Cellular Cl^- transport in cultured cystic fibrosis airway epithelium

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WILLUMSEN, NIELS J., C. WILLIAM DAVIS, AND RICHARD C. BOUCHER. *Cellular Cl^- transport in cultured cystic fibrosis airway epithelium.* Am. J. Physiol. 256 (Cell Physiol. 25): C1045–C1053, 1989.—Cultured human nasal epithelia derived from cystic fibrosis (CF) patients were studied with double-barreled, Cl^--selective microelectrodes to measure membrane potentials and intracellular Cl^- activity (a_c^{Cl}). The a_c^{Cl} of CF cultures was 46.5 ± 2.5 mM (n = 28), a value not significantly different from a_c^{Cl} of normal human nasal cells. Reduction of the luminal [Cl^-] from 120 to 3 mM failed to reveal any apical Cl^- permeability (conductive or nonconductive) in CF cultures. Bumetanide (10^{-4} M, serosal) led to a 10 mM decrease in a_c^{Cl} without affecting the electrical parameters of the cells. Reduction of serosal [Cl^-] led to a marked decrease in a_c^{Cl} (from 58.0 ± 6.7 to 26.8 ± 2.9 mM) that could partly be blocked by bumetanide. Reduction of serosal [Cl^-] led to a rapid depolarization (5.4 ± 0.7 mV) of the basolateral membrane potential (V_b), a decrease of the fractional apical membrane resistance (0.03 ± 0.01), and an increase (34 ± $\Omega \cdot cm^2$) in the transepithelial resistance (R_t). We conclude that *1*) the apical membrane of CF airway epithelia is impermeable to Cl^-, and *2*) Cl^- transport across the basolateral membrane occurs mainly through a bumetanide-inhibitable cotransport system but also through a Cl^- conductance, neither of which appears to be affected by CF.

human nasal epithelium; apical membrane; basolateral membrane; amiloride; bumetanide; chloride substitution

CYSTIC FIBROSIS (CF) is an inheritable human disease that affects multiple epithelia. In airways, intestine, and sweat gland ducts, CF is associated with a reduced Cl^- permeability of the apical epithelial cell membranes (2, 5, 10, 11, 20). Patch-clamp studies confirmed the apical Cl^- impermeability in CF airway epithelia (7, 18) and provided evidence that the CF defect reflects an abnormal Cl^- channel regulation rather than the absence of Cl^- channels (15). Recently, we demonstrated that it is possible to activate an apical Cl^- conductance in CF epithelia by exposure to the Ca^{2+} ionophores A23187 and ionomycin but not by activators of adenosine 3′,5′-cyclic monophosphate (cAMP)-dependent kinase or protein kinase C (21). It is not known whether the defect in the conductive Cl^- permeability of the apical membrane is the only Cl^- transport defect in the CF epithelium. Recently, possible defects have been assigned to the basolateral membrane (1) and the paracellular path (12). Furthermore, because no measurements of intracellular Cl^- activity (a_c^{Cl}) in CF airway epithelia have been reported, it is not known whether the CF defect in apical Cl^- permeability, or other defects, affect the a_c^{Cl}. Changes in a_c^{Cl} in CF could be important because a_c^{Cl} determines in part other transport processes, e.g., Cl^--HCO_3^- exchange and potentially the activity of regulatory molecules (G proteins; 8).

Therefore, we addressed the following questions: *1*) are there other Cl^- pathways, i.e., nonconductive permeabilities, in the CF cell apical membrane, that can be detected; *2*) are the paths for Cl^- translocation across the basolateral membrane (cotransport and conductive paths) affected by CF; and *3*) is the a_c^{Cl} affected by CF? These studies employed double-barreled, Cl^--selective intracellular microelectrodes in combination with inhibitors and ion substitution protocols to estimate individual membrane CF permeabilities and to estimate a_c^{Cl}. Primary cultures of CF nasal epithelia were employed because of increased availability and quantitative retention of transepithelial transport activities.

MATERIALS AND METHODS

Tissue specimens. Cystic fibrosis nasal specimens were obtained from 15 cystic fibrosis subjects diagnosed by typical clinical criteria, including raised sweat Cl^- concentrations (mean age = 13.4 ± 1.4 yr, range 4–27 yr; 9 males, 6 females). The specimens were polyps that were removed to relieve nasal obstruction. Most specimens were obtained at CF centers in the continental United States and shipped to the University of North Carolina at Chapel Hill in cell-dispersion media (see below) via overnight carriers.

All procedures were approved by the University of North Carolina Committee for the Protection of the Rights of Human Subjects.

Tissue culture, microelectrodes, and Ussing chamber technique. The techniques for tissue culturing and electrophysiological measurement have been described in details elsewhere (22a). In brief, the CF nasal epithelial cells were grown to confluency on a collagen matrix on the bottom of a polycarbonate cup by standard techniques (23). The polycarbonate cup was directly inserted into a modified Ussing chamber that was configured for transepithelial measurements. The cultures were maintained at 37°C by means of a heating jacket, and both the apical (upward) and the basolateral surfaces were continuously perfused with a Krebs-bicarbonate Ringer solution.

The cultures were impaled from the luminal side with double-barreled, Cl^--selective microelectrodes. These electrodes were made from longitudinally fused borosilicate glass capillaries with outer diameters of 1.2 and 1.5 mm for the reference and Cl^- selective barrel, respectively. The tip diameters of the electrodes were ~0.2 μm. The tip of the Cl^--selective barrel was filled with a liquid Cl^- exchanger (Corning, 477913) and both barrels were backfilled with 3 M KCl. The slope of the Cl^--selective electrodes were -51 ± 1 mV, and the response time was <2 s.

The criteria for a successful impalement are listed in an accompanying paper (22a).

The apical membrane potential (V_a) is throughout the paper referenced to the luminal (grounded) bath, whereas the basolateral membrane potential (V_b) and the transepithelial potential (V_t) are referenced to the serosal bath. The electrochemical driving forces ($\Delta\tilde{\mu}_a^{Cl}/zF$ and $\Delta\tilde{\mu}_b^{Cl}/zF$) are defined positive when a_c^{Cl} is above equilibrium across the respective membrane.

Solutions and drugs. The composition of the Ringer solutions employed are the following (in mM): Krebs-bicarbonate Ringer (KBR): 140 Na^+, 120 Cl^-, 5.2 K^+, 25 HCO_3^-, 2.4 HPO_4^{2-}, 0.4 $H_2PO_4^-$, 1.1 Ca^{2+}, 1.2 Mg^{2+}, 5.2 glucose; low [Cl^-] Ringer: as KBR except all but 3 mM Cl^- substituted by gluconate; high [K^+] Ringer: as KBR except [K^+] was increased to 120.2 mM and [Na^+] reduced to 25 mM.

In the amiloride experiments, 10^{-5} amiloride (a gift from Merck Sharpe & Dohme, West Point, PA) was added to KBR. In the bumetanide experiments, 10^{-4} M bumetanide (a gift from Leo Pharmaceuticals, Copenhagen, Denmark) was added to either KBR or low [Cl^-] Ringer. All solutions were warmed to 30°C and bubbled with 95% O_2-5% CO_2 before use. The pH was maintained at 7.4.

Statistical analysis. The changes in parameter in response to experimental maneuver were analyzed by a two-tailed, paired Student's t test. Significances were accepted if $P < 0.05$. Significance levels are given in the tables. The differences between control bioelectric parameters for normal and CF cultures were analyzed by a Student's t test for independent means assuming identical variances. Unless otherwise indicated, all values are given as mean ± SE.

RESULTS

The V_t in CF cultures averaged -29.2 ± 4.4 mV (Table 1), which is increased in comparison to normal cultures by almost a factor of three (Table 1; 22a). R_t was similar to R_t of normal nasal epithelia. Thus the equivalent short-circuit current (I_{eq}) was increased in CF relative to normal cultures three-fold.

To investigate the correlates of the raised V_t at the cellular level and to determine their relationship to Cl^- transport, we studied CF cells with Cl^--selective, double-barreled microelectrodes. Figure 1 depicts illustrative impalements of CF cultures. Note that in this preparation V_a is positive. Table 1 summarizes the data collected from impalements of 271 cells in 28 cultures.

V_a varied from negative to positive with a mean of -15.6 mV (Fig. 2*A*). Also, Fig. 2*A* demonstrates that there was no marked tendency for changes in V_a during longer lasting impalements (up to 60 s). The V_a in the CF cultures were distributed unimodally (Fig. 2*A*), indicating that the cells with positive apical membrane po-

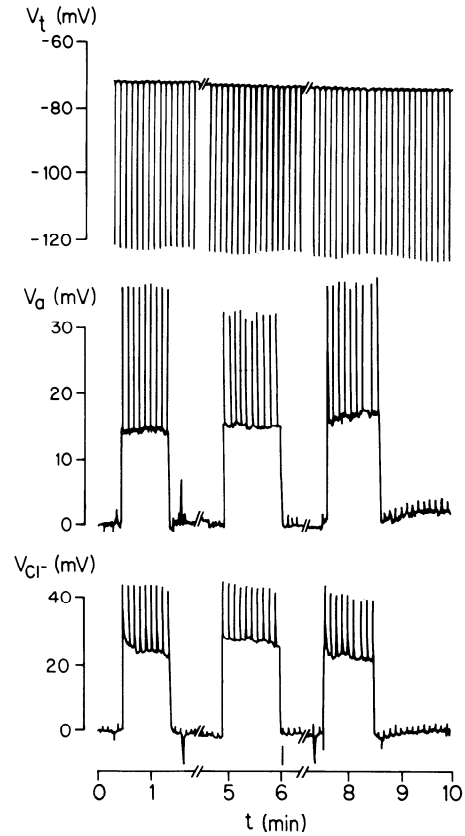

FIG. 1. Illustrative tracings of transepithelial potential (V_t), apical membrane potential (V_a), and chloride electrode signal (V_{Cl}) during 3 sequential impalements of a cystic fibrosis (CF) culture under control conditions.

TABLE 1. *Bioelectric parameters for cystic fibrosis nasal epithelial cultures under control conditions*

	V_t, mV	V_a, mV	V_b, mV	R_t, $\Omega \cdot cm^2$	I_{eq}, $\mu A/cm^2$	fR_a	a_c^{Cl}, mM	$\Delta\tilde{\mu}_a/zF$, mV	$\Delta\tilde{\mu}_b/zF$, mV	n
CF	−29.2	−15.6	−44.8	435	−76.2	0.54	46.5	−2.5	26.8	28 (271)
	±4.4	±3.1	±2.2	±42	±12.9	±0.03	±2.5	±2.7	±2.9	
Normal	−10.1	−26.1	−36.2	400	−28.2	0.46	42.7	6.0	16.2	34 (353)
	±1.1	±1.2	±1.2	±35	±3.0	±0.02	±2.0	±1.4	±1.6	
P	<0.005	<0.005	<0.005	NS	<0.005	<0.025	NS	<0.005	<0.005	

Values are means ± SE compared with those from normal cultures (22a). n, no. of cultures. Total no. of impalements is given in parentheses. NS, no significance. See text for definitions.

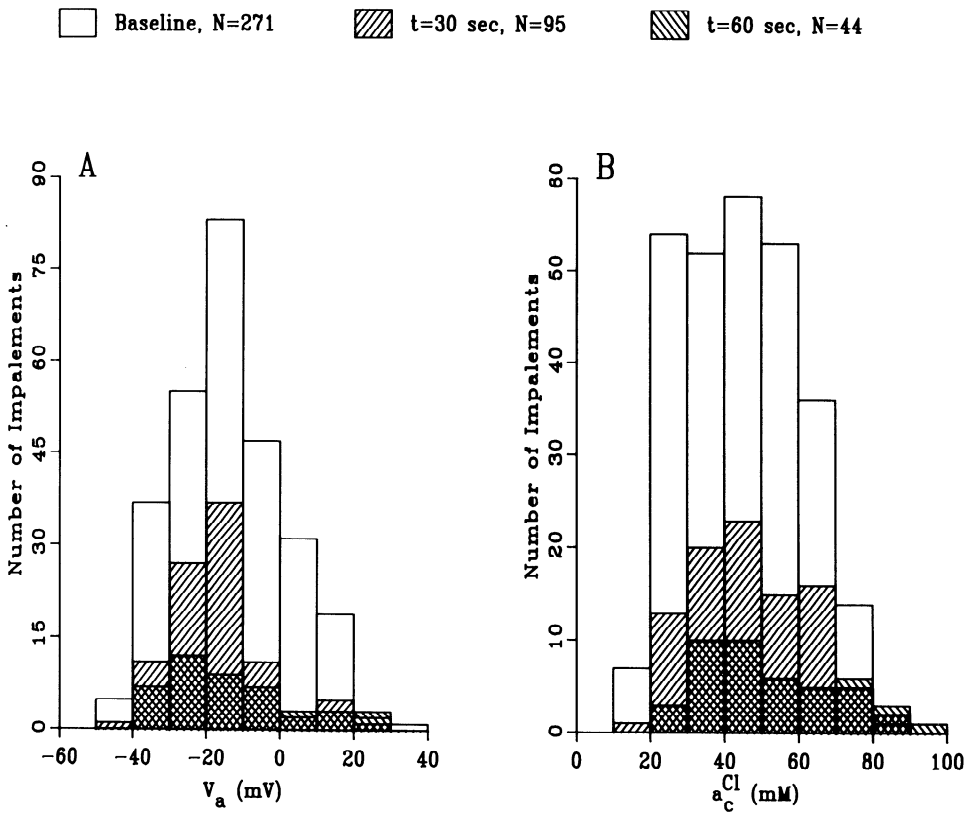

FIG. 2. Frequency distribution of apical membrane potential (V_a) (A) and intracellular Cl^- activity (a_c^{Cl}) (B) for impalements of 271 cystic fibrosis (CF) cells. Hatched bars, distribution of V_a and a_c^{Cl} measured in continuous impalements at 30 and 60 s, respectively.

tentials are part of a single population of cells. Both the V_a and the V_b were significantly higher than in the normal preparations. The fractional resistance was 0.54 (Table 1).

The a_c^{Cl} was distributed through a relatively wide range as illustrated in Fig. 2B. Figure 2B also shows that a_c^{Cl} was not affected by the impalement within 60 s. The mean a_c^{Cl} was similar to that reported for normal nasal epithelia (22a). Interestingly, the driving force for Cl^- across the apical membrane was inwardly directed (from lumen to cell). If a correction of a_c^{Cl} of 8 mM for interfering anions is made (22a), a_c^{Cl} would be 10 mV below equilibrium across the apical membrane.

Effect of reduction of luminal Cl^- concentration. To study apical membrane Cl^- permeabilities, $[Cl^-]$ of the luminal bathing solution was reduced from 120 to 3 mM in nine cultures. A typical experiment is depicted in Fig. 3. A small (5 mV) hyperpolarization of V_t and a 13% increase in R_t were observed. V_a and chloride electrode signal (V_{Cl}) changed by 5 and 6 mV (both in the positive direction), respectively, indicating little change in a_c^{Cl}. The fractional apical membrane resistance (fR_a) remained constant (0.55). After reduction of the $[Cl^-]$ in the luminal solution, no change in a_c^{Cl} was observed within 15 min. Furthermore, no significant change was observed in the electrophysiological properties of the cells (Table 2). Of particular interest is that a_c^{Cl} remained constant despite the fact that the outwardly directed driving force increased from 3 to 90 mV.

Effect of amiloride. To study the effects of blocking the conductive Na^+ pathway on Cl^- transport, 10^{-5} M amiloride was added to the luminal medium in 10 CF cultures. Figure 4 depicts a representative tracing of the amiloride effects on CF cultures. V_t decreased rapidly from −25 to 0 mV. This change was accompanied by a 39-mV hyperpolarization of the apical membrane. V_a and V_{Cl} changed at the same rate and by the same amount, reflecting a constant a_c^{Cl}. A marked increase of fR_a from 0.51 to 0.81 was observed in this culture.

The steady-state results of the amiloride exposure are summarized in Table 3. V_t depolarized by 36 mV, and the residual V_t in the new steady state was only 1 mV (not significantly different from 0). The change in V_t was due largely to a hyperpolarization of V_a. R_t almost doubled and I_{eq} was practically abolished by amiloride. The fR_a increased from 0.57 to 0.71. The a_c^{Cl} did not change despite a change in $\Delta\tilde{\mu}_a^{Cl}/zF$ from −7 to +28 mV (steady-state values).

Generally, the effect of amiloride was fully reversible within 10–20 min.

Effect of bumetanide. To test for the activity of a basolateral cotransport system in CF cells, the effect of serosal application of 10^{-4} M bumetanide was studied in six cultures. The time course of the reduction in a_c^{Cl} was slow in CF (Fig. 5), and a_c^{Cl} decreased maximally by 10 mM in response to bumetanide exposure.

The steady-state results for a_c^{Cl} bioelectric parameters are summarized in Table 4. Bumetanide had no effects on membrane potentials, transepithelial electrical parameters, and induced only trivial change in the fractional resistance of CF cultures. The decrease in a_c^{Cl} is expected to increase the inwardly directed $\Delta\tilde{\mu}_a^{Cl}/zF$ by 7 mV and decrease $\Delta\tilde{\mu}_b^{Cl}/zF$ by the same amount. Such trends were observed (Table 4).

Effect of reduction of serosal Cl^- concentration. In seven CF cultures, we studied the effect of reducing the Cl^-

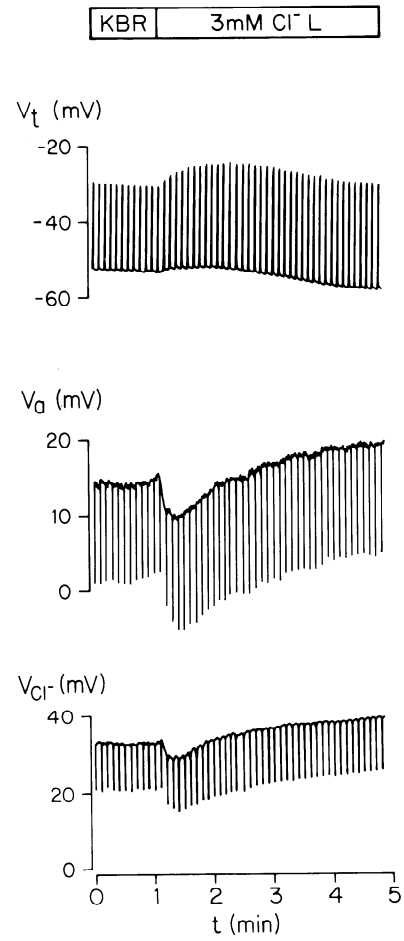

FIG. 3. Effects of luminal [Cl^-] reduction from 120 to 3 mM on V_t, V_a, and V_{Cl} in a CF human nasal epithelial culture. Tracings are representative of 9 CF preparations. See Fig. 1 for definitions.

concentration in the serosal bathing solution from 120 to 3 mM. This maneuver led to a rapid decrease in V_t that was due solely to a depolarization of the basolateral membrane (Figs. 6 and 7). The time course of the change in V_b (Fig. 7) parallels the time course for a complete shift of the serosal solution. R_t increased slightly (34 ± 15 $\Omega \cdot cm^2$, significantly different from 0, $P < 0.05$) within the first 80 s, and fR_a decreased consistently (Fig. 7). No acute changes in a_c^{Cl} were noted. The initial ($t < 80$ s) response to a reduction of serosal [Cl^-] was similar to that reported from normal cultures (22a).

Continuous exposure to reduced serosal [Cl^-] led to a substantial decrease in a_c^{Cl} in CF cultures. The time course of the decrease in a_c^{Cl} is illustrated in Fig. 8. After 30 min, a_c^{Cl} had not yet reached steady state.

The values of the bioelectric parameters recorded at 10–40 min after serosal [Cl^-] reduction are presented in Table 5. The decrease in V_t reflected the combined effect of a small decrease in V_a and a larger decrease in V_b. The change in the loop current induced by the imposed transepithelial Cl^- gradient probably accounts for the depolarization of the apical membrane. R_t increased 106 $\Omega \cdot cm^2$ and the transepithelial current was reduced by 24 $\mu A/cm^2$, presumably because of the imposed inward flow of Cl^- caused by the asymmetric solutions. No change in fR_a was observed. The a_c^{Cl} was reduced by 31 mM.

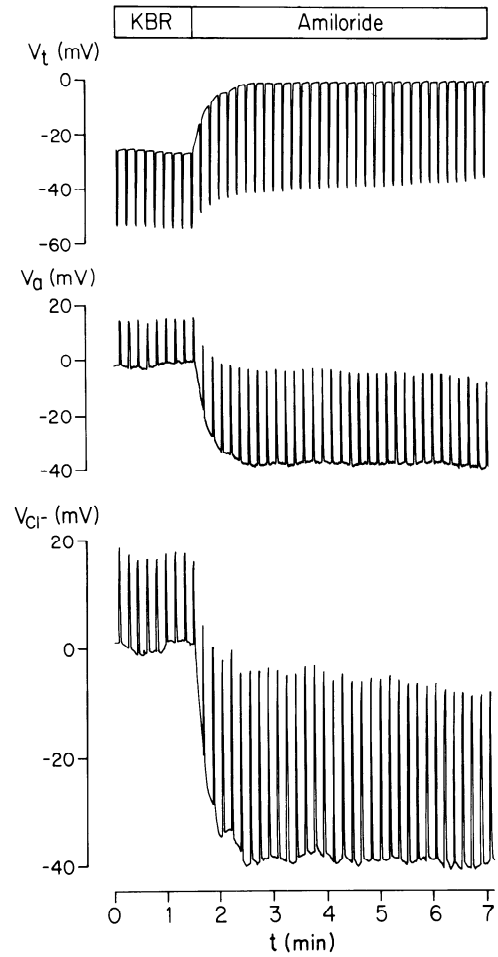

FIG. 4. Effects of application of 10^{-5} M amiloride to luminal bathing solution on V_t, V_a, and V_{Cl} of a CF culture. Tracings are representative of 10 CF preparations. See Fig. 1 for definitions.

TABLE 2. *Effect of reduction of luminal [Cl^-] to 3 mM on steady-state bioelectric parameters of cystic fibrosis human nasal epithelial cultures*

	V_t, mV	V_a, mV	V_b, mV	R_t, $\Omega \cdot cm^2$	I_{eq}, $\mu A/cm^2$	fR_a	a_c^{Cl}, mM	$\Delta\tilde{\mu}_a/zF$, mV	$\Delta\tilde{\mu}_b/zF$, mV	n
Control	−27.7	−20.0	−47.8	469	−63.8	0.52	48.3	3.1	30.8	9 (67)
	±9.2	±6.1	±3.4	±107	±17.6	±0.06	±4.8	±4.7	±5.4	
3 mM Cl^-	−31.0	−17.1	−48.1	494		0.54	50.1	89.5	32.1	9 (46)
lumen ($t > 5$ min)	±9.5	±5.5	±4.4	±100		±0.05	±4.9	±7.2	±4.0	
P	NS	NS	NS	NS		NS	NS	<0.005	NS	

Values are means ± SE; n, no. of cultures. Total no. of impalements is given in parentheses. NS, no significance. See text for definitions.

TABLE 3. *Effect of 10^{-5} M amiloride on steady-state bioelectric parameters of cystic fibrosis human nasal epithelial cultures*

	V_t, mV	V_a, mV	V_b, mV	R_t, $\Omega \cdot cm^2$	I_{eq}, $\mu A/cm^2$	fR_a	a_c^{Cl}, mM	$\Delta\tilde{\mu}_a/zF$, mV	$\Delta\tilde{\mu}_b/zF$, mV	n
Control	−37.4	−8.2	−45.6	427	−93.1	0.57	52.1	−6.7	30.8	10 (102)
	±8.4	±5.0	±4.5	±74	±26.8	±0.03	±4.3	±4.8	±5.9	
Amiloride	−1.2	−43.6	−44.8	769	−3.4	0.71	51.3	28.3	29.5	10 (38)
($t > 5$ min)	±1.1	±4.0	±3.5	±178	±1.31	±0.04	±5.2	±3.8	±3.5	
P	<0.01	<0.005	NS	<0.025	<0.01	<0.005	NS	<0.005	NS	

Values are means ± SE; n, no. of cultures. Total no. of impalements is given in parentheses. NS, no significance. See text for definitions.

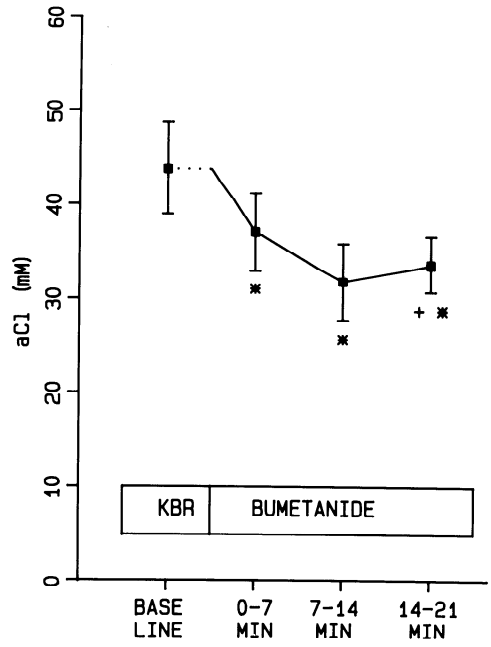

FIG. 5. Time dependence of a_c^{Cl} of CF cultures ($n = 6$) after application of 10^{-4} M bumetanide to serosal bathing solution. * Mean a_c^{Cl} value of the 14- to 21- min period was significantly different from baseline (KBR) value ($P < 0.05$). + Mean a_c^{Cl} of 14- to 21-min period was not significantly different from mean a_c^{Cl} of 7- to 14-min period. See Fig. 2 for definitions.

Effect of reduction of serosal Cl^- concentration in presence of bumetanide. To separate Cl^- transport via a bumetanide-sensitive pathway from that occurring via conductive pathways across the basolateral membrane, we exposed six CF cultures to bumetanide (10^{-4} M, basolateral) for 20 min and then reduced the Cl^- concentration from 120 to 3 mM (containing 10^{-4} M bumetanide) in the basolateral bath for 30 min. The steady-state results are presented in Table 6 and the time course of the changes in a_c^{Cl} in Fig. 9. A decrease in a_c^{Cl} of only 7 mM was observed after 30 min in response to serosal [Cl^-] reduction (Table 6).

Effect of increasing luminal K^+ concentration. In two CF preparations, the K^+ concentration in the luminal bath was increased from 5.2 to 120.2 mM in the presence of 10^{-5} M amiloride to test whether the apical cell membrane has a measurable K^+ permeability. This protocol only trivially affected the bioelectrical parameters. Most importantly, no changes were detected in V_a ($\Delta V_a = -2.0 \pm 0.8$ mV, SD) or fR_a ($\Delta fR_a = 0.02 \pm 0.02$ mV, SD) after an increase in the luminal K^+ concentration.

DISCUSSION

Control bioelectric parameters. Under control conditions, CF nasal cultures were characterized by a higher mean V_t than reported for normal cultures. The V_t measurements reported here for cultured cells are in reasonable agreement with in vivo observations in CF nasal epithelia (inferior turbinate) where V_t ranged from −42 to −77 mV (9) and tended to exceed values for V_t of freshly excised CF polyps (−11.7 ± 3.1 mV; Ref. 10).

The difference in V_t between CF and normal cultures was due both to a reduced V_a and to an increased V_b in CF cultures. Both V_t and the individual membrane potentials varied over a much larger range in CF cultures as compared with normal cultures (22a); however, the unimodal distribution of V_a in both groups suggests that the impaled cells belong to the same type and/or that they are electrically coupled.

I_{eq} was substantially increased in the CF cultures (-76.2 ± 12.9 $\mu A/cm^2$) compared in normals [-28.7 ± 3.0 $\mu A/cm^2$, (22a)], indicating an increased rate of transepithelial ion transport. The increased I_{eq} in CF is also characteristic of the intact tissue (5).

Lack of Cl^- pathways in apical membrane. The electrochemical driving force for Cl^- flow across the apical membrane was perturbed either by reduction of the luminal Cl^- concentration to 3 mM or by application of amiloride to the luminal perfusate. No significant effects of luminal [Cl^-] reduction on electrical parameters were observed in CF cells (Table 2). The absence of response of bioelectric parameters is similar to that observed in human CF sweat duct cells (3). Luminal [Cl^-] reduction increased the electrochemical driving force for Cl^- exit by 86 mV (outwardly directed). The lack of a decrease in a_c^{Cl} after luminal [Cl^-] reduction strongly supports the notion that neither conductive nor electrically silent Cl^- pathways are present in the apical membrane of CF cells.

Amiloride effect. In normal human airway epithelia, amiloride abolishes Na^+ absorption and induces Cl^- secretion. The effects of amiloride on the bioelectric properties of CF preparations are qualitatively similar to normal tissues but quantitatively larger. In the CF cultures in the present study, amiloride hyperpolarized the apical membrane and increased the $\Delta\tilde{\mu}_a^{Cl}/zF$ by 35 mV but had no significant effect on a_c^{Cl} (Table 3). The observation that the driving force for Cl^- secretion across the apical membrane after amiloride is even higher than in normal cultures (17.4 ± 3.2; Ref. 23), coupled with the nearly complete abolition of I_{eq} by amiloride, support the

TABLE 4. *Effect of 10^{-4} M bumetanide on bioelectric parameters of cystic fibrosis human nasal epithelial cultures measured 20–40 min after bumetanide application*

	V_t, mV	V_a, mV	V_b, mV	R_t, $\Omega \cdot cm^2$	I_{eq}, $\mu A/cm^2$	fR_a	a_c^{Cl}, mM	$\Delta\tilde{\mu}_a/zF$, mV	$\Delta\tilde{\mu}_b/zF$, mV	n
Control	−20.4	−18.1	−38.5	489	−47.2	0.53	44.6	−0.6	19.9	6 (53)
	±4.7	±5.1	±5.1	±98	±11.0	±0.06	±4.6	±2.6	±4.1	
Bumetanide	−19.4	−17.8	−37.2	489	−44.9	0.48	34.5	−7.7	11.8	6 (46)
($t > 10$ min)	±4.0	±5.5	±4.3	±100	±9.6	±0.05	±4.1	±4.0	±2.0	
P	NS	NS	NS	NS	NS	<0.01	<0.05	NS	NS	

Values are means ± SE; n, no. of cultures. Total no. of impalements is given in parentheses. NS, no significance. See text for definitions.

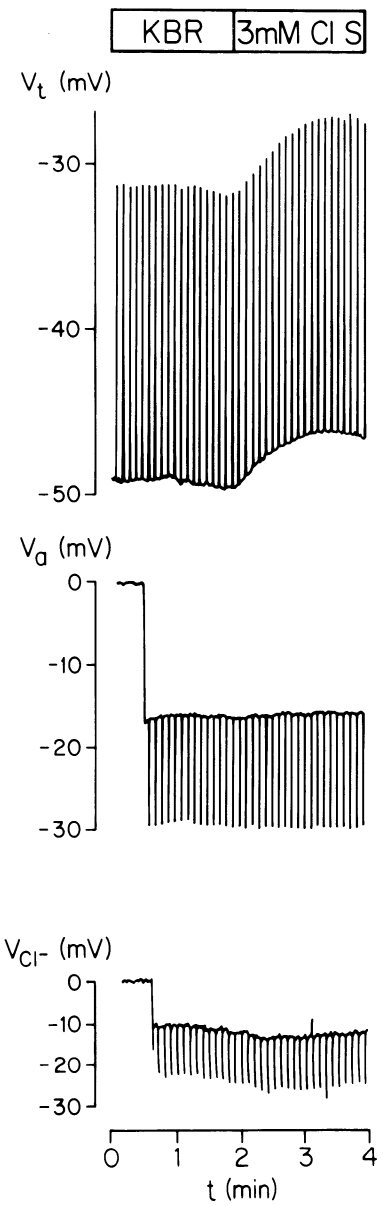

FIG. 6. Effect of reducing serosal [Cl^-] from 120 to 3 mM in a CF culture on V_t, V_a, and V_{Cl}. Tracings are representative of 7 preparations. See Fig. 1 for definitions.

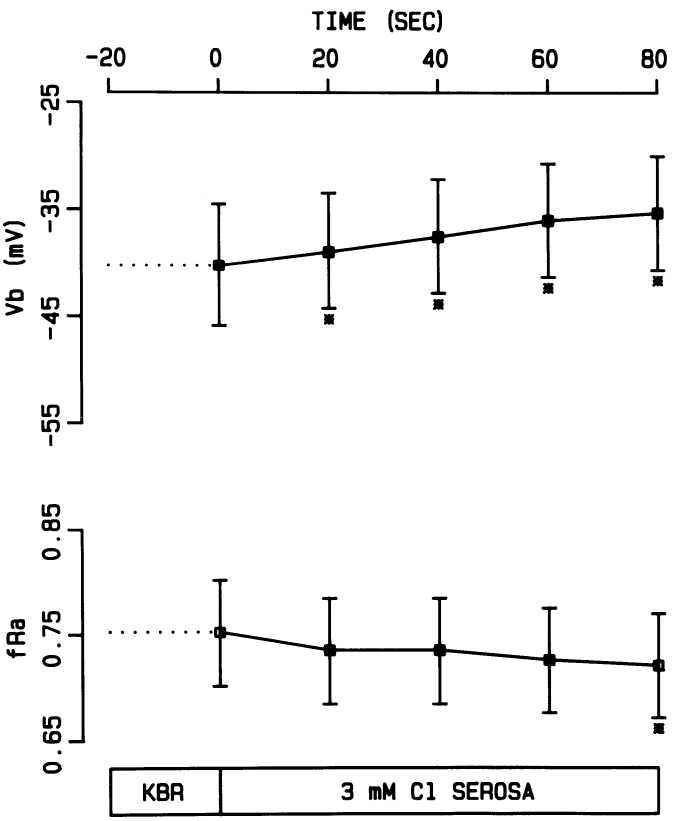

FIG. 7. Acute ($t < 80$ s) depolarization of basolateral membrane (V_b) and decrease in fractional apical membrane resistance (fR_a) of CF ($n = 8$) cultures after reduction of serosal [Cl^-] from 120 to 3 mM.

notion that the conductive Cl^- pathway in the apical membrane in CF cultures is negligible.

Amiloride increased fR_a to 0.71, corresponding to a ratio between apical and basolateral membrane resistances (R_a/R_b) of 2.5. Boucher et al. (4) reported an fR_a of 0.80 ($R_a/R_b = 4$) under the same conditions. Such an fR_a value might seem surprisingly low if the apical membrane is permeable only to Na^+. The low luminal [Cl^-] experiments yielded no evidence for an apical Cl^- conductance in CF cells. Possible explanations for the low fR_a during amiloride exposure are *1*) amiloride does not inhibit the apical Na^+ conductive permeability completely, *2*) there is a conductive pathway for another ion species in the apical membrane (most probable candidate on a quantitative basis is K^+) *3*) a significant resistive layer resides beneath the impaled epithelial layer, and *4*) R_b changes in parallel with R_a due to a dynamic (Na^+ transport dependent) basolateral K^+ permeability.

Direct experimental evidence indicates that the first two possibilities are unlikely. First, the fact that amiloride almost completely abolished I_{eq} indicates that the elimination of the conductive apical Na^+ permeability by amiloride is close to 100%. Second, experiments in which

TABLE 5. *Effect of reduction of serosal [Cl^-] on steady-state bioelectric parameters of cystic fibrosis human nasal epithelial cultures*

	V_t, mV	V_a, mV	V_b, mV	R_t, $\Omega \cdot cm^2$	I_{eq}, $\mu A/cm^2$	fR_a	a_c^{Cl}, mM	$\Delta\tilde{\mu}_a/zF$, mV	$\Delta\tilde{\mu}_b/zF$, mV	n
Control	−27.4	−17.6	−45.1	522	−50.0	0.55	58.0	5.8	33.1	7 (29)
	±8.2	±3.4	±6.2	±122	±13.7	±0.02	±6.7	±3.7	±7.8	
3 mM Cl^-	−19.0	−10.8	−29.8	639		0.55	26.8	−21.2	86.1	7 (51)
serosa ($t > 20$ min)	±7.8	±5.3	±5.0	±151		±0.06	±2.9	±7.0	±4.6	
P	<0.025	<0.05	<0.01	<0.025		NS	<0.01	<0.01	<0.005	

Values are means ± SE; n, no. of cultures. Total no. of impalements is given in parentheses. NS, no significance. See text for definitions.

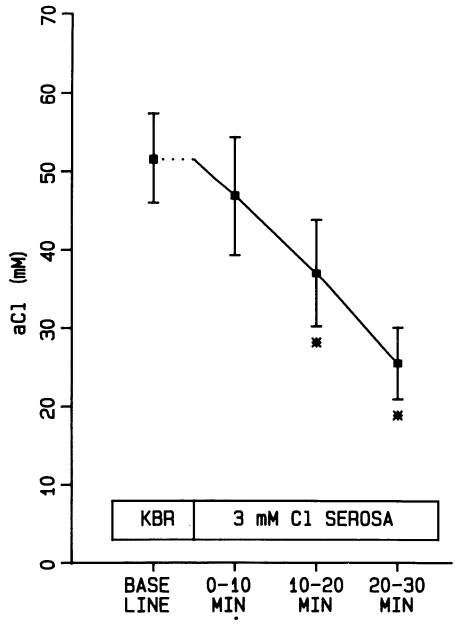

FIG. 8. Time dependence of a_c^{Cl} of CF ($n = 6$) cells after reduction of serosal [Cl^-] from 120 to 3 mM. * Mean a_c^{Cl} was significantly different ($P < 0.05$) from base-line (KBR) value. See Fig. 2 for definitions.

luminal [K^+] was raised from 5.2 to 120.2 mM in the presence of amiloride failed to detect any changes in V_a or fR_a indicative of a significant apical K^+ conductance. This observation is in agreement with studies of the tracheal epithelium in which no evidence for an apical K^+ conductance was detected by intracellular microelectrode techniques (17).

The low fR_a could be caused by the presence of a subepithelial resistive layer. When this possibility was analyzed using an appropriate equivalent circuit representation,[1] the resistance of the hypothetical resistive layer was calculated to be 212 $\Omega \cdot cm^2$. This value is not only unreasonably high (~50% of R_t under control conditions), but we have been unable to find any evidence for the presence of such a resistance: the resistance of the supporting collagen membrane is negligible (in the order of $10\Omega \cdot cm^2$) and when the tip of the microelectrode was advanced through the epithelial cell layer, we invariably found that $fR_a \cong 1.0$.

If the basolateral K^+ permeability is dynamic and regulated in parallel with the rate of Na^+ entry across the apical membrane or with the pump rate of the basolateral Na^+-K^+-ATPase as reported for other Na^+ absorbing epithelia (14, 6, 19), then the increase in fR_a due to blockade of a luminal Na^+ conductance would be reduced because of parallel increases in R_b (reduction of the K^+ conductance). In this case, the total resistance of the cellular pathway would approach infinity and R_t would approach R_s and even minute leak currents around the microelectrode tip would lead to a markedly decreased apparent fR_a. Such leak currents will influence fR_a only when the V_a is increased toward infinity. In fact, in response to amiloride, R_t increases from 427 to 769 $\Omega \cdot cm^2$, which is intermediate between the value of R_s estimated by Boucher et al. (987 $\Omega \cdot cm^2$; Ref. 4) and the value estimated from an equivalent electrical circuit analysis, based on control data (605–615 $\Omega \cdot cm^2$; Ref. 22). Therefore, the findings of an fR_a significantly less than unity in amiloride-treated CF cultures probably reflects a coupled increase in the resistance of both membranes in combination with small leak current at the site of the impalement.

In conclusion, by perturbing the $\Delta\tilde{\mu}_a^{Cl}/zF$ and by monitoring the subsequent changes in V_a and a_c^{Cl}, we have provided evidence that the apical cell membrane of CF cells express neither a conductive, nor an electrically silent, Cl^- permeability in this membrane.

Cl^- transport across basolateral membrane. We designed experimental protocols to determine whether the CF nasal epithelia retain a bumetanide-sensitive cotransport system and a conductive permeability for Cl^- translocation across the basolateral membrane.

Under control conditions, Cl^- was above equilibrium across the basolateral membrane by 29 mM. This is equivalent to a driving force of 27 mV directed from cell to serosa. Bumetanide added to the basolateral bath led to a slow (Fig. 5) decrease in a_c^{Cl} of ~10 mM, which was not paralleled by any change in the membrane potentials or R_t. This finding indicates that a bumetanide-sensitive system for cell Cl^- accumulation is present in the basolateral membrane.

Further evidence in favor of a normal cotransport activity in CF cells was observed in the Cl^--substitution experiments. Reduction of Cl^- alone in the basolateral solution induced ~0.9 mM/min loss in cell Cl^- >30 min.

[1] The magnitude of a hypothetical resistor was determined from a standard equivalent circuit model for epithelia (see Ref. 22) equipped with an additional serial-connected resistor (R_x). If fR'_a denotes the apparent fractional apical membrane resistance (measured as $\Delta V_a/\Delta V_t$) then

$$R_x = R_t \left(1 - \frac{fR_a'}{fR_a}\right)$$

Assuming the true fR_a is approximately one (say 0.98), we obtain R_x = 212 $\Omega \cdot cm^2$ when using $fR_a' = 0.71$ and $R_t = 769\ \Omega \cdot cm^2$ (Table 3).

TABLE 6. *Effect of reduction of serosal [Cl^-] to 3 mM in presence of 10^{-4} M bumetanide on steady-state bioelectric parameters of cystic fibrosis human nasal epithelial cultures*

	V_t, mV	V_a, mV	V_b, mV	R_t, $\Omega \cdot cm^2$	I_{eq}, $\mu A/cm^2$	fR_a	a_c^{Cl}, mM	$\Delta\tilde{\mu}_a/zF$, mV	$\Delta\tilde{\mu}_b/zF$, mV	n
Bumetanide	−19.5	−17.3	−36.8	507	−43.1	0.50	33.9	−8.4	10.9	6 (33)
	±3.7	±5.4	±4.2	±94	±9.4	±0.06	±3.5	±3.5	±2.2	
3 mM Cl^-	−16.7	−13.3	−30.0	707		0.50	27.0	−19.3	26.1	6 (24)
+bumetanide ($t > 20$ min)	±5.6	±6.2	±4.3	±113		±0.04	±2.9	±5.6	±3.4	
P	NS	NS	<0.025	<0.025		NS	<0.025	NS	<0.005	

Values are means ± SE; n, no. of cultures. Total no. of impalements is given in parentheses. NS, no significance. See text for definitions.

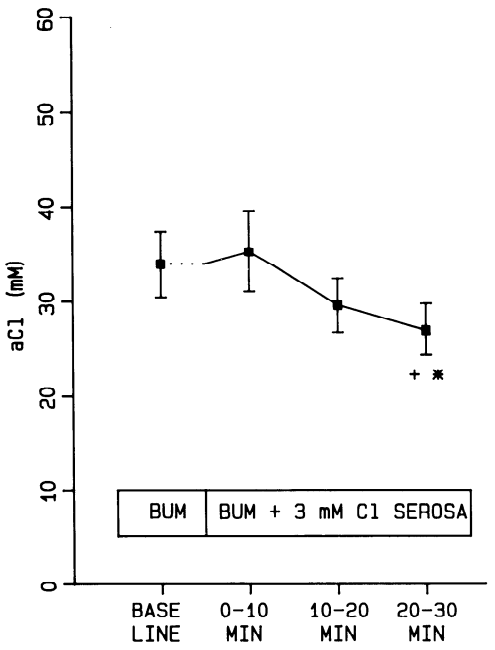

FIG. 9. Time dependence of a_c^{Cl} of CF cultures ($n = 6$) after reduction of serosal [Cl^-] from 120 to 3 mM in presence of 10^{-4} M bumetanide (BUM) in serosal bathing solution. * Mean a_c^{Cl} was different ($P < 0.05$) from base-line (KBR) value. + Mean a_c^{Cl} of 20- to 30-min period was not significantly different ($P > 0.05$) from the 10- to 20-min period. See Fig. 2 for definitions.

Reduction of the serosal [Cl^-] concentration from 120 to 3 mM in the presence of bumetanide greatly slowed the loss of cellular Cl^- as compared with the loss induced by Cl^- substitution alone (Fig. 9). Thus these observations support the notion that an electrically silent Cl^--accumulating cotransport mechanism is located in the basolateral membrane and indicate that this path is the major path for Cl^- movement across the basolateral membrane of CF airway epithelia.

Like the normal human airway epithelium, V_b in the basal state does not approximate the electromotive force of the basolateral membrane (approximately −80 mV assuming an intracellular K^+ activity of 80 mM), suggesting that another conductive permeability is expressed in the basolateral membrane. Analysis of continuous cellular impalements monitoring changes in response to Cl^- substitution at the basolateral membrane (Fig. 7) indicates this conductance is for Cl^-. The simplest interpretation of the depolarization of V_b (5.4 ± 0.7 mV, $n = 8$), coupled with the small but significant decrease in fR_a (0.03 ± 0.01) and increase in R_t (34 ± 15 $\Omega \cdot cm^2$), in response to serosal Cl^- replacement is that a conductive Cl^- pathway is present in the basolateral membrane. Other alternative possibilities, including changes in membrane permeabilities secondary to changes in cell volume and/or pH, however, cannot be ruled out.

In summary, our experiments indicate that unlike the apical membrane, paths for Cl^- translocation are operational in the basolateral membrane in CF airway epithelia. The overall permeability, as estimated by the rate of loss of Cl^- in response to serosal Cl^- substitution (0.9 vs. 0.4 mM/min for CF and normal, respectively) might appear higher for CF. However, the greater net loss in CF probably reflects the failure of Cl^- to enter across the luminal membrane to replace a fraction of the Cl^- lost to the serosal bath. The notion is supported by the observation that a_c^{Cl} decreases measurably after reduction of serosal [Cl^-] in bumetanide pretreated preparations in CF but not normal airway epithelia (22a).

a_c^{Cl} under control conditions. The a_c^{Cl} measured by the Cl^--selective microelectrode technique is in reasonable agreement with the Cl^- concentration of cultured human CF epithelium measured with chemical techniques (56.9 mM) by Stutts et al. (16) assuming an intracellular activity coefficient of 0.74 (13). The a_c^{Cl} is similar to that of normal cultures (42.7 ± 2.0 and 46.5 ± 2.5 mM in normal and CF cells, respectively). Thus it appears that the existence of paths for Cl^- translocation expressed only on the basolateral barrier are sufficient to regulate a_c^{Cl} to normal values in CF airway epithelia.

Defective Cl^- transport in CF. In conclusion, the present study has defined normal and defective Cl^- permeabilities in the cultured CF cells. In contrast to normal nasal epithelia, the introduction of a large outwardly directed electrochemical driving force across the apical membrane by amiloride application did not induce Cl^- secretion in CF epithelia, indicating the absence of a Cl^- conductance in this barrier. Removal of Cl^- from the luminal medium did not reduce the a_c, confirming that the apical membrane has no Cl^- conductance, and further indicating that no other major electrically silent Cl^- transport pathway exists in this barrier.

We identified the following two basolateral Cl^--transport pathways: *1*) a bumetanide-inhibitable cotransport system; and *2*) a small Cl^--conductive pathway. Both these pathways appeared similar to those identified in normal nasal epithelial cells (22a). The observation that a functioning basolateral Cl^- conductance is expressed

in both CF and normal epithelia indicates that this conductance reflects the activity of a different channel than expressed in the apical membrane or a similar channel that is regulated differently. Importantly, despite the lack of apical Cl^- permeability, CF cells are able to maintain a normal a_c^{Cl} suggesting that the transport mechanisms that regulate a_c^{Cl} are unaffected by the CF defect.

Finally, under open-circuit conditions the CF nasal epithelium absorbs Cl^- at an increased rate relative to normal epithelia (22a). Because of an absence of an apical Cl^- permeability, this Cl^- absorption probably occurs through a paracellular path. This notion was supported by the finding of a large shunt conductance (22) in combination with an increased transepithelial electrical driving force.

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