The identical clinical picture and heterozygote carrier frequency found among these two populations, one defined by geography, the other by ethnicity, has led to the suggestion that the two groups bear identical molecular lesions, perhaps arising through marriage of an Ashkenazi Jew into the French Canadian group (*5*). Our results showing a generally intact α-chain gene in Ashkenazi patients but a deletion in the α-chain gene of the French Canadian patients of 5 to 8 kb at the 5′ end, demonstrate that in the cell lines studied this is not the case. However, generalizations about this finding cannot be made until larger numbers of patients and carriers from each group are analyzed at the DNA level to assess the homogeneity of the mutations in these two populations.

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An Apical-Membrane Chloride Channel in Human Tracheal Epithelium

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The mechanism of chloride transport by airway epithelia has been of substantial interest because airway and sweat gland–duct epithelia are chloride-impermeable in cystic fibrosis. The decreased chloride permeability prevents normal secretion by the airway epithelium, thereby interfering with mucociliary clearance and contributing to the morbidity and mortality of the disease. Because chloride secretion depends on and is regulated by chloride conductance in the apical cell membrane, the patch-clamp technique was used to directly examine single-channel currents in primary cultures of human tracheal epithelium. The cells contained an anion-selective channel that was not strongly voltage-gated or regulated by calcium in cell-free patches. The channel was also blocked by analogs of carboxylic acid that decrease apical chloride conductance in intact epithelia. When attached to the cell, the channel was activated by isoproterenol, although the channel was also observed to open spontaneously. However, in some cases, the channel was only observed after the patch was excised from the cell. These results suggest that this channel is responsible for the apical chloride conductance in airway epithelia.

CHLORIDE SECRETION BY AIRWAY epithelia and other epithelia is a two-step process. Chloride enters the cell across the basolateral membrane by means of an electrically neutral, Na^+-coupled cotransport step; the coupling to Na^+ energizes intracellular accumulation of Cl^- above electrochemical equilibrium. Chloride then leaves the cell passively, moving down a favorable electrochemical gradient by means of an electrically conductive apical exit step (*1*). Apical Cl^- conductance is a primary regulatory step that controls the rate of transepithelial Cl^- secretion and the response to neurohumoral mediators. Although most agents that stimulate secretion increase intracellular levels of cyclic AMP and cytosolic Ca^{2+} concentrations, the identity of the second messenger that directly regulates the apical Cl^- permeability is uncertain.

In cell-attached and excised patches of membrane, I observed unitary current steps with the patch-clamp technique (*2, 3*). Figure 1 shows the current-voltage (*I–V*) relation of a Cl^- channel in an excised, inside-out cell-free patch bathed with symmetrical 145 m*M* NaCl solutions. The *I–V* relation rectified strongly; conductance increased with outward currents. The slope conductance measured at 0 mV with symmetrical 145 m*M* Cl solutions was 25 ± 2 picosiemens (pS) (mean ± SEM, n = 37 patches). No consistent differences were observed for channels obtained from different tracheas. Figure 1 also shows that unilateral changes in the Cl^- concentration shifted the reversal potential in the direction expected for a Cl^- channel. The selectivity of the channel for Cl^- over cations and the sulfate and gluconate anions is a property similar to that of the apical Cl^- conductance (*1*).

Isoproterenol stimulates Cl^- secretion by increasing the apical Cl^- conductance in intact and cultured epithelia (*1, 4*). Figure 2 shows that isoproterenol also activated previously quiescent channels in a cell-attached patch. In six patches that appeared to contain only a single channel (*5*), the probability (P) of finding the channel open before addition of isoproterenol was zero. However, at a mean time of 59 ± 13 seconds after the addition of isoproterenol, the value of P was at a peak of 0.46 ± 0.16. In five other multichannel patches in which isoproterenol increased activity, the value of P in three patches was zero during the 56 ± 14 seconds of observation before addition of isoproterenol (Fig. 2). In the other two patches, there was a brief burst of activity of one channel before the addition of isoproterenol, followed by the opening of several channels after isoproterenol addition. Although Fig. 2 shows a dramatic activation of the Cl^- channel by isoproterenol, this was not always observed. From 40 patches in which the Cl^- channel was observed, the channel was activated by isoproterenol in 28% and opened spontaneously in the cell-attached mode of untreated cells in 30%. However, in 42% of the patches, the channel was observed to open only after excision of the patch from the cell.

Agents that stimulate secretion increase the intracellular Ca^{2+} concentration (*6, 7*) and produce small changes (5 to 15 mV) in apical membrane voltage (*1*). However, I found no consistent effect of internal Ca^{2+} on channel activity in excised, inside-out, cell-free patches (Fig. 3). In five excised patches that contained either one or two channels, the value of P was 0.73 ± 0.06 with 1 m*M* Ca^{2+} bathing the internal surface of the patch and 0.78 ± 0.07 when the

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internal concentration of Ca^{2+} was less than 1 n*M* (measured at +50 mV). This apparent lack of Ca^{2+} regulation contrasts with the regulation of the basolateral K^+ conductance by Ca^{2+} (*6*). Figure 3 also shows that changes in membrane voltage had little consistent effect on channel activity. With large changes in voltage, the value of *P* increased in some cases, decreased in others, but was usually unchanged. In nine excised patches that contained a single channel, *P* was 0.76 ± 0.6 at +50 mV and 0.69 ± 0.10 at −50 mV, which indicated that the channel

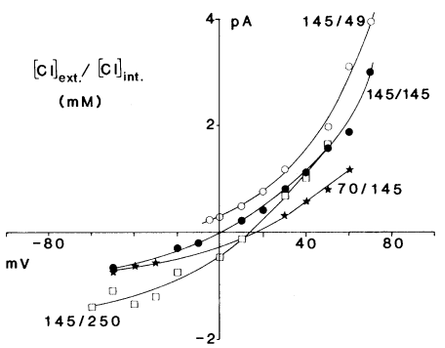

Fig. 1. Representative single-channel current-voltage (*I-V*) relations obtained in excised, inside-out patches. Closed circles represent the *I-V* relation obtained in symmetrical NaCl solutions (pipette solution, 145 m*M* NaCl and 1 m*M* Ca^{2+}; bath solution, 145 m*M* NaCl and 0.1 m*M* Ca^{2+}). Open circles show the *I-V* relation when the internal Cl^- concentration was decreased to 49 m*M* in exchange for SO_4^{2-} (expected reversal potential for a Cl^--selective channel of −27.6 mV). Squares show *I-V* relation when the internal NaCl concentration was increased to 250 m*M* (expected reversal potential, +13.8 mV). Stars show *I-V* relation when external (pipette) NaCl concentration was decreased to 70 m*M* (expected reversal potential, +18.5 mV; $n = 3$). (Lines were fit by eye.) The ratio of Cl^- to Na^+ permeabilities, $P(Cl^-/Na^+)$, from the Goldman-Hodgkin-Katz equation, in four experiments in which there was a NaCl gradient across the patch, yielded a value of 6.7 ± 1.0 (mean ± SEM). In other experiments, Na_2SO_4 or sodium gluconate was substituted for one-half to two-thirds of the NaCl in the internal solution; the apical membrane of tracheal epithelium is impermeable to SO_4^{2-} and gluconate. If one assumes that these two anions do not permeate the channel, then I calculate values for $P(Cl^-/Na^+)$ of 7.6 ± 1.2 ($n = 3$) during SO_4^{2-} substitutions and of 6.9 ($n = 2$) during gluconate substitutions. Moreover, when external Cl^- was replaced by gluconate, outward currents were never observed. The inability of gluconate to carry current and the agreement between values of $P(Cl^-/Na^+)$ calculated from NaCl gradients and partial replacement of Cl^- by SO_4^{2-} or gluconate indicate that this channel is impermeable to SO_4^{2-} and gluconate. In contrast to anion replacement, substitution of Na^+ by K^+ or Cs^+ in either external or internal solution had no effect on reversal potential. Although the values of reversal potential (and therefore $P(Cl^-/Na^+)$ are not highly accurate because of the small amplitudes and current rectification, the data indicate that this is an anion channel.

was not strongly voltage-gated. The observation that neither Ca^{2+} nor voltage is the main regulator of the Cl^- conductance is consistent with recent studies on cultured and intact epithelia (*7, 8*).

Another way to show that a specific channel is responsible for a physiologic property is to examine the effect of blockers. Although no highly specific blockers have been reported, high concentrations of anthracene-9-carboxylic acid (9-AC) and diphenylamine-2-carboxylate (DPC) inhibit the apical Cl^- conductance (*9*). Figure 4 shows that 9-AC applied to the internal bathing solution decreased single-channel conductance in a dose-dependent and reversible manner; DPC had a similar effect. In three patches, 4 m*M* 9-AC decreased single-channel current measured at +60 mV to 70 ± 6% of control values. In comparison, 6 m*M* 9-AC inhibited Cl^- secretion by 50% in intact tracheal epithelium (*9*). In four patches, 1 m*M* DPC decreased single-channel current measured at +60 mV to 59 ± 10% of control values. In comparison, 0.7 m*M* DPC decreased Cl^- secretion by 50% in intact tracheal epithelium. The data do not allow me to address the specific kinetics or mechanism by which these agents affect the channel. For example, 9-AC and DPC might produce a rapid, "flickering" block that is beyond the frequency response of our recording system. However, these agents have similar effects on Cl^- secretion in the intact epithelium and on the Cl^- channel: their effect on the channel is reversible, they have a similar rank-order of potency, and inhibitory concentrations are similar for the intact epithelium and the isolated channel. These findings suggest that the channel is responsible for the apical Cl^- conductance.

These results demonstrate a new type of Cl^- channel that is different from Cl^- channels described in other preparations. In other epithelia, an anion channel has been described in A-6 and Madin-Darby Canine Kidney renal epithelial cell lines, in cultured rabbit urinary bladder cells, and in cultured pulmonary alveolar type II cells (*10*). However, those large channels (approximately 400 pS) were voltage-gated, poorly selective for anions over cations, and inhibited by stilbene derivatives, properties not consistent with the Cl^- conductive properties of tracheal epithelium. The conductive properties and regulation of the tracheal Cl^- channel also differ from those reported for Cl^- channels in a variety of nonepithelial excitable cells (*11*). This Cl^- channel may be similar to Cl^- channels reported for two other chloride-secreting epithelia. In canine tracheal epithelial cells, swollen in high K^+ solutions, a 26-pS channel and a 150-pS channel were seen (*12*); the 26-pS channel may be similar to that reported here. In perfused tubules from shark rectal gland (*13*), the apical membrane contained a 20- to 50-pS channel that was blocked by internal DPC.

The Cl^- channel in tracheal epithelium is regulated by an intracellular second messenger because isoproterenol activated Cl^- channels in the cell-attached configuration in which the drug is not accessible to the external side of the channel. However, the

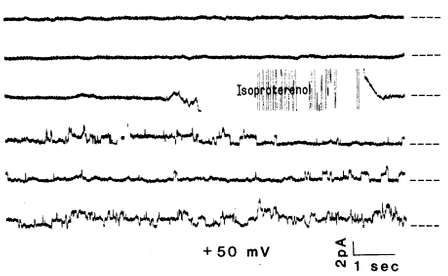

Fig. 2. Effect of isoproterenol on Cl^- channel activity in a cell-attached patch. Tracing is a continuous record. Isoproterenol was squirted at the time indicated (noise artifacts) into the chamber at a final concentration of 5 μ*M*. The holding potential was +50 mV (membrane depolarized). Pipette solution was the same as the bath, 145 m*M* Cl^-. Upward deflections represent outward current. Dashed lines indicate the current levels when all channels are closed. There are at least three channels in the patch.

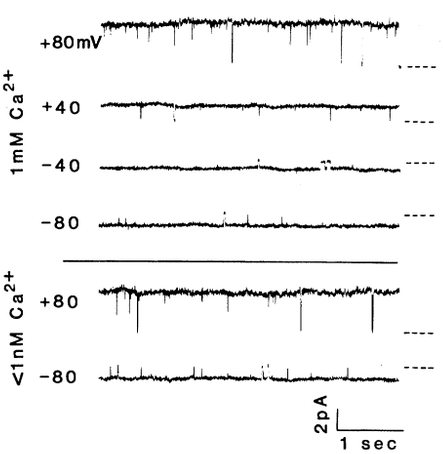

Fig. 3. Effect of internal Ca^{2+} concentration and membrane voltage on channel activity. Representative tracings from one channel at Ca^{2+} concentrations of 1 m*M* and less than 1 n*M* (nominal Ca^{2+} and 5 m*M* EGTA) are shown at different membrane voltages. Pipette and bath solutions contained 145 m*M* NaCl. Pipette solution contained 1 m*M* Ca^{2+}. Similar observations were made in most experiments; I routinely used solutions with Ca^{2+} concentrations of less than 1 n*M* (Fig. 4). Dashed lines indicate current levels when no channels are open. The results indicate that there was not a major Ca^{2+}-activation or voltage-gating of the channel, although the methods would prevent observation of subtle changes in kinetics.

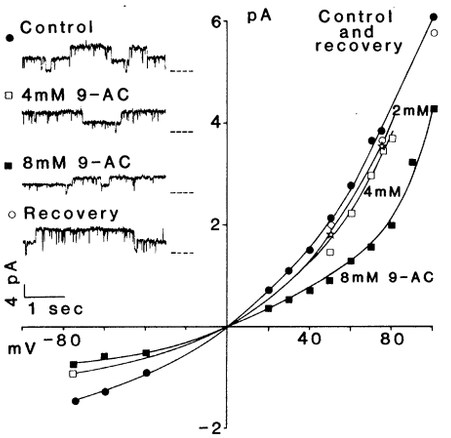

Fig. 4. Effect of anthracene-9-carboxylic acid (9-AC) on single-channel current-voltage relation. *I-V* relations were obtained when the internal surface was bathed with the 9-AC concentrations indicated. Pipette (external) solution was 145 m*M* NaCl and 1 m*M* $CaCl_2$. Bath (internal) solution contained 145 m*M* NaCl and 5 m*M* EGTA. There was no effect of the vehicle, dimethyl sulfoxide, which was added alone. The currents at +75 mV under the conditions indicated are shown at left; dashed lines indicate zero current levels. Stars indicate *I-V* relation with 2 m*M* 9-AC. This patch contained at least two channels. In studies on intact epithelia (*9*) these agents were added to the solution bathing the external surface of the cells, while in the present studies the agents were added to the internal surface of the membrane. However, given the lipid solubility of these agents, it is possible that they readily cross the lipid membrane.

identity of the regulator is unclear; neither cytosolic Ca^{2+} nor membrane voltage appears to be the primary or sole regulator under these conditions. The observation that in over one-third of the cases the channel did not open until after the patch was excised, even in cells that had been treated with isoproterenol, raises the possibility that the channel may be tonically inhibited by some intracellular substance. Removal of the channel from the cell may remove the inhibition.

These findings may prove to be of value in understanding the abnormalities of ion transport in cystic fibrosis. Both the sweat gland duct epithelium and airway epithelia are Cl^--impermeable (*14*), and, at least in airway epithelia, this results from a decreased or absent apical membrane Cl^- conductance (*15*). Whether the defect results from faulty regulation of the channel, or an abnormality of the channel itself, remains to be seen.

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