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Chloride Transport in Apical Membrane Vesicles from Bovine Tracheal Epithelium: Characterization Using a Fluorescent Indicator

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Summary. Cl transport in apical membrane vesicles derived from bovine tracheal epithelial cells was studied using the Cl-sensitive fluorescent indicator 6-methoxy-N-(3-sulfopropyl) quinolinium. With an inwardly directed 50 mM Cl gradient at 23°C, the initial rate of Cl entry (J_{Cl}) was increased significantly from 0.32 ± 0.12 nmol · sec^{-1} · mg protein^{-1} (mean ± SEM) to 0.50 ± 0.07 nmol · sec^{-1} · mg protein^{-1} when membrane potential was changed from 0 to +60 mV with K/valinomycin. At 37°C, with membrane potential clamped at 0 mV, there was a 34 ± 7% (n = 5) decrease in J_{Cl} from a control value of 0.37 ± 0.03 nmol · sec^{-1} · mg protein^{-1} upon addition of 0.2 mM diphenylamine-2-carboxylate. The following did not alter J_{Cl} significantly (J_{Cl} values given as percent change from control): 50 mM *cis* Na (−1 ± 5%), 0.1 mM furosemide (−3 ± 4%), 0.1 mM furosemide in the presence of 50 mM *cis* Na (−5 ± 2%), 0.1 mM H_2DIDS (−18 ± 9%), a 1.5 pH unit inwardly directed H gradient (−7 ± 7%), and 0.1 mM H_2DIDS in the presence of a 1.5 unit pH gradient (4 ± 18%). With inward 50 mM anion gradients, the initial rates of Br and I entry (J_{Br} and J_I, respectively) were not significantly different from J_{Cl}. J_{Cl} was a saturable function of Cl concentration with apparent K_d of 24 mM and apparent V_{max} of 0.54 nmol · sec^{-1} · mg protein^{-1}. Measurement of the temperature dependence of J_{Cl} yielded an activation energy of 5.0 kcal/mol (16–37°C). These results demonstrate that Cl transport in tracheal apical membrane vesicles is voltage-dependent and inhibited by diphenylamine-2-carboxylate. There is no significant contribution from the Na/K/2Cl, Na/Cl, or Cl/OH(H) transporters. The conductive pathway does not discriminate between Cl, Br, and I and is saturable. The low activation energy supports a pore-type mechanism for the conductance.

Key Words trachea · ion permeability · chloride channel · fluorescence

Introduction

Active secretion of Cl is part of the normal function of a variety of epithelia [10, 27, 32]. Of these, perhaps the best characterized is the tracheal epithelium, which secretes Cl by a mechanism dependent on two processes: 1) entry of Cl across the basolateral membrane via Na/K/2Cl cotransport and 2) exit of Cl across the apical membrane via a Cl conductance [27]. The initial evidence leading to the development of this schema was based on electrical measurements with Ussing chambers and intracellular microelectrodes [22, 24, 27]. More recently, single-channel recording techniques have provided basic information about the unitary conductance, selectivity, opening probability, and regulation of the apical membrane chloride channels [8, 9, 25, 26].

Apical and basolateral membrane vesicles have been useful systems for studying membrane transport, and have provided information complementary to that from studies in the intact epithelium [11, 18, 31]. A method has been described for preparing apical membrane vesicles from the bovine tracheal epithelium [15]. The major transport processes of the intact tracheal apical membrane are a Cl conductance and an amiloride-sensitive Na conductance. Flux studies have shown both to be preserved in apical membrane vesicles [15, 16]. Recently, the Cl-sensitive fluorescent dye 6-methoxy-N-(3-sulfopropyl)quinolinium (SPQ) has been used to study Cl transport in several vesicle systems [3, 4, 12, 13]. The temporal resolution of this fluorescence assay (on the order of milliseconds and seconds in a stop-flow apparatus and a conventional fluorimeter, respectively) enables continuous monitoring of the effects on early phases of Cl influx of ion and pH gradients, temperature and inhibitors. We report here the characterization of Cl transport in apical membrane vesicles isolated from bovine tracheal epithelial cells using the SPQ fluorescence quench technique.

Materials and Methods

Vesicles were prepared as described previously [15], except that all solutions used were buffered with Tris-sulfate rather than HEPES-Tris. Epithelial cells were scraped off the trachea without prior dissection from the cartilage, and scraped cells were

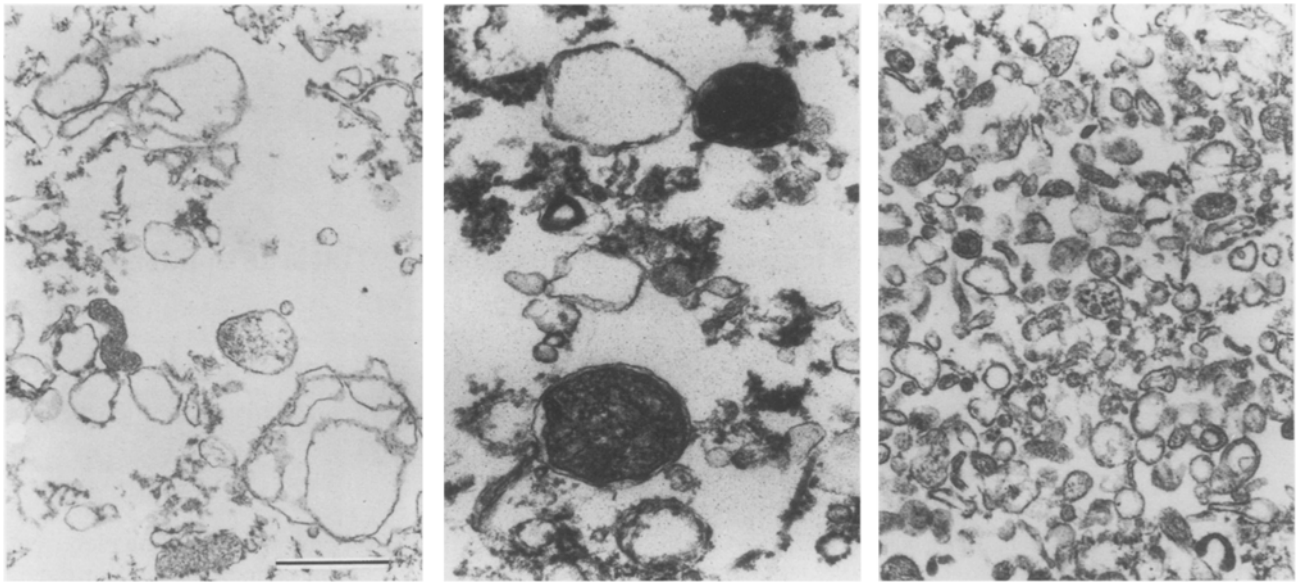

Fig. 1. Electron micrograph of membrane fractions. *Left:* Crude microsomes; *Middle:* Mg^{2+}-precipitated fraction; *Right:* Apical membrane vesicles. Note uniformity in size and morphology. Scale bar equals 0.5 μm

homogenized in a Sorvall Omni-Mixer (Dupont Instruments, Newtown, CT) at 0.8 × top speed for 2 min, at 4°C. The Mg^{2+} precipitation and differential centrifugation steps were identical to those described previously [15]. About 1% of total cell protein was recovered in the apical membrane pellet as determined by the technique of Smith et al. [23] using bovine serum albumin as a standard. Apical membrane vesicle purity was assessed from the enrichment of alkaline phosphatase [19] (20-fold over initial homogenate), and the depletion of DNA [2] and succinic dehydrogenase [1] (accumulation ratios of 0.2 and 0.1, respectively). There was a slight accumulation of Na,K-ATPase [20] (fivefold). These results are similar to those of Langridge-Smith et al. [15]. Electron micrographs (Fig. 1) of our preparation depict the homogeneity of our final apical membrane vesicles relative to the crude microsomes and the Mg^{2+}-precipitated fraction (basolateral and other contaminating membranes). The vesicles were suspended in 100 mM mannitol, 2 mM Tris-sulfate, pH 7.5, and stored at −80°C until use. Storage at −80°C had no effect on transport characteristics.

SPQ was synthesized as described previously [30]. Vesicles (~8 mg protein/ml) were loaded with 10 mM SPQ and appropriate buffers by incubation for > 24 hr at 4°C. Buffers contained 100 mM sucrose, 10 mM HEPES-Tris, X mM K-gluconate, and (150 − X) mM N-methyl-D-glucamine gluconate (NMG-gluconate), pH 7.0. X = 50 mM in experiments where membrane potential (ψ) was clamped at 0 mV; X = 5 mM when ψ was set to +60 mV. Vesicles were washed 3 times with SPQ-free buffer (60,000 × g, 4°C for 10 min), and used within 4 hr. Valinomycin (Sigma Chemical Co., St. Louis, MO) was added at a concentration of 25 μg/mg of membrane protein immediately after vesicle washing to clamp the vesicles at the K equilibrium potential. Dye leakage (<5%/hr) was minimized by maintaining vesicles at 4°C until the time of the experiment.

External buffers had the following composition: 100 mM sucrose, 10 mM HEPES-Tris, and a total of 150 mM salt, 50 mM of which was always K-anion. The remaining 100 mM salt was NMG-gluconate except in studies testing the effect of *cis* Na, when 50 mM Na-gluconate and 50 mM NMG-gluconate were used. For all experiments pH was 7.0, except those in which pH gradients were employed. Gluconic acid (1 mM) was used to titrate to pH 5.5. Internal and external buffers were balanced for both osmolarity and ionic strength. All buffers were filtered twice with 0.22 μm Millipore (Bedford, MA) or Nucleopore (Pleasanton, CA) filter discs to remove dust particles which scatter light.

Chloride transport blockers and their sources were: H_2DIDS (dihydro-4,4′-diisothiocyano-2,2′-disulfonic stilbene), Molecular Probes (Eugene, OR); furosemide, Hoechst-Roussel Pharmaceuticals, Inc. (Somerville, NJ); and DPAC (diphenylamine-2-carboxylate), ICN Biomedicals (Plainview, NY). Vesicles were preincubated with H_2DIDS or furosemide (both 0.1 mM) for at least an hour. DPAC was added directly from a 100 mM ethanolic stock to a final concentration of 0.2 mM. Addition of an equal volume (4 μl) of ethanol had no effect.

SPQ fluorescence was measured continuously and averaged at 1-sec intervals with an SLM 4800 fluorimeter (SLM, Urbana, IL) interfaced to an IBM PC/XT computer. Acrylic cuvettes (Sarstedt, FRG) were used in all experiments. Excitation wavelength was 350 nm (8 nm bandpass); emission fluorescence was measured with a Schott KV 408 cut-on filter (Duryea, PA). Two ml of uptake buffer was added to a cuvette positioned in a thermostatically controlled holder and stirred continuously. To initiate the experiments, apical membrane vesicles (40 μg protein) were added to the cuvette. Fluorescence was monitored for 800 sec, at which time the fluorescence signal remained constant and was regarded as representative of complete equilibration of external Cl with the intravesicular space. In some experiments, complete equilibration was achieved by the addition of Triton X-100. There was no decrease in fluorescence upon Triton addition after 800 sec.

Chloride influx, d[Cl]/dt (mM/sec), was calculated from the initial rate of change of SPQ fluorescence and a two-point calibration method using an SPQ fluorescence versus [Cl] curve as described previously [13]. Briefly, the fluorescence of SPQ can be related to the concentration of Cl by the Stern-Volmer equation:

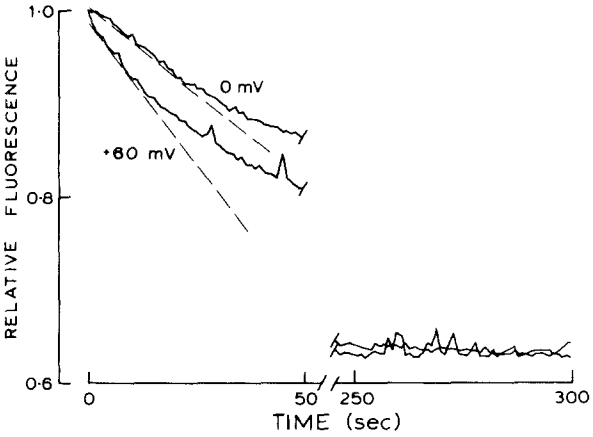

Fig. 2. Effect of changing membrane potential on the time course of intravesicular SPQ fluorescence in response to a 50 mM Cl gradient. Upper curve is time course under control conditions (0 mV membrane potential; intravesicular and external [K] = 50 mM). Lower curve is time course in the presence of a +60 mV membrane potential (intravesicular [K] = 5 mM; external [K] = 50 mM). In these runs, Triton X-100 was added at 150 sec to obtain final SPQ fluorescence. Initial rates of fluorescence decrease (dashed lines) were determined by a computerized exponential fit to the first 90 points. Records are paired runs from the same vesicle preparations

$$F = F_{ex} + F_o/(1 + K[Cl]) \quad (1)$$

where K is the Stern-Volmer quench coefficient, F_{ex} is the time-independent fluorescence of extravesicular SPQ, F is the fluorescence of intravesicular SPQ in the presence of Cl, and F_o is the fluorescence of intravesicular SPQ in the absence of Cl. This equation can be differentiated and rearranged to yield:

$$(d[Cl]/dt)_{t=0} = F_o/[K(F - F_{ex})^2] \cdot (dF/dt)_{t=0}. \quad (2)$$

Knowing the initial rate of change of SPQ fluorescence ($(dF/dt)_{t=0}$), F_o, F_{ex}, and F, $d[Cl]/dt$ (mM/sec) at $t = 0$ can be determined. F_o and F_{ex} are determined as described elsewhere [13]. J_{Cl} in nmol · sec^{-1} · mg protein^{-1} was calculated by multiplying $d[Cl]/dt$ (mM/sec) by the glucose space of the vesicles (2.4 μl/mg protein; *unpublished data*). J_{Br} and J_I were calculated analogously [13].

The Stern-Volmer quench coefficient used to calculate $d[Cl]/dt$ in the presence of gluconate has been determined previously [13]. For an internal gluconate of 150 mM, Stern-Volmer quench constants of 0.058, 0.097, and 0.138 mM^{-1}, respectively, were used for calculations of J_{Cl}, J_{Br}, and J_I [13]. Statistical significance was determined with Student's t-test. P of <0.05 was considered statistically significant. Data are expressed as mean ± SEM.

Results

Figure 2 shows the time course of fluorescence quenching on addition of SPQ-loaded vesicles to a medium containing 50 mM Cl. The initial rate of quenching was increased in the presence of a +60 mV membrane potential at 23°C. In seven experiments on two sets of vesicles, a 60 mV inside-positive membrane potential increased J_{Cl} significantly from a control value of 0.32 ± 0.12 nmol · sec^{-1} · mg protein^{-1} (when $\psi = 0$ mV) to 0.50 ± 0.07 nmol · sec^{-1} · mg protein^{-1} (Fig. 3).

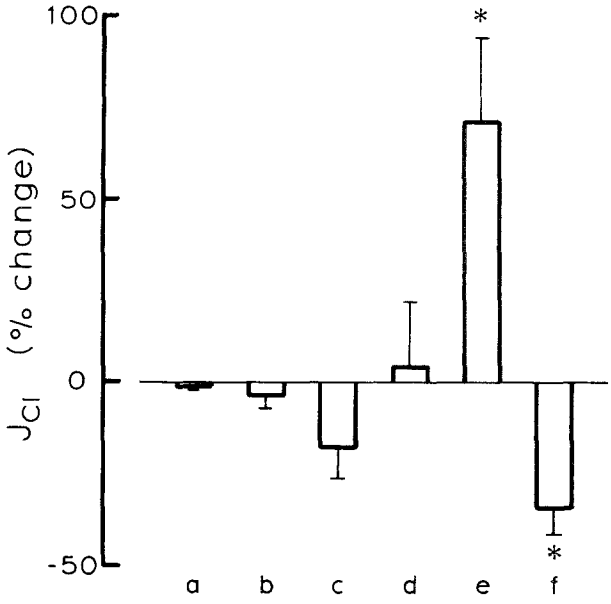

Fig. 3. Effects of *a)* 50 mM *cis*-Na, *b)* 0.1 mM Furosemide, *c)* 0.1 mM H_2DIDS, *d)* 1.5 pH unit inwardly directed proton gradient and 0.1 mM H_2DIDS, *e)* +60 mV membrane potential, and *f)* 200 μM DPAC on J_{Cl}. Experiments were performed at 0 mV internal potential difference and 37°C, except that experiments comparing 0 and +60 mV were performed at 23°C. Data are presented as the mean percentage change from control J_{Cl} (±SEM). Asterisks denote significance ($P < 0.05$)

Diphenylamide-2-carboxylate (DPAC) has been shown to inhibit Cl transport in a variety of epithelia [5]. In cultured canine tracheal epithelial cells, DPAC inhibits single-channel current and short-circuit current (I_{sc}) with a K_d of about 1 mM [26]. We have shown DPAC to inhibit I_{sc} in monolayers cultured from bovine tracheal epithelial cells with a similar K_d (*data not shown*). As shown in Fig. 3, DPAC (0.2 mM)[1] significantly inhibited J_{Cl} by 34 ± 7% ($n = 5$), compared to an inhibition of about 20% predicted from the K_d. These data support the presence of a DPAC-sensitive Cl conductance similar to that demonstrated in the apical membranes of the intact tracheal epithelial cells.

Other possible pathways for Cl influx were examined. To determine whether Na/K/2Cl cotransport was present, Na was added to the external buffer. There was no significant enhancement of J_{Cl}

[1] Higher concentrations of DPAC were not used because of its high extinction coefficient (~6900 M^{-1} cm^{-1}) at 350 nm. The concentration used, 0.2 mM, minimized this complicating effect.

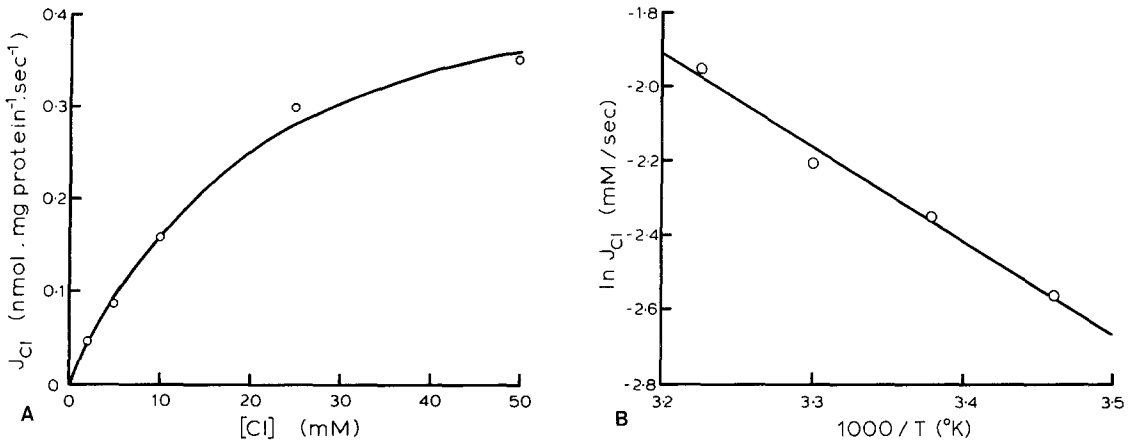

Fig. 4. (*A*) Dependence of J_{Cl} on Cl concentration. Curve is the best least-squares fit to an Eadie-Hofstee plot of data, with a K_d = 24 mM and V_{max} = 0.54 nmol · sec^{-1} · mg protein^{-1}. Points are means of triplicate estimates; standard errors were approximately the same size as the symbols used. (B) Temperature dependence of J_{Cl}. The calculated E_a was 5.0 ± 1.3 kcal/mole. Data points are means of triplicate estimates. Similar results were obtained in three other sets of experiments

by *cis*-Na (mean percent change = −0.9 ± 4.9%; n = 6; Fig. 3) over the control rate of 0.38 ± 0.03 nmol · sec^{-1} · mg protein^{-1}. Furthermore, the loop diuretic furosemide (0.1 mM) had no effect on J_{Cl} in both the presence and absence of external Na. These results indicate the absence of Na/K/2Cl and Na/Cl cotransport in apical membrane vesicles from bovine trachea. Cl/OH(H) transport has been shown to be an important mechanism of Cl transport in several systems. However, an inwardly directed proton gradient (pH_{ext} = 5.5, pH_{int} = 7.0) had no effect on J_{Cl} (test J_{Cl} = 0.31 ± 0.02 nmol · sec^{-1} · mg protein^{-1}; control J_{Cl} = 0.29 ± 0.02 nmol · sec^{-1} · mg protein^{-1}; n = 2). Preincubation with the stilbene anion exchange inhibitor, H_2DIDS (0.1 mM), did not alter J_{Cl} significantly (−18 ± 9% from control; n = 4). Finally, a pH gradient and H_2DIDS in combination did not change J_{Cl}. These results indicate absence of Cl/OH(H) transport in our membrane preparation.

To characterize further the basic properties of the Cl conductance, the effects of external Cl concentration and temperature on J_{Cl} were examined. The results (Fig. 4*A*) show saturation of J_{Cl} with increasing external Cl. The apparent K_d was 24 mM with a V_{max} of 0.54 nmol · sec^{-1} · mg protein^{-1}. Figure 4*B* shows the temperature dependence of J_{Cl} in the form of an Arrhenius plot. The plot yields an activation energy (E_a) for Cl influx of ~5 kcal/mol.

Initial rates of anion transport in response to 50 mM gradients of Cl, Br, and I were compared. J_{Br} was similar to J_{Cl} (0.37 ± 0.02 nmol · sec^{-1} · mg protein^{-1} *vs.* 0.48 ± 0.03 nmol · sec^{-1} · mg protein^{-1}, n = 7). There was no difference between J_I and J_{Cl} (0.41 ± 0.11 nmol · sec^{-1} · mg protein^{-1} and 0.47 ± 0.05 nmol · sec^{-1} · mg protein^{-1}, respectively; n = 6). In one set of vesicles, halide fluxes were determined in the presence of a +60 mV internal potential difference. Again, no difference in fluxes of the three halides was detected (*data not shown*).

Discussion

We report here the characterization of the transport properties of apical membranes prepared from bovine tracheal epithelial cell using a fluorescence assay. Other studies utilizing this fluorescence technique in renal brush border [3] and basolateral [4] membranes, as well as red blood cell ghosts [13] and placental brush border membranes [12] have established the accuracy of Cl flux measurements using SPQ. Compared to isotopic methods, the SPQ fluorescence assay affords greater sensitivity and temporal resolution, while requiring less membrane protein.

Compared to carriers or pumps, ion channels have very high turnover numbers and correspondingly low membrane densities. This creates two potential problems in studying their properties using membrane vesicles. Firstly, because of their small size, many vesicles may lack channels. Secondly, ionic transport in those vesicles which contain channels may be too rapid, and prove difficult to detect. Simple calculations suggest that these concerns have little bearing on the experiments described here.

Tracheal epithelium treated with indomethacin has an apical membrane conductance (G_a) of 0.44 mS · cm^{-2}, as revealed by equivalent circuit analysis [28]. Under these conditions there is no net Cl

secretion and I_{sc} is equal to net Na absorption. When maximal Cl secretion is induced by epinephrine, G_a increases to 3.64 mS · cm^{-2} though Na absorption remains unchanged. This change in conductance is not seen in Cl-free medium. Thus the additional 3.24 mS · cm^{-2} probably represents opening of Cl channels. Apical membrane Cl channels have unitary conductances of 20–50 pS [8, 25, 26]. In tissues stimulated with cAMP, the probability of opening (P_o) for Cl channels is about 0.15.[2] Our vesicles have a diameter of approximately 0.2 μm, corresponding to a surface area of 0.13 μm^2. These figures yield an average of one Cl channel per vesicle.

The half-time for influx can be estimated from:

$$t_{1/2} = (U_{max} \cdot \ln 2)/(n \cdot P_o \cdot J) \quad (3)$$

where J is the influx in ions · sec^{-1} for an open channel, U_{max} is the maximal Cl uptake (ions), n is the number of channels per vesicle, and P_o is the probability of opening.

The diffusional flux in the absence of a membrane potential in μEq · hr^{-1} is within 1% of the conductance in mS at 37°C [17, 21]. Thus, a 30 pS channel corresponds to an influx (J) of 5×10^6 ions per second. Fifty mM Cl in a vesicle of 0.2 μm diameter corresponds to 120,000 ions (= U_{max}). Substitution of these values, together with $n = 1$ and $P_o = 0.15$, into the above equation gives a $t_{1/2}$ of 111 msec for the cAMP-stimulated state.

Our experiments, as well as the Cl efflux studies of Dubinsky and Monti [6], yield a value for $t_{1/2}$ of ~10 sec. Therefore, the resting P_o is about one hundredth of the value of P_o in the stimulated state, or ~0.0015. This low value for P_o in the absence of cAMP-dependent activation is consistent with both equivalent circuit and patch-clamp data. In an equivalent circuit analysis of dog tracheal epithelium, Welsh et al. [28] found that elevation of cAMP levels with epinephrine produced an apical membrane electromotive force (E_a) that was essentially equal to the equilibrium potential for Cl across this membrane. With cAMP levels lowered by prolonged treatment with indomethacin, the apical membrane resistance increased tenfold. This membrane now behaved as if perfectly Na-selective, with E_a being approximately the same as E_{Na}, the equilibrium potential for Na [28][3]. In cell-attached patches, prior to addition of isoproterenol, all [9] or almost all [25, 26] Cl channels from either human or dog showed no openings.

The Cl fluxes measured in this study permit the calculation of the absolute Cl permeability (P_{Cl}). Using the glucose space of 2.4 μl · mg protein^{-1} and the surface area-to-volume ratio for a 0.2 μm diameter sphere of 3×10^5 cm^{-1}, our control J_{Cl} of ~0.35 nmol · sec^{-1} · mg protein^{-1} (37°C) is equivalent to a transmembrane flux of 0.49 pmol · cm^{-2} · sec^{-1}. With a membrane potential of 0 mV and a [Cl] of 50 mM, this corresponds to a P_{Cl} of 9.8×10^{-9} cm · sec^{-1}. The conditions of the experiments of Langridge-Smith et al. [16] do not permit the rigorous calculation of P_{Cl} because valinomycin (5 μg/ml) was present in the absence of intravesicular K. Thus, the electrochemical driving force is not defined. However, these investigators obtained an initial rate of Cl flux of 0.18 nmol · sec^{-1} · mg protein^{-1} with a 20 mM Cl gradient. Once this value is normalized to the 50 mM Cl gradient used in our experiments, this is in close agreement with our data. Dubinsky and Monti [6], using a Cl-sensitive electrode to measure Cl efflux from vesicles loaded with 150 mM Cl, identified a valinomycin-stimulated efflux of 4 nmol · mg protein^{-1} · sec^{-1} as being via a conductive pathway. However, in these experiments valinomycin (1 μg/ml) was present and the vesicles contained 160 mM K with no external K. The vesicles must therefore have possessed a considerable interior negative membrane potential, which would drive Cl efflux.

Our results exclude the presence of Na/K/2Cl (or Na/Cl) cotransport in tracheal apical membrane vesicles, as indicated by the lack of effect on J_{Cl} by *cis*-Na and/or 0.1 mM furosemide. These findings support the existing model for tracheal epithelial cell Cl secretion [27], which localizes Na/K/2Cl cotransport to the basolateral, rather than the apical membrane of the cell [29].

In our preparation, Cl/OH(H) transport does not contribute significantly to J_{Cl}; an inwardly di-

[2] In T_{84} cells stimulated to secrete by raising intracellular cAMP, P_o has been estimated as 0.15 [8]. Welsh [26] found Cl channels in 43 excised, inside-out patches of apical membrane from cultured dog tracheal epithelial cells. In the cell-attached mode, only 8 of these patches showed Cl channels following addition of isoproterenol. Thus, even in the stimulated state, the overall P_o for this population of channels is <0.2. Similar results have been obtained for human tracheal cells in culture; only a small fraction of Cl channels open in the cell-attached mode in response to isoproterenol, and once open, the P_o is ~0.5 [25]. From these figures, and similar results of Frizzell et al. [9], P_o after elevation of cAMP can be estimated to be ~0.15.

[3] An estimate for the resting G_{Na} of the apical membrane can be determined from the relation: $I_{Na} = G_{Na}(\psi_a - E_{Na})$, where I_{Na} is the I_{sc} in the resting state (which is due entirely to Na transport). Using values for I_{Na} of 30–60 μA · cm^{-2}, ψ_a of −50 mV, and E_{Na} of +50 mV, one obtains estimates for G_{Na} of 0.3–0.5 mS · cm^{-2}. This is similar to the estimate of G_a in resting tissues of ~0.4 mS · cm^{-2} obtained by Welsh et al. [28] again suggesting that most of the resting G_a is due to Na.

rected proton gradient, 0.1 mM H_2DIDS, and a combination of the two did not change J_{Cl} significantly. Our findings are consistent with observations in cultured bovine tracheal epithelia, where H_2DIDS had no effect on I_{sc} (*data not shown*). Neither SITS nor DIDS affects ^{36}Cl uptake into apical membrane vesicles [16].

The data obtained in this study support the presence of a voltage-driven Cl conductance; K-valinomycin voltage clamping of the membranes to a potential of +60 mV resulted in a significant increase in J_{Cl}. In cultured (*data not shown*) and intact [14] bovine tracheal epithelium, addition of DPAC or related compounds to the mucosal side of the tissue decreased Cl transport. DPAC also has been reported to attenuate the current amplitude of Cl channels in excised, inside-out patches from cultured canine epithelial cells [26]. In our studies, 0.2 mM DPAC blocked J_{Cl} by approximately 34%, providing further support for conductive Cl entry.

The activation energy for Cl transport, 5.0 kcal/mole, is comparable to that for free diffusion in water, supporting transport via a pore, rather than a carrier. ^{36}Cl fluxes into apical membrane vesicles are temperature-dependent [16]; however, difficulties in determining accurate initial rates of tracer uptake did not permit calculation of the activation energy.

Values for J_{halide} as calculated from SPQ fluorescence were similar for Cl, Br, and I. Recently, Frizzell [7] has reported that the two different apical membrane Cl channels of tracheal epithelium (50 and 20 pS) have differing selectivity: I (1.6), Br (1.3), Cl (1) and Cl (1), Br (0.4), I (0.4), respectively. Our results (Cl $\doteq$ I $\simeq$ Br) may reflect an average sequence from these two channel types.

In summary, Cl transport in vesicles derived from the apical membranes of bovine tracheal epithelial cells proceeds via a pathway that is conductive and DPAC-sensitive. No significant contribution by electroneutral Na/K/2Cl cotransport and Cl/OH(H) transport was observed. The dependence of initial flux rates on Cl concentration shows saturation. The calculated E_a supports the existence of a pore, rather than a carrier-type conductive mechanism. The influx pathway has similar selectivity for Cl, Br, and I. Finally, these results provide further support for the use of vesicles from bovine tracheal epithelium in studies of the apical membrane chloride channel and its regulation.

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