# $Na^+$ Transport in Cystic Fibrosis Respiratory Epithelia

## Abnormal Basal Rate and Response to Adenylate Cyclase Activation

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## Abstract

**The transepithelial potential difference (PD) of cystic fibrosis (CF) airway epithelium is abnormally raised and the $Cl^-$ permeability is low. We studied the contribution of active $Na^+$ absorption to the PD and attempted to increase the $Cl^-$ permeability of CF epithelia. Nasal epithelia from CF and control subjects were mounted in Ussing chambers and were short-circuited. The basal rate of $Na^+$ absorption was raised in CF polyps compared with control tissues. Whereas beta agonists induced $Cl^-$ secretion in normal and atopic epithelia, beta agonists further increased the rate of $Na^+$ absorption in CF epithelia without inducing $Cl^-$ secretion. This unusual effect is not due to an abnormal CF beta receptor because similar effects were induced by forskolin, and because cAMP production was similar in normal and CF epithelia. We conclude that CF airway epithelia absorb $Na^+$ at an accelerated rate. The abnormal response to beta agonists may reflect a primary abnormality in a cAMP-modulated path, or a normal cAMP-modulated process in a $Cl^-$ impermeable epithelial cell.**

## Introduction

Ion transport by airway epithelia from subjects with cystic fibrosis (CF)[1] is abnormal. Previously we reported that the transepithelial electric potential difference (PD) was raised across CF respiratory epithelia and speculated that this abnormality might reflect an increased rate of $Na^+$ absorption (1). Subsequently, we found that $Cl^-$ permeability was abnormally low and noted that this defect might contribute to the raised PD in respiratory epithelia (2, 3). Quinton (4) has reported decreased chloride permeability of sweat ductal epithelium and also suggested that $Cl^-$ impermeability is the primary defect that characterizes the CF epithelial dysfunction (5). Either raised $Na^+$ absorption or decreased $Cl^-$ secretion could account for the lower water content of CF pulmonary secretions and airways surface liquid (6–8).

The present study was initiated with two goals: (*a*) to compare the rate of $Na^+$ absorption across excised and short-circuited airway epithelia from a large number of CF patients, with that of tissue obtained from normal and disease control subjects; and (*b*) to test in CF respiratory epithelia the effects of agents, e.g., beta adrenergic agonists, that stimulate chloride secretion in normal respiratory epithelia (9–11). Because these agents are thought to work by changing cell cAMP concentrations (10–11), we also investigated the effects of beta adrenergic agonists on the rate of cAMP production in tissue biopsies of CF and normal nasal epithelia. Excised nasal epithelium was studied because these tissues are frequently available, relatively free of infection, and exhibit defects expressed in lower airways (1).

## Methods

*CF subjects.* All patients with cystic fibrosis (age range, 6–27 yr; mean age, 13.1±5.0 yr; 14 males, 13 females) were characterized by traditional clinical criteria and raised sweat $Cl^-$ values. 27 subjects, comprising our entire experience, were used for the population comparisons of short-circuit current (Isc). Tissues from 25 subjects were used for isotope flux studies to quantitate baseline ion transport activities. Tissues from 14 patients were used for studies of adrenergic agents. Only two of these 14 patients had received oral methyl-xanthines or inhaled beta agonists in the month before surgery. 6 of these 14 patients had received nasal steroids; each patient discontinued therapy at least 2 wk before surgery.

*Control subjects.* Control subjects underwent turbinate reductions for nasal reconstruction or sleep apnea. 53 subjects (age range, 9–62 yr; mean age, 31.2±12.6 yr; 29 males, 24 females), accounting for all normal tissues studied for the previous 40 mo in our laboratory, were used for population studies of Isc. Tissues from 41 subjects were employed in the assessment of baseline ion transport activities utilizing isotope flux measurements. The tissues from 20 normal subjects were used for studies of adrenergic agents. No control subjects were receiving medication (oral or nasal) before surgery.

*Atopic subjects.* The 18 patients (age range, 10–68 yr; mean age, 32.1±13.8 yrs; 11 males, 7 females) were characterized by seasonal rhinitis, a raised concentration of circulating eosinophils, and nasal polyps. All 18 subjects were used for population studies of Isc. Tissues from nine subjects were employed in the studies of baseline ion transport activities. Most patients were treated with topical nasal steroids, but therapy was usually discontinued 2 wk before the operation. Two subjects were taking systemic steroids at the time of study and two were taking methyl-xanthines. The six patients that were used for studies of adrenergic agents were receiving no adrenergic agents.

*Operative techniques.* All patients that had polypectomies and more than half of the patients that underwent reconstructive surgery were exposed to general anesthesia. Topical anesthetic was not administered before excision of any tissue.

*Tissue preparation, bioelectric measurements, and measurement of solute permeabilities.* All studies were performed in a laboratory adjacent to the operating room and were often performed by the authors at institutions other than the University of North Carolina (see Acknowledgments). Freshly excised tissue was carried directly to a laboratory, dissected free of submucosal tissue, and mounted in Ussing flux chambers (surface area, 0.26 or 0.53 $cm^{-2}$) within 15 min of excision (3, 9). The tissues were dissected and maintained in Ussing chambers in a Krebs-

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1. *Abbreviations used in this paper:* CF, cystic fibrosis; G, conductance; Isc, short-circuit current; M, mucosal; PD, potential difference; $PGE_1$, prostaglandin $E_1$; S, submucosal.

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bicarbonate Ringer solution (KBR) with a composition of (in millimoles per liter): 140 $Na^+$, 120 $Cl^-$, 5.2 $K^+$, 25 $HCO_3^-$, 2.4 $HPO_4^-$, 0.4 $H_2PO_4^-$, 1.2 $Mg^{++}$, 1.1 $Ca^{++}$, 5.6 glucose. The tissues were continuously short-circuited (transepithelial PD = 0 mV) except for a 45-s interval every 15 min when the open circuit potential was recorded. Conductance (G) was calculated from Ohm's law.

Unidirectional solute permeabilities ($^{22}Na^+$, $^{36}Cl^-$, and $^{14}C$-mannitol, all obtained from New England Nuclear, Boston, MA) were measured in the mucosal (M) to submucosal (S) direction and in the S → M direction in separate tissues. Samples for radiocounting were obtained every 15 min. Gamma and beta scintillation counting and the calculation of solute permeabilities were performed as described previously (3) and reflect steady-state values. When more than one preparation per patient specimen was obtained (range, 1–4), tissues were generally allocated to opposite flux directions. However, we could not routinely measure fluxes across conductance matched preparations from the same patient specimen because of the frequency of single preparations per specimen and the occurrence of a G disparity of >10% for two preparations from the same patient specimen. Consequently, we matched the G and Isc (within 8%) cumulatively as tissues were obtained in each flux direction (M → S, S → M) for specimens from each group of patients. The flux intervals between 45 and 75 min, before any drug addition, were used for the estimates of baseline parameters. For the assessment of the effects of isoproterenol on solute fluxes across nasal epithelia, after steady state measurements were made (45–75 min), tissues were exposed to indomethacin ($10^{-6}$ M/liter in both bathing solutions) for 60 min followed by the addition of isoproterenol ($10^{-4}$ M/liter) for 75 min. Amiloride ($10^{-4}$ M/liter) was then added to the mucosal solution.

Similar protocols were generally employed for studies of the bioelectric effects of other drugs that may alter cell cAMP concentrations (10, 11). 75 min after mounting, the tissues were usually pretreated with indomethacin ($10^{-6}$ M), a maneuver designed to minimize baseline $Cl^-$ permeability (12, 13). After 60-min exposure to indomethacin, the tissues were exposed to a second drug; 60 or 75 min later a third agent was added. One protocol (see Fig. 6) required addition of a fourth drug. All drugs except amiloride were added to both (mucosal and submucosal) bathing solutions. Amiloride was added only to the mucosal solution. Except for indomethacin and bumetanide, the concentration of each drug was increased in log steps from $10^{-9}$ M to a final concentration of $10^{-6}$ M (prostaglandin $E_1$; $PGE_1$), $10^{-5}$ M (forskolin), or $10^{-4}$ M (isoproterenol, amiloride).

All tissues were fixed in the flux chamber after study for histologic characterization (3).

*Measurement of cAMP.* Small (<1 mg) mucosal biopsies were obtained by sharp dissection from excised tissues and maintained in KBR at 37°C under an atmosphere of 95% $O_2$–5% $CO_2$. For time course studies, biopsies were exposed to maximal effective concentrations of isoproterenol ($10^{-4}$ M) for periods of 2, 5, 10, 20, and 60 min. For dose-effect studies, biopsies were exposed to a beta agonist (isoproterenol, $10^{-9}$–$10^{-4}$ M), isoproterenol ($10^{-4}$ M) and sotalol ($10^{-4}$ M), or a direct stimulator of adenylate cyclase, forskolin (14), ($10^{-7}$–$10^{-5}$ M) for 2 min at 37°C. cAMP content of a TCA (10%) extract of the tissue was measured by radioimmunossay as described by Steiner et al. (15). The TCA-precipitated pellet was solubilized in 200 μl of a 0.05% sodium dodecyl sulfate (SDS) and 0.05% NaOH solution, and protein content was measured by the method of Bradford (16). Each exposure of tissue from a patient to a drug was carried out in triplicate and the results for each drug concentration were averaged. The recovery of added radiolabeled cAMP exceeded 90%. All cAMP levels were expressed as picomolars per milligram protein.

*Drugs and solutions.* KBR was the standard solution (9). Indomethacin, $PGE_1$, and isoproterenol HCl were obtained from Sigma Chemical Co., St. Louis, MO; forskolin was obtained from Calbiochem-Behring Corp., La Jolla, CA, and bumetanide was from Leo Pharmaceuticals, Copenhagen, Denmark. Amiloride was a gift of Merck, Inc., Rahway, NJ.

*Data analysis.* We discarded data from specimens with G>25 $mS \cdot cm^{-2}$. This was comprised of 20% of CF specimens, 28% of atopic specimens, and 31% of specimens from normal subjects. This selection criterion reflects two considerations. First, no tissue with a G that exceeded 25 $mS \cdot cm^{-2}$ exhibited a PD that exceeded 2 mV. Because 2 mV represents 10–15% or less of the in vivo values for PDs in CF or normal subjects (1), these specimens are unlikely to reflect in situ function. Second, as G of the tissue approaches G of the bathing solution (~40 $mS \cdot cm^{-2}$), small errors in bathing solution fluid resistance compensation will induce significant errors in measuring Isc and G. The transport rate of tissues from different populations (Isc) that met this criterion was compared by averaging the maximal Isc under basal conditions that was sustained for 15 min of all tissues from each patient. Because Isc values for subjects within a group were not normally distributed, nor were group variances equal, the differences between groups were compared by the Wilcoxon rank sum test.

For comparisons of baseline unidirectional and net solute permeabilities and bioelectric properties, the mean steady state values for the 45–75 min intervals, before drug exposure, were employed. If more than one specimen was obtained from a subject in a flux direction, the values were averaged to provide a single estimate per subject. Because the differences in G and Isc between the flux directions were <8% in all groups, and were not significantly different, these values were meaned for presentation (Tables I and II). In contrast to the data for Isc, the values of G and the unidirectional fluxes were normally distributed for all groups (Shapiro-Wilks Test of Normality). Therefore, values for unidirectional fluxes were compared within each group with *t* tests for independent means. Values for G and unidirectional solute permeabilities for different groups were compared with *t* tests for independent means. Comparison of net fluxes ($J_{Na^+}^{net}$) for different groups was performed with a *t* test for independent means employing data only from patients where sufficient tissue was obtained to allocate a specimen to each flux direction (17 CF patients, 11 normal subjects, 8 atopic subjects). Drug-induced changes in unidirectional solute permeabilities and bioelectric properties were compared with paired *t* tests.

*Table I. Baseline Bioelectric Properties and Solute Flows Across Excised and Short-circuited Nasal Epithelium From Normal, Cystic Fibrosis (CF), and Atopic Subjects**

| Bioelectric properties | Normal | CF | Atopic |
|---|---|---|---|
| | *mean±SEM* | *mean±SEM* | *mean±SEM* |
| PD (*mV*) | 4.6±.4 | 10.9±1.1‡ | 5.6±1.2 |
| Isc ($\mu A \cdot cm^{-2}$) | 67.4±3.5 | 150.0±12.2‡ | 86.5±13.2 |
| G ($mS \cdot cm^{-2}$) | 15.4±0.6 | 14.0±1.0 | 15.5±1.4 |
| Ion fluxes ($J$, $\mu eq \cdot cm^{-2} \cdot h^{-1}$) | | | |
| $J_{Na^+}^{MS}$ | 7.34±.46 | 10.01±.69‡ | 8.12±.76 |
| $J_{Na^+}^{SM}$ | 5.02±.28 | 4.68±.61 | 6.16±.56 |
| $J_{Na^+}^{net}$ | 2.32±.51§ | 5.33±.95§‖ | 1.96±.92¶ |
| $J_{Cl^-}^{MS}$ | 7.34±.39 | 5.31±.40‡ | 8.20±.79 |
| $J_{Cl^-}^{SM}$ | 7.12±.31 | 4.79±.61‡ | 8.11±.92 |
| $J_{Cl^-}^{net}$ | 0.22±.49 | 0.52±.70 | 0.09±1.23 |
| Mannitol permeability ($P$, $\times 10^{-7}$ $cm \cdot s^{-1}$) | | | |
| $P_{man}^{MS}$ | 35.4±3.2 | 33.0±3.8 | 38.7±10.6 |
| $P_{man}^{SM}$ | 35.8±5.1 | 30.0±4.3 | 39.9±7.5 |

* The mean data reflect the contribution of patients as follows: Normal, MS flux direction, 27 patient specimens, 38 preparations; SM 29 patient specimens, 39 preparations; 41 patients in total. CF, MS, 22 patient specimens, 34 preparations; SM, 18 patient specimens, 26 preparations; 25 patients in total. Atopic, MS, 8 patient specimens, eight preparations; SM, nine patient specimens, nine preparations; nine patients in total. The comparison for $J_{Na^+}^{net}$ employed data for 17 CF patients, 11 normal subjects, and eight atopic subjects.
‡ Different from normal or atopic ($P < 0.01$).
§ Different from zero ($P < 0.001$).
‖ Different from normal or atopic ($P < 0.05$).
¶ Different from zero ($P < 0.05$).

*Table II. Bioelectric Properties and Solute Flows at Short-circuit Conditions Across Excised Nasal Epithelium From Normal and Cystic Fibrosis Subjects That Were Exposed to Indomethacin ($10^{-6}$ M) and Isoproterenol ($10^{-4}$ M)*

| | Normal (n = 9) | | | Cystic fibrosis (n = 6) | | |
|---|---|---|---|---|---|---|
| | Control | Indomethacin | Isoproterenol | Control | Indomethacin | Isoproterenol |
| Bioelectric properties | | | | | | |
| Isc ($\mu A \cdot cm^{-2}$) | 65.8±9.3* | 60.1±6.9 | 80.6±9.4¶ | 157.3±24.8 | 139.5±19.9 | 176.4±29.8¶ |
| G ($mS \cdot cm^{-2}$) | 14.0±0.7 | 13.5±0.7 | 15.8±0.9¶ | 13.2±1.7 | 13.4±1.2 | 13.8±1.3 |
| Solute flows $J(\mu eq \cdot cm^{-2} \cdot h^{-1})$ | | | | | | |
| $J^{MS}_{Na^+}$ | 6.58±0.65* | 6.63±0.70 | 6.48±0.83 | 10.39±1.39 | 10.03±1.03‖ | 11.43±1.37¶ |
| $J^{SM}_{Na^+}$ | 4.38±0.50 | 4.35±0.48 | 4.65±0.52 | 3.89±0.83 | 4.13±0.80 | 4.60±0.70 |
| $J^{net}_{Na^+}$ | 2.20±0.82‡ | 2.28±0.85‡ | 1.93±0.98 | 6.50±1.62§ | 5.90±1.30§ | 6.83±1.53§ |
| $J^{MS}_{Cl^-}$ | 7.13±0.93* | 7.04±0.89 | 7.14±0.95 | 5.17±0.82 | 4.99±0.74 | 5.15±0.85 |
| $J^{SM}_{Cl^-}$ | 7.31±0.49* | 7.28±0.51 | 8.74±0.63¶ | 4.12±0.86 | 4.67±0.87 | 4.84±0.69 |
| $J^{net}_{Cl^-}$ | −0.18±1.05 | −0.24±1.03 | −1.60±1.13 | 1.05±1.19 | 0.32±1.14 | 0.31±1.09 |
| Mannitol permeability ($P$, $\times 10^{-7}$ $cm \cdot s^{-1}$) | 37.1±4.6 | 35.8±4.4 | 36.1±5.1 | 32.0±5.3 | 30.1±3.4 | 36.6±3.6 |

$-J_{net}$, secretion. * Different than CF ($P < 0.05$). ‡ Different from zero ($P < 0.05$). § Different from zero ($P < 0.005$). ‖ Different than control value ($P < 0.05$). ¶ Different from Indomethacin value ($P < 0.05$).

## Results

The frequency distribution of mean Isc of tissues excised for each subject from CF, allergic, and control patients is shown in Fig. 1. Although considerable overlap in Isc exists, CF tissues exhibit a significantly greater Isc than do tissues from normal ($P < 0.001$) or atopic patients ($P < 0.001$). No significant difference in Isc was noted between normal and allergic patients. As previously reported, the superficial epithelium of tissues from each group was characterized by ~80% ciliated cells (3).

The baseline bioelectric parameters and solute permeabilities for tissues of normal, CF, and atopic subjects are shown in Table I. The values for normal subjects ($n$ = 41 subjects) for all parameters are similar to those reported previously (3). Net $Na^+$ absorption is the major active ion flow across the excised and short-circuited normal nasal epithelium and accounts for >90% of the measured Isc. The $Cl^-$ fluxes are equal. The sum of the passive ion flows for $Na^+$ and $Cl^-$ (partial ionic conductances) was ~80% of the measured G. The disparity between the partial ionic conductances and G may represent the contribution of unmeasured solution ions (e.g., $HCO_3$), the conductances associated with the active $Na^+$ path (apical $G_{Na^+}$, basolateral $G_K$ [17, 18]), or both. Although there is a trend for Isc to be slightly increased in atopic polyp epithelia, no systematic differences between any baseline parameters of atopic polyp ($n$ = 8 subjects) and normal epithelia were detected.

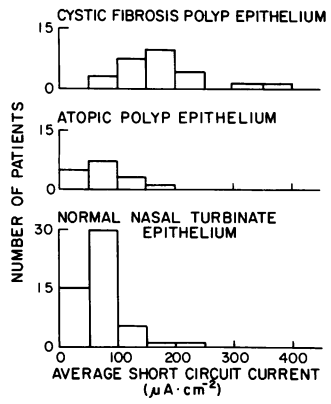


*Figure 1.* Distribution of short-circuit current of tissues excised from cystic fibrosis (*A*), allergic (*B*), and normal subjects (*C*). The average number of tissues studied per patient was 3.0±1.0 for CF, 2.1±0.5 for allergic, and 1.8±0.6 for normal subjects.

CF polyp epithelium could be distinguished from normal or atopic epithelia by the higher resting current (Isc) and PD. Examination of the unidirectional fluxes reveals that the increased Isc of CF tissues reflects a greater rate of net $Na^+$ absorption. This increase in net $Na^+$ absorption principally reflects an increased flux in the active ($J^{MS}_{Na^+}$) direction. Unidirectional $Cl^-$ fluxes across CF epithelia were smaller than measured in normal or atopic tissues. No net $Cl^-$ translocation was detected. Despite the smaller $Cl^-$ fluxes, G in CF tissues was not significantly smaller in CF than normal or atopic tissues. The partial ionic conductances for $Na^+$ and $Cl^-$ ($J^{SM}_{Na^+}$, $J^{MS}_{Cl^-}$) accounted for ~70% of G. We do not know whether the greater disparity between the sum of the partial ionic conductances for $Na^+$ and $Cl^-$ and G in CF tissues reflects the contribution of an active component to G, e.g., associated with the greater $Na^+$ transport rate, or an unmeasured ion. Mannitol permeability coefficients, a marker of paracellular permeability (19, 20), were not different for any of the three groups.

The response of short-circuit current to sequential exposure to indomethacin, isoproterenol, and amiloride is shown in Fig. 2. Indomethacin exerted little effect on Isc (or PD or G; see Table II) of normal turbinate but isoproterenol ($10^{-4}$ M) initially raised Isc and G. The increase in current decreased slowly over 75 min, and amiloride then further reduced Isc by ~40%. The response of atopic polyp tissues was similar to normal turbinate: isoproterenol raised Isc by 27.5±4.1 $\mu A \cdot cm^{-2}$ and raised G by 2.9±1.0 $mS \cdot cm^{-2}$. Isc was subsequently decreased by amiloride ($10^{-4}$ M) by 31.6±3.2% ($n$ = 3). The pattern of response of CF epithelia to isoproterenol exhibited three distinct differences from normal or atopic tissues. First, the isoproterenol-induced increase in Isc in CF tissues was slower to develop and more sustained. Second, G did not significantly change (+0.4±0.5 $mS \cdot cm^{-2}$, Table II). Third, during exposure to isoproterenol the Isc could be completely abolished by amiloride. A similar response was noted for three CF nasal tissues that were not indomethacin pretreated and for excised bronchial epithelium from two CF patients.

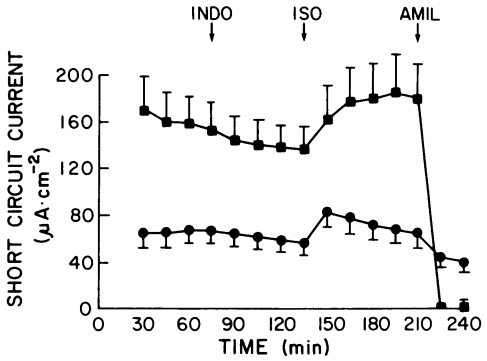


*Figure 2.* Effect of sequential addition of indomethacin ($10^{-6}$ M), isoproterenol ($10^{-4}$ M), and amiloride ($10^{-4}$ M) on short-circuit current of nasal tissues excised from CF (■) and normal (●) subjects. Each point is the mean of 12 CF or 12 normal tissues. Bars represent SEM.

Similar responses in both CF and normal tissues were observed with lower concentrations of isoproterenol ($10^{-6}$ M/liter). Responses of both CF and control tissues to isoproterenol were blocked by pretreatment with sotalol ($10^{-4}$ M). Exposure to vehicles alone was not associated with significant changes in CF polyp or normal turbinate Isc.

Analysis of solute and ion permeabilities for these tissues confirmed that isoproterenol induced qualitatively different effects on ion flow across CF and normal tissues. In the basal state, or after indomethacin, net $Na^+$ absorption accounted for >90% of the Isc of both CF and normal tissue (Table II). Net $Na^+$ absorption was two- to threefold greater in CF tissues. Isoproterenol induced a significant increase in unidirectional secretory (S → M) $Cl^-$ flow in normal tissues but no significant change in $Na^+$ translocation or mannitol permeability (P, × $10^{-7}$ cm·s$^{-1}$). In contrast, isoproterenol raised the rate of $Na^+$ flow in the absorptive (M → S) direction but did not affect $Cl^-$ flow across CF tissues.

Amiloride pretreatment of normal tissues may increase the $Cl^-$ secretory response to beta agonists in part by increasing the electrical gradient that favors chloride exit from the cell (apical membrane hyperpolarization). The magnitude of the isoproterenol-induced increase in Isc after amiloride pretreatment, which reflects $Cl^-$ secretion, is likely to be influenced by this process (Fig. 3). A similar response was noted in atopic tissues (Δ Isc induced by isoproterenol after amiloride = 37.1±4 μA·cm$^{-2}$, $n$ = 4). Since isoproterenol did not stimulate short-circuit current of CF tissues pretreated with amiloride, it appears that the lower $Cl^-$ permeability of the CF epithelium cannot be reversed by beta agents. These results also support the notion that isoproterenol selectively raises the rate of $Na^+$ absorption in CF tissue.

Because beta adrenergic agents induced a $Na^+$ flux response in CF tissues, it seemed likely that dysfunction of CF tissue was not linked to a defect in beta receptor function. We tested this possibility more rigorously by two approaches. First, tissues were exposed to agents that induce a $Cl^-$ secretion in normal tissues that is independent of beta receptor stimulation. For example, $PGE_1$ ($10^{-6}$ M), an agent which raises apical chloride conductance in non-CF airway epithelia (21), increased the Isc of normal, atopic and CF tissues by 22±8.4% ($n$ = 4), 34±7.1% ($n$ = 3), and 31±5.9% ($n$ = 4), respectively. The entire Isc (100%) of CF tissues ($n$ = 4) was sensitive to amiloride, whereas Isc fell by only 38±5.9% ($n$ = 4) and 35±6.8% ($n$ = 3) in normal and atopic tissues, respectively. Consequently, interaction of another agonist with a separate population of receptors, i.e., the $PGE_1$ receptor, mediates the same change in $Na^+$ transport in CF tissues as does activation of the beta receptor. Moreover, exposure of tissues to forskolin, a direct stimulator of adenylate cyclase activity, also induced the same pattern of response by CF and normal tissues (Fig. 4).

We also measured intracellular concentrations of cAMP as an index of beta receptor activation. The time course of cAMP concentration in biopsy specimens after exposure to a maximal concentration of isoproterenol ($10^{-4}$ M) is shown in Fig. 5 *A*. Basal concentrations are similar to those reported by others (22). No differences between CF and normal in the peak concentration or time course were noted. Log dose-effect relationships for the increase in tissue cAMP induced by a 2-min exposure to isoproterenol were also explored (Fig. 5 *B*). The maximal increases in cAMP for CF and control epithelia were again similar at $10^{-4}$ M isoproterenol (16.8±9.4 pM/mg protein, $n$ = 5; 18.4±11.1 pM/mg protein, $n$ = 4), respectively. The concentration of isoproterenol that induced the half-maximal increase in cAMP ($EC_{50}$) was similar for CF and normal epithelia: 5 × $10^{-7}$; 8 × $10^{-7}$ M, respectively. Sotalol ($10^{-4}$ M) blocked the increase in cAMP induced by isoproterenol. Forskolin ($10^{-7}$–$10^{-4}$ M) induced larger increases in cAMP in CF and normal tissues. The maximal concentration accumulated after forskolin was similar for CF and normal tissues: 39.9±16.1 pM/mg protein, $n$ = 4; and 37.3±11.5 pM/mg protein, $n$ = 4, respectively. The $EC_{50}$s

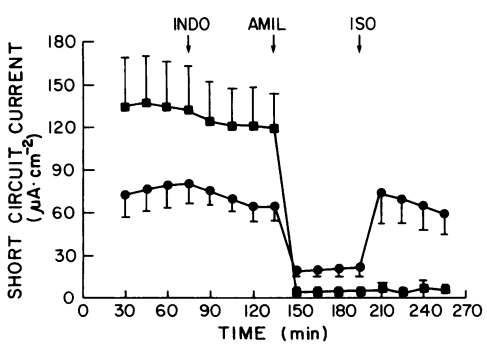


*Figure 3.* Effect of sequential addition of indomethacin ($10^{-6}$ M), amiloride ($10^{-4}$ M), and isoproterenol ($10^{-4}$ M) on short-circuit current of nasal tissues excised from CF (■) and normal (●) subjects. Each point is the mean of six CF or four normal tissues. Bars represent SEM.

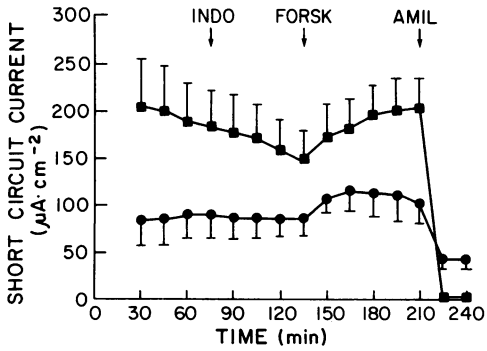


*Figure 4.* Effect of sequential addition of indomethacin ($10^{-6}$ M), forskolin ($10^{-5}$ M), and amiloride ($10^{-4}$ M) on short-circuit current of nasal tissues excised from CF (■) and normal (●) subjects. Each point is the mean of four CF or three normal tissues. Bars represent SEM.

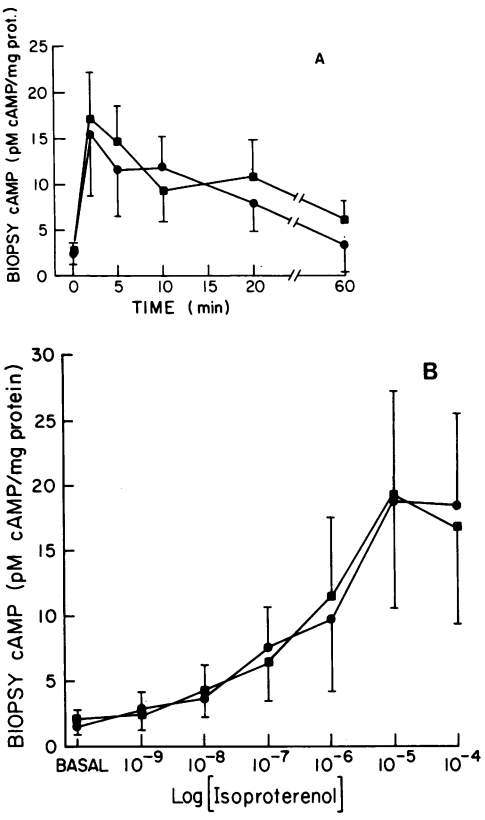


*Figure 5.* (*A*) Time course of cAMP concentration in biopsies from CF (■) and normal nasal specimens (●), exposed to maximal ($10^{-4}$ M) concentration of isoproterenol. Each point is the mean of four tissues; bars depict SEM. (*B*) Dose-effect relationship between log concentration of isoproterenol and cAMP concentrations in biopsies from CF (■) and normal nasal specimens (●). All specimens were exposed to isoproterenol for 2 min. Each point reflects the mean of four normal and five CF patients, bars depict SEM.

for forskolin were also similar for CF and normal tissues: $2 \times 10^{-6}$ M/liter; $9 \times 10^{-7}$ M/liter, respectively. Biopsies with epithelium removed produced unmeasurable quantities of cAMP in response to isoproterenol or forskolin.

Finally, we attempted to reduce cell $Cl^-$ permeability of normal tissues by pretreatment with indomethacin and bumetanide, a loop diuretic that inhibits coupled Na/Cl movement across the basolateral membrane and consequently inhibits the capacity for $Cl^-$ secretion (23; and Fig. 6). Bumetanide appeared to reduce the $Cl^-$ secretory capacity of normal tissue because the effects of isoproterenol on Isc were blunted and the residual Isc in the presence of amiloride, which reflects the induction of $Cl^-$ secretion, was smaller (compare Fig. 2 and Fig. 6). This effect was probably not accompanied by a change in resting $Na^+$ absorption because Isc was constant during the period of exposure to indomethacin and bumetanide (120–185 min). Furthermore, bumetanide did not influence the effect of isoproterenol on the Isc of CF tissue, again suggesting that the response of CF tissues to isoproterenol does not involve a $Cl^-$ pathway.

## Discussion

Like our previous report (3), unidirectional $Cl^-$ fluxes across freshly excised and short-circuited CF polyp epithelia were

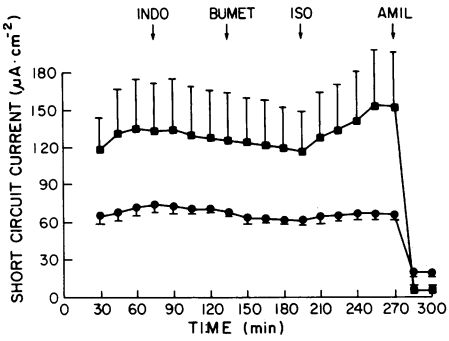


*Figure 6.* Effect of sequential addition of indomethacin ($10^{-6}$ M), bumetanide ($10^{-4}$ M), isoproterenol ($10^{-4}$ M), and amiloride ($10^{-4}$ M) on short-circuit current of nasal tissues excised from CF (■) and normal (●) subjects. Each point is the mean of four CF or five normal tissues. Bars represent SEM.

smaller than $Cl^-$ fluxes measured across nasal epithelium from normal subjects (Table I). The smaller $Cl^-$ fluxes appear not to reflect differences in tissue architecture (polypoid morphology) nor chronic inflammation because polyps excised from allergic patients do not share this feature. The permeability of a probe of the paracellular path, [$^{14}$C]mannitol (19, 20), was not different for the three subject groups. Thus, it is probable that the decreased $Cl^-$ permeability of the CF polyp epithelium is located in a cellular path. However, the data shown in Fig. 1 and Table I differ in two important respects from those reported previously (3). First, in the present study we observed that CF nasal epithelia absorb $Na^+$ under short-circuit conditions at a rate approximately twofold greater than nasal epithelia of normal or atopic individuals. Second, the transepithelial slope conductance (G) of CF tissues in this study is equal to rather than smaller than the G of the normal or disease control tissues.

The apparent discrepancies between the two studies reflect the difficulties in comparing baseline functions of epithelia freshly excised from human subjects. We have attempted to identify and analyze variables that may specifically contribute to the differences in $Na^+$ transport rate and G noted in these studies. Certain variables are relatively easy to identify and quantitate, e.g., patient age, medication history, and distribution of cell types in the resected specimen. These variables did not differ in the two studies, nor as discussed in the previous report, are they likely to account for the differences in function noted in the CF epithelia (3). Consequently, it may not be possible to identify the specific variable(s) that account for the discrepency between this study and our earlier, smaller sample. However, two other variables may be pertinent to the specific differences between the two studies but are more difficult to quantitate.

First, the interval between operative excision and mounting in a well-gassed perfusion chamber represents a period of relative tissue hypoxia that likely impairs tissue function. Because of increased experience, this interval routinely decreased by 50% in this as compared with our previous study. Recently, we have reported that hypoxia or metabolic inhibitors rapidly reduce Isc, G, active $Na^+$ absorption, and unidirectional $Cl^-$ fluxes in respiratory epithelia (24). These findings parallel studies by others where hypoxia has been shown to reduce Isc, apical membrane $Na^+$ conductance ($G_{Na^+}$), transepithelial G, and $Na^+$ absorption (25). Therefore, it is possible that the increased Isc and $Na^+$ absorption of CF tissues in the present study reflect better pres-

ervation of function of the $Na^+$ transport path due to more rapid tissue mounting. It is also possible that the increased G noted in the present study reflects in part an increase in cell G associated with the greater rate of $Na^+$ transport (increased apical $G_{Na^+}$ and basolateral $G_K$). The observation from a recent microelectrode study that the near-instantaneous change in G induced by amiloride blockade of active $Na^+$ absorption was threefold greater in CF than normal epithelia ($\Delta G = 3.1$ mS·cm$^{-2}$ for CF; $G = 0.8$ mS·cm$^{-2}$ for normal tissues) supports this notion (26).

Second, excised specimens can be physically damaged during acquisition and during clamping in a flux chamber. The PDs of excised CF and normal tissues are proportionately lower than those measured in vivo (3). We do not know the permeability of the paracellular path to mannitol in situ. However, the relatively high $P_{mannitol}$ values ($30–40 \times 10^{-7}$ cm·s$^{-1}$) measured in nasal epithelia as compared with excised canine or human bronchi ($15–20 \times 10^{-7}$ cm·s$^{-1}$) (9, 24), raise the possibility that physical damage may have occurred to the specimens during the operative excision and/or mounting procedure. Such damage would create nonselective leaks in the epithelial barrier that would serve to partially shunt the transepithelial PD. The addition of a leak path to the native tissue passive permeability paths complicates any comparison of G for excised tissues. However, the mannitol permeabilities in the present study were not significantly different from those reported previously (3), making it unlikely that damage alone can account for the increased G noted in the CF tissues in this series. Note that a nonspecific leak adds a component to unidirectional ion fluxes. However, the net ion fluxes measured under equilibrium conditions and their associated short-circuit current, Isc, are not systematically altered by the leak component added to the unidirectional fluxes. Indeed, the rate of net $Na^+$ absorption across either CF or control tissues was not related to the magnitude of the leak component, i.e., the difference between $J^{MS}_{Na^+}$ vs. $J^{SM}_{Na^+}$ was independent of $P_{mannitol}$ over a wide range.

In addition to the experimental variables encountered in these studies, there must be some selection criteria employed for inclusion of tissues for data analysis. We elected to use G as a criterion for selecting tissues on the basis of previous precedent established by a number of studies of respiratory epithelia (9, 10, 23, 27). The specific limits for G (<25 mS·cm$^{-2}$) were selected as described in Methods (Data analysis). Because CF polyp G may be expected to be low secondary to a reduced $Cl^-$ permeability, a bias might have been introduced into our data analysis by virtue of the selection of conductance as our criterion. However, the number of tissues excluded by this criterion was similar in each group (ranging from 20% for CF to 31% for normals), making the potential contribution of this bias to our results probably small.

Based on the above analysis, certain conclusions about basal function of freshly excised CF epithelia as compared with normal or disease control tissues appear justified. First, the absolute rate of $Na^+$ absorption is increased. The rate of $Na^+$ transport cannot be overestimated consequent to any of the variables analyzed above. Whereas these studies do not identify the mechanism(s) involved in raising the rate of $Na^+$ absorption across CF epithelia, recent microelectrode studies indicate that the increased rate does not reflect simple changes in the electrical driving force for $Na^+$ across the apical membrane consequent to reduced $Cl^-$ permeability (26). Indeed, the magnitude of the apical membrane PD in CF is smaller (−15 mV) than for normal tissues (−30 mV). Second, the problem of tissue damage may generate sufficient variability to make it difficult to reach firm conclusions about differences in G. However, it appears probable that the absolute G of CF tissues may closely approximate that of normal tissues but the distribution of the total conductance between the $Na^+$ conductance and the $Cl^-$ conductance differs (26).

The responses to isoproterenol also identify a clear difference in the nature of electrolyte transport by CF airway epithelia. Isoproterenol raised Isc of epithelia from each population of subjects. Such a response, however, could reflect either induction (or an increase) of $Cl^-$ secretion or increased $Na^+$ absorption in respiratory epithelia. Our experiments show that isoproterenol increased $Cl^-$ secretion in normal nasal epithelia. First, the increase in Isc (Fig. 2), was accompanied by an increase of $Cl^-$ flux in the secretory direction without a change in the magnitude of $Na^+$ absorption (Table II). Second, amiloride pretreatment, which blocks $Na^+$ absorption and inhibits basal Isc, actually enhanced the Isc response to isoproterenol (Fig. 3). Third, pretreatment with an inhibitor of $Cl^-$ secretion, bumetanide, blocked the response to isoproterenol (Fig. 6). Accordingly, although the magnitude of $Cl^-$ secretion in response to adrenergic stimulation is smaller in nasal epithelium than in some other proximal airway epithelia (10, 11), the pattern of the response is typical.

In contrast, the increased Isc of CF tissues induced by isoproterenol (Fig. 2) reflects an increase in the rate of $Na^+$ absorption. The observation that the entire Isc after isoproterenol stimulation was sensitive to amiloride (Fig. 2) provided the first clue that isoproterenol increased the rate of $Na^+$ absorption rather than induced $Cl^-$ secretion in CF tissue. The observations that pretreatment with amiloride (Fig. 3) but not bumetanide (Fig. 6) completely abolished the response of CF tissues to isoproterenol is in agreement with this notion. The most direct evidence, however, comes from analysis of flux data, which show that isoproterenol increased the rate of $Na^+$ flux in the absorptive direction ($J^{MS}_{Na^+}$) but induced no change in unidirection $Cl^-$ flows (Table II).

It has been proposed that the interaction between beta receptor and adenylate cyclase and the production of cAMP is abnormal in CF (28–31). Our data suggest that the basis of the different response noted in CF respiratory epithelia does not lie in an abnormality in this receptor-enzyme system. CF tissues respond to beta agonists, indicating that an agonist-receptor interaction occurred, but the nature of the response is different. Moreover, stimulation of adenylate cyclase by different receptors, e.g., $PGE_1$ receptors, or direct stimulation of adenylate cyclase itself by forskolin (Fig. 4), induces the same pattern of response in CF tissues, suggesting that the major ion transport defect is not attributable to an abnormality in this system. Finally, the increase in cAMP production induced by isoproterenol or forskolin was similar in CF and control tissues. These results suggest that adenylate cyclase function is comparable in epithelia from both groups and is coupled to functional beta receptors. Because the quantities of cAMP induced by isoproterenol or forskolin in normal tissue are not the same but the maximal bioelectric responses are comparable, differences between the responses between CF and normals probably cannot be accounted for by differences in the cell concentration of "second" messenger. Consequently, it appears that the abnormal response of CF tissues reflects a dysfunction of a late process "distal" (to the production of cAMP) in the cAMP stimulatory pathway of the epithelial cell.

Our findings vary somewhat with those of Sato (32) in the sweat gland acinus. As did we, Sato found that tissue cAMP accumulation was similar in CF and normal tissues after iso-

proterenol exposure. The functional difference described between CF and normal sweat gland acinii exposed to beta agonists was increased $Cl^-$ secretion in normal as compared with no response in CF tissues. In contrast, we noted responses in both normal and CF epithelia but a qualitative difference in the nature of the response.

A single defect, e.g., reduced chloride permeability, may account for the dysfunction in the CF sweat duct (4). However, the present study has shown that the ion transport dysfunction in CF airways is more complicated. In addition to lower $Cl^-$ permeability, CF airway tissue is characterized by an abnormally raised resting rate of $Na^+$ absorption and a qualitatively different response to beta stimulation. Because of the absence of satisfactory $Cl^-$ channel blockers, we at present cannot distinguish between the possibilities that all the defects in CF airway epithelia reflect (*a*) the consequence of chronic reduction of cell $Cl^-$ permeability or (*b*) an abnormality in a cAMP dependent regulatory system.

In conclusion, our data indicate that excised and short-circuited CF nasal epithelia exhibit a raised rate of $Na^+$ absorption that contributes to the raised PD characteristic of airway epithelia in this disease. Further, the rate of $Na^+$ absorption is increased by exposure to beta agonists, a response different from normal tissues or tissues from disease controls. A preliminary report (33) indicates that the rate of $Na^+$ absorption across the nasal epithelium under open circuited conditions is also increased. Thus, it can be speculated that an increased rate of $Na^+$ absorption, coupled with fluid movement, may contribute to the relative dehydration of airway secretions that characterizes the CF lung disease (6, 7).

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