl⁻ channels also have been demonstrated to play important roles in other tissues. For example, in portions of the kidney nephron, ClC-Ka/ and ClC-Kb/barttin channels provide pathways for basolateral Cl⁻ absorption (11). In absorptive intestinal epithelia, CIC-2 is reported to mediate basolateral Cl⁻ transport (28). In the sweat duct, CFTR accounts for both apical and basolateral Cl⁻ conductances (33).

The goal of this study was to better understand the pathway for Cl⁻ exit across the basolateral membrane. We studied well-differentiated primary cultures of human airway epithelia, because they provide a model for both normal airway epithelial function and abnormalities in CF (19, 43). Moreover, the finding that CF airway epithelia manifest a reduced rate of transepithelial Cl⁻ absorption in ASL homeostasis. We studied the basolateral Cl⁻ current in epithelia, rather than in isolated cells, to assign the current specifically to the basolateral membrane. Our results suggest that the basolateral membrane of human airway epithelia contains a regulated Cl⁻ channel.

MATERIALS AND METHODS

Human airway epithelia. Airway epithelial cells were obtained from trachea and bronchi of lungs removed for donation and prepared by the University of Iowa In Vitro Models and Cell Culture Core using methods previously described (19, 43). Cells were isolated by enzyme digestion and seeded onto collagen-coated Millicell polycarbonate filters (Millipore, Bedford, MA). Epithelia were maintained at 37°C in a humidified atmosphere of 5% CO₂ and air. The culture medium consisted of a 1:1 mix of Dulbecco’s modified Eagle’s medium and Ham’s F-12, 2% Ultroser G (Biosepra SA, Cergy-Saint-Christophe, France), penicillin (100 U/ml), streptomycin (100 µg/ml), gentamicin (50 µg/ml), fluconazole (2 µg/ml), and amphotericin B (1.25 µg/ml). Twenty-four hours after plating, the apical medium was removed, and the cells were maintained at the air-liquid interface. These cultures differentiate and develop a ciliated surface within 14 days of seeding. All studies used epithelia at least 2 wk after seeding.

Transgenic mice airway epithelia. C1c-2 –/– and C1c-3 –/– mice were generated as previously described (8, 27). Airway epithelia were isolated from mouse trachea and seeded onto permeable supports as described earlier (44). In brief, mouse tracheas were cut lengthwise and then immersed at 4°C in dissociation medium containing 15 mg/ml of Pronase per 10 ml of Ham’s F-12 medium. After 24 h, clumps of epithelial cells were harvested and resuspended in medium containing a 50:50 ratio of DMEM-Ham’s F-12 supplemented with 4 mM glutamine, 10 µg/ml insulin, 5 µg/ml transferrin, 62 µg/ml cholera toxin, 5 ng/ml epidermal growth factor, 0.03 mg/ml bovine pituitary interferon, 10⁻⁸ M retinoic acid, 5% fetal bovine serum, and 20 U·µg⁻¹·ml⁻¹ Pen-Strep. Following dissociation, the cell harvest was preincubated in a Primaria culture dish in a CO₂ incubator at 37°C for 2–3 h to adhere fibroblasts from the cell harvest. Epithelial cells were seeded onto rat tail collagen precoated Costar Transwell membrane. Four days after seeding, an apical air interface was established. Once formed, the polarized culture epithelia were maintained in medium containing 50:50 DMEM-Ham’s F-12 supplemented with 4 mM glutamine, 10⁻⁸ M retinoic acid, 2% V/V NuSerum, and 20 U·µg⁻¹·ml⁻¹ Pen-Strep. For studies in Ussing chambers (see below), mouse airway epithelia were treated identically to human epithelia.

Chemicals. All chemicals used for Ussing chamber studies were purchased from Sigma Chemical (St. Louis, MO). 4-[4-Oxo-2-bisoxo-3-(3-trifluoromethylphenyl)thiazolidin-5-ylidenemethyl]benzoic acid (CFTRinh-172) (24) was a generous gift from the Cystic Fibrosis Foundation.

Ussing chamber measurements. Short-circuit current (Isc) and transepithelial potential (V) were measured in modified Ussing chambers (Jim’s Instruments, Iowa City, IA). Transepithelial voltage was clamped to zero for all studies except where specified. A 1-s voltage pulse of 5 mV was given every 50 s. For conditions with symmetrical Cl⁻ concentrations, solutions on both surfaces of the epithelia contained (in mM) 135 NaCl, 5 HEPES, 1.2 MgCl₂, 1.2 CaCl₂, 2.4 K₂HPO₄, and 0.6 KH₂PO₄. To generate a Cl⁻ concentration gradient, Cl⁻ was replaced with gluconate on the basolateral side, and the Ca²⁺ concentration was increased to 5 mM due to the Ca²⁺ buffering capacity of gluconate. Cl⁻ in the apical buffer was replaced with Br⁻ or I⁻ in the halide permeability studies. In the hypo/hypertonicity studies, the concentration of NaCl or Na-gluconate was maintained at 105 mM and osmolality was adjusted with mannitol. For hypertonicity studies in nonpermeabilized epithelia, the NaCl or Na-gluconate concentration was 135 mM and osmolality was adjusted by adding dry mannitol directly to the Ussing chambers. For hypertonicity studies in nonpermeabilized epithelia, the concentration of NaCl or Na-gluconate was maintained at 90 mM and osmolality was adjusted with mannitol.

After measuring baseline current, we performed one of two protocols. In the first protocol, we added the following agents sequentially: (1) apical amiloride (10⁻⁴ M), which inhibits apical Na⁺ channels and hyperpolarizes the apical membrane, thereby generating a driving force for a Cl⁻ secretory Isc; (2) forskolin (10⁻⁵ M) and 3-isobutyl-1-methylxanthine (IBMX; 10⁻⁴ M), which increase cellular levels of cAMP, leading to phosphorylation of CFTR by protein kinase A; (3) basolateral 4,4’-diisothiocyanostilbene-2,2’-disulfonic acid (DIDS; 10⁻⁴ M), which inhibits Cl⁻ channels and/or transporters in the basolateral membrane; and then (4) basolateral bumetanide (10⁻⁴ M), which inhibits basolateral NKCC.

In the second protocol, we measured current generated by a Cl⁻ concentration gradient after clamping voltage across the basolateral membrane to zero. We performed the following interventions sequentially: (1) apical amiloride (10⁻⁴ M); (2) basolateral bumetanide (10⁻⁴ M); (3) basolateral ouabain (10⁻⁴ M), which inhibits the Na⁺–K⁺–ATPase, thereby inhibiting most transepithelial electrolytic transport; (4) replacement of the basolateral solution with a Cl⁻-free buffer that contained the same concentration of bumetanide and ouabain, thereby generating a transepithelial Cl⁻ concentration gradient; (5) apical nystatin (0.36 mg/ml) to permeabilize the apical membrane; and finally, (6) basolateral DIDS (10⁻⁴ M) or basolateral N-phenylanthranilic acid (DPC, 10⁻³ M) were added after the current stabilized (5–10 min).

To determine the current-voltage (I-V) relationship of the basolateral Cl⁻ conductance, we performed the second protocol up to the third step, followed by nystatin without a Cl⁻ concentration gradient. Voltage was then clamped for 1 s every 10 s at 10- or 20-mV steps from −80 to +80 mV. DIDS was then added to the basolateral buffer, and the voltage steps were repeated.

Data analysis. Statistical significance was determined using Student’s t-test. P values <0.05 were considered statistically significant. Data are means ± SE.

RESULTS

A basolateral Cl⁻ conductance in airway epithelia. Primary cultures of differentiated airway epithelia grown at the air-liquid interface exhibit many properties that are similar to in vivo epithelia (19, 43), thus providing a model for assessing transepithelial ion transport in human epithelia. To study Cl⁻ transport, we mounted the cells in Ussing chambers, inhibited...
Na⁺ transport with amiloride, and recorded either $I_{sc}$ or transepithelial current in the presence of an ion concentration gradient ($I$). For all studies, we used HCO₃⁻-free buffer to study Cl⁻ transport without a contribution from HCO₃⁻ transporters (20, 23, 35).

To test for the presence of a basolateral Cl⁻ conductance, we examined the effect of DIDS. DIDS blocks many different Cl⁻ channels and is reported to inhibit basolateral Cl⁻ conductance in Calu-3 epithelia (17, 37). To study Cl⁻ current across the basolateral membrane, we first blocked electrogenic transepithelial ion transport by treating the cells with amiloride and bumetanide. We added the Na⁺-K⁺-ATPase inhibitor ouabain to eliminate the possibility that it would contribute to basolateral current. We then imposed a Cl⁻ concentration gradient by substituting gluconate for Cl⁻ (20, 23, 35). Removing Cl⁻ from both apical and basolateral solutions eliminated the current (Fig. 1A). Removing Cl⁻ from both apical and basolateral solutions eliminated the current (Fig. 1B), suggesting that it resulted from Cl⁻ flow from the apical to the basolateral solution. We obtained qualitatively similar results when we replaced Na⁺ in the solution with K⁺ (data not shown). To test whether the apical membrane was permeabilized, we applied nystatin (0.36 mg/ml for 5 min) to the apical surface and then added amiloride (Fig. 1C); nystatin eliminated the effect of amiloride. The CFTR inhibitor CFTRinh-172 (24) also failed to inhibit current under these conditions (not shown). These results indicate that nystatin had permeabilized the apical membrane. However, with time after addition, nystatin progressively increased transepithelial electrical conductance ($G_i$) (note the increase in current required for the 5-mV pulses, Fig. 1, C and D). This progressive increase in $G_i$ precluded an accurate assessment of basolateral Cl⁻ channels by measuring changes in $G_i$. We therefore assayed transepithelial current as a more sensitive measure and as has been done by others (37). Nystatin addition also generated a transient current increase (Fig. 1). This current likely represents a transient Na⁺ current that occurs when the apical membrane is permeabilized; it did not occur in the absence of Na⁺ (not shown).

The effect of DIDS on basolateral current was dose dependent, with $10^{-4}$ M blocking ~75% of current (Fig. 1E). DPC, another nonspecific Cl⁻ channel blocker, also inhibited current (Fig. 1F), further suggesting the existence of Cl⁻ channel(s) and/or electrogenic transporter(s) in the basolateral membrane.

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**Fig. 1.** 4,4′-Diisothiocyanostilbene-2,2′-disulfonic acid (DIDS)-sensitive basolateral Cl⁻ current. Unless otherwise indicated, transepithelial transport was blocked by the addition of apical amiloride ($10^{-4}$ M), basolateral bumetanide ($10^{-4}$ M), and basolateral ouabain ($10^{-4}$ M). Cl⁻ in the basolateral solution was then replaced by gluconate to generate a transepithelial Cl⁻ concentration gradient, and nystatin (0.36 mg/ml) was added to permeabilize the apical membrane. Ap, apical; bl, basolateral. **A:** effect of DIDS ($10^{-4}$ M) in the presence of a Cl⁻ concentration gradient. Note that the initial peak in positive current after nystatin addition is probably due to Na⁺ redistribution during permeabilization (also see B) ($n > 10$). **B:** effect of DIDS ($10^{-4}$ M) in the absence of Cl⁻ ($n = 4$). Calu-3 epithelia [baseline transepithelial conductance ($G_i$) = 1.94 ms/cm²] were bathed in symmetrical saline, and amiloride was added to control cells or to cells with the apical membrane permeabilized with nystatin ($n = 3$). $I_{sc}$, short-circuit current. **D:** effect of prolonged nystatin treatment ($n = 3$). **E:** effect of DIDS concentration on basolateral Cl⁻ current. Experiment as in A was repeated with 3 different concentrations of DIDS added to parallel cultures ($n = 4$ each). **F:** effect of $N$-phenylanthranilic acid (DPC; $10^{-5}$ M) added to the basolateral solution ($n = 5$).
In contrast, treatment with the carbonic anhydrase inhibitor acetazolamide had no effect on DIDS-sensitive current (92 ± 8% of control, n = 3), suggesting that HCO₃⁻ generated by epithelia did not contribute to the current.

**Effect of DIDS on transepithelial ion transport.** The ability of DIDS to block basolateral Cl⁻ channels raised the question of how it would affect transepithelial Cl⁻ current. If a portion of Cl⁻ transported into the cell by NKCC recycled back across the basolateral membrane through basolateral Cl⁻ channels, then we predicted that basolateral DIDS would increase transepithelial Cl⁻ secretion. To stimulate transepithelial Cl⁻ transport, we blocked apical Na⁺ channels with amiloride, thereby hyperpolarizing the apical membrane and producing a driving force for apical Cl⁻ exit. We then increased intracellular cAMP concentrations with forskolin and IBMX, which leads to CFTR phosphorylation by cAMP-dependent protein kinase and the subsequent addition of bumetanide to block CFTR-dependent Cl⁻ secretion. To study the basolateral anion conductance, we blocked apical Na⁺ channels with amiloride, thereby hyperpolarizing the apical membrane and producing a driving force for apical Cl⁻ exit. We then increased intracellular cAMP concentrations with forskolin and IBMX, which leads to CFTR phosphorylation by cAMP-dependent protein kinase and the opening of these channels. Applying DIDS to the basolateral surface increased Cl⁻ secretion (Fig. 2, A and C), and subsequent addition of bumetanide to block NKCC inhibited Cl⁻ secretion. Conversely, applying DIDS to the apical surface had a minimal effect on Cl⁻ secretion (Fig. 2, B and C), suggesting that apical channels other than CFTR contributed little to Cl⁻ secretion. As an additional test of whether DIDS-stimulated Cl⁻ secretion was CFTR dependent, we tested the effect of the CFTR inhibitor CFTRinh-172 (24). CFTRinh-172 blocked the majority of CFTR current and the DIDS-stimulated current (Fig. 2, D and E). These results suggest that DIDS increased CFTR-dependent transepithelial Cl⁻ secretion.

Our data do not exclude other ways that DIDS might affect transepithelial transport. For example, two different cell types might contribute: secretory cells that have NKCC in the basolateral membrane and absorptive cells that have basolateral DIDS-sensitive Cl⁻ channels. In this scenario, Cl⁻ might be transported between the two different cell types through gap junctions. A portion of Cl⁻ transported into secretory cells by NKCC could recycle back across the basolateral membrane through basolateral Cl⁻ channels in absorptive cells. By blocking Cl⁻ recycling, DIDS would increase transepithelial Cl⁻ secretion. There are other potential effects of DIDS. It might have activated a signaling cascade that activated CFTR. DIDS might have increased the activity of basolateral NKCC, or DIDS might have enhanced Na⁺-K⁺-ATPase activity. Our studies of the effect of DIDS when the apical membrane was permeabilized make these alternatives less likely.

**Properties of the basolateral Cl⁻ conductance.** We examined the selectivity of the basolateral anion conductance by repeating the experiment in Fig. IA after substituting Br⁻ for Cl⁻ in the apical solution. The halide conductance sequence was Cl⁻ > Br⁻ > I⁻ (Fig. 3A). This sequence was different from that reported for CFTR (Br⁻ ≥ Cl⁻ > I⁻) (1), suggesting that CFTR is not a major contributor to the basolateral anion conductance.

To determine the I-V relationship of the basolateral Cl⁻ conductance, we recorded basolateral current after clamping the membrane at different voltages before and after adding DIDS. DIDS reduced the conductance, and the DIDS-sensitive I-V relationship was almost linear with only slight outward rectification (Fig. 3B).

**Regulation of basolateral Cl⁻ conductance.** To further understand the basolateral Cl⁻ conductance and its function, we examined its regulation. Because elevating cellular levels of...
cAMP leads to CFTR activation, we asked whether it also would increase the basolateral Cl\textsuperscript{-}/H\textsubscript{11002} current. Applying forskolin and IBMX increased basolateral DIDS-sensitive current by 161\% (Fig. 4 A). This result suggests coordinated regulation of apical and basolateral Cl\textsuperscript{-} conductance by cAMP-dependent pathways. Because CFTR is also regulated by PKC, we tested the effect of 4-phorbol 12-myristate 13-acetate (PMA) (6). PMA increased DIDS-sensitive current by 188\% (Fig. 4 A), providing an additional potential mechanism for coordinately regulating Cl\textsuperscript{-} conductances at the two membranes.

In airway epithelia, intracellular Ca\textsuperscript{2+} has been reported to activate basolateral K\textsuperscript{+} channels (25), some apical non-CFTR Cl\textsuperscript{-} channels (2), and basolateral Cl\textsuperscript{-} channels (10, 37). To test whether the basolateral Cl\textsuperscript{-} conductance was similarly regulated, we treated cells with the Ca\textsuperscript{2+}-ionophore ionomycin. The lack of an effect (Fig. 4 A) suggests that Ca\textsuperscript{2+}-activated Cl\textsuperscript{-} channels did not contribute to the basolateral Cl\textsuperscript{-} current.

Some Cl\textsuperscript{-} channels have been reported to be sensitive to a reduced pH (18). To test whether basolateral Cl\textsuperscript{-} conductance was similarly regulated, we treated cells with the Ca\textsuperscript{2+}-ionophore ionomycin. The lack of an effect (Fig. 4 A) suggests that Ca\textsuperscript{2+}-activated Cl\textsuperscript{-} channels did not contribute to the basolateral Cl\textsuperscript{-} current.
basolateral buffer conditions. DIDS-sensitive current was not significantly different under any of the conditions tested (Fig. 4B), suggesting that swelling-activated Cl⁻ channels were not a major contributor to the basolateral Cl⁻ conductance. Swelling-activated Cl⁻ channels can be blocked by tamoxifen (3). However, applying tamoxifen did not block the basolateral Cl⁻ current of permeabilized epithelia (data not shown) further suggesting that swelling-activated Cl⁻ channels were not responsible for the basolateral Cl⁻ current. To exclude a possible effect of nystatin on cell volume that masked the effect of mannitol-induced changes in cell volume, we measured DIDS-sensitive current in the absence of nystatin (Fig. 4C). Under these conditions, current was smaller than that recorded in the presence of nystatin suggesting that apical CFTR limited transepithelial current. Adding 100 mM mannitol to both the apical and basolateral solutions had no effect on DIDS-sensitive current (104 ± 13% of control, n = 3). In separate experiments, switching the apical and basolateral solutions to hypotonic buffers in the absence of nystatin had no effect on DIDS-sensitive current (110 ± 18% of control, n = 3). These data further suggested that basolateral Cl⁻ conductance was not regulated by cell volume.

Basolateral Cl⁻ conductance in airway epithelia from patients with CF and from gene-targeted mice. CFTR mediates Cl⁻ transport across both the apical and the basolateral membranes of sweat gland ducts (33). To test whether CFTR contributed to basolateral Cl⁻ transport in airway epithelia, we studied CF airway epithelia. CF airway epithelia showed no transepithelial Cl⁻ transport, and applying basolateral DIDS had no effect on the $V_{sc}$ (Fig. 5A, compare with Fig. 2A). After applying a Cl⁻ concentration gradient and then permeabilizing the apical membrane, we observed a DIDS-sensitive basolateral Cl⁻ current like that in non-CF epithelia (Fig. 5B). Moreover, CFTRinh-172 did not block basolateral current in non-CF epithelia (Fig. 5C). These data suggest that CFTR did not contribute to basolateral conductance. These results also suggest that unlike in the sweat gland duct, Cl⁻ transport across the basolateral membrane in airway epithelia is not disrupted in CF.

In the intestine, CIC-2 is reported to be responsible for Cl⁻ absorption across the basolateral membrane (28). Because CIC-2 is expressed in airway epithelia (22), we tested whether it was responsible for the basolateral Cl⁻ current. We studied airway epithelia cultured from wild-type and Clc-2 −/− mice; the $I_{sc}$ tended to be smaller than in human airway epithelia, but the qualitative responses to amiloride and to forskolin and IBMX were similar (Fig. 6A). The effect of basolateral DIDS was maintained in the knockout mice (Fig. 6, A and B), suggesting that CIC-2 is not a major contributor of basolateral Cl⁻ conductance. CIC-3 is another Cl⁻ channel expressed in airway epithelia (22). The effect of basolateral DIDS was also maintained in airway epithelia from Clc-3 −/− mice (Fig. 6C). Thus neither CIC-2 nor CIC-3 appear to make a major contribution to basolateral Cl⁻ conductance.

**DISCUSSION**

Our results indicate the presence of a basolateral Cl⁻ current in differentiated primary cultures of human airway epithelia. The data begin to identify the characteristics and regulation of these channels.
secretion. Bestrophin-1 is a Ca\textsuperscript{2+}-activated Cl\textsuperscript{-} channel with a halide permeability of I\textsuperscript{-} > Br\textsuperscript{-} > Cl\textsuperscript{-} (36). Our experiments indicate that the basolateral Cl\textsuperscript{-} conductance in human airway epithelia is not activated by Ca\textsuperscript{2+} and has a halide permeability opposite that of bestrophin-1. Thus bestrophin-1 is not likely to play a major role in the basolateral Cl\textsuperscript{-} conductance of human airway epithelia.

Other studies in Calu-3 cells suggested the presence of an outwardly rectifying, depolarization-activated basolateral Cl\textsuperscript{-} channel (37, 42). The channel was sensitive to DIDS and had a halide permeability of Cl\textsuperscript{-} > Br\textsuperscript{-} > I\textsuperscript{-}. We found that the basolateral anion current in airway epithelia had a similar halide permeability sequence, but the I-V relationship was not strongly outwardly rectifying. Although these comparisons suggest that the outwardly rectifying Cl\textsuperscript{-} channel in Calu-3 cells is not the major channel in the basolateral membrane of human airway epithelia, it might contribute some portion of the basolateral Cl\textsuperscript{-} conductance.

In rat airway epithelia, one study found an outwardly rectifying, depolarization-activated basolateral Cl\textsuperscript{-} channel (16). Like the current in human airway epithelia, the channel was sensitive to DIDS. However, its reported halide selectivity (Br\textsuperscript{-} = I\textsuperscript{-} > Cl\textsuperscript{-}) differed, suggesting that channel was not responsible on its own for the basolateral Cl\textsuperscript{-} current of airway epithelia. This difference could be due to species differences or perhaps to differences in the experimental approach.

Uyekubo et al. (39) studied cAMP-dependent Cl\textsuperscript{-} absorption across bovine airway epithelia. They found a cAMP-stimulated basolateral Cl\textsuperscript{-} channel that was predicted to be important for transepithelial Cl\textsuperscript{-} absorption. Unlike the current we found, the I-V relationship was inwardly rectifying. However, the activation by agents that increase cAMP suggests it could contribute to the basolateral Cl\textsuperscript{-} current.

Studies in other epithelia may be informative. For example, Hanrahan et al. (14) reported a high-conductance anion channel in the basolateral membrane of rabbit urinary bladder epithelium. The channel had a linear I-V relationship and was sensitive to DIDS. The identity of the channel remains unknown; however, the similarity in properties suggests that a similar basolateral Cl\textsuperscript{-} channel might contribute to the airway epithelial conductance. Studies in rat inner medullary collecting duct epithelia also report results similar to what we observed. Basolateral DIDS increased cAMP-stimulated Cl\textsuperscript{-} secretion, thus suggesting a DIDS-sensitive basolateral Cl\textsuperscript{-} conductance (46). The identity of the channel is unknown, but it is thought to be important for Cl\textsuperscript{-} absorption.

We also considered the possibility that Cl\textsuperscript{-} exit across the basolateral membrane might occur through a transporter rather than a channel. Known Cl\textsuperscript{-} transporters fall into three main families: cation-coupled Cl\textsuperscript{-} cotransporters, anion exchangers, and Cl\textsuperscript{-}/H\textsuperscript{+} exchangers. Cation-coupled Cl\textsuperscript{-} cotransporters are electroneutral (15), and the absence of HCO\textsubscript{3}\textsuperscript{-} buffering eliminates the involvement of an electrogenic anion exchanger. Mammalian CIC-4 and CIC-5 were recently reported to be Cl\textsuperscript{-}/H\textsuperscript{+} exchangers that are activated by acidic pH (26, 29); however, CIC-4 and -5 are strongly rectifying and are localized to intracellular organelles, making them unlikely candidates for basolateral Cl\textsuperscript{-} conductance (31). Therefore, it is more likely that basolateral Cl\textsuperscript{-} current is due to a channel rather than a transporter.

Thus, although our data do not reveal the identity of the basolateral Cl\textsuperscript{-} channels in differentiated human airway epithelia, they do describe characteristics that can be used in their identification. The increase in current by reduced pH and the lack of activation by Ca\textsuperscript{2+} or cell swelling suggest that a CIC-like channel might contribute. However, our studies suggest that the loss of CIC-2 or CIC-3 does not eliminate the basolateral Cl\textsuperscript{-} current. One limitation of this conclusion is that our studies of CIC-2 and -3 were performed in mouse airway epithelia. It also remains possible that more than one channel type might contribute to Cl\textsuperscript{-} flow across the basolateral membrane. If so, the aggregate of their properties could confuse attempts to identify a single channel type.

**Coordinated regulation of Cl\textsuperscript{-} transport.** Coordinated regulation of apical CFTR and basolateral Cl\textsuperscript{-} channels may be important for efficient absorption. The simultaneous activation...
of CFTR and basolateral Cl\(^{-}\) channels could provide a pathway for Cl\(^{-}\) to accompany Na\(^{+}\) through the cell. Our results suggest that the cAMP and PKC signaling pathways may regulate Cl\(^{-}\) conductances at both membranes. During Cl\(^{-}\) secretion, we found that inhibiting the basolateral Cl\(^{-}\) current with DIDS further stimulated secretion. Apparently blocking the basolateral Cl\(^{-}\) conductance prevented Cl\(^{-}\) that had entered the cell through NKCC from recycling back across the basolateral membrane.

Finally, in submitting this report, we noted that H. Fischer et al. (11a) had just published the results of an investigation of the basolateral membrane Cl\(^{-}\) conductance in human airway epithelia. That study shows some results with our study, although each also investigates different aspects of the basolateral membrane Cl\(^{-}\) current. The only point of difference between the two studies is that our data suggest that the conductance is not primarily volume regulated, whereas their data suggest that hypertonicity inhibits the Cl\(^{-}\) conductance. The reason for this difference is not apparent.

Identifying the channels responsible for basolateral Cl\(^{-}\) conductance is of major importance to further understanding of airway surface liquid regulation. We speculate that aberrant regulation of basolateral Cl\(^{-}\) conductance might have deleterious consequences on the quantity or composition of airway surface liquid and detrimental effects on lung function.

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