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Mechanism of Cl Secretion in Canine Trachea: Changes in Intracellular Chloride Activity with Secretion

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Summary. Cl-sensitive microelectrodes were employed to investigate the mechanism of Cl secretion by canine tracheal epithelium. In control tissues with a mean calculated short-circuit current (I_{sc}) of 18.1 μA/cm^2, the intracellular Cl activity (a^i_{Cl}) was 47.2 mM. This value is 30.1 mM (or 27.0 mV) above the electrochemical equilibrium for Cl across the apical membrane. Epinephrine, which stimulates Cl secretion, increased the calculated I_{sc} to 160 μA/cm^2 and decreased a^i_{Cl} to 32.2 mM, a value only 11.2 mM (or 10.9 mV) above equilibrium for the apical membrane. These results indicate a secretagogue induced decrease in the impedance to Cl exit from the cell via the apical membrane. From these and prior measurements we calculate that epinephrine-induced Cl efflux from the cell can occur by simple diffusion across the apical membrane. Further implications of these calculations are also discussed.

Key Words intracellular Cl activity · Cl transport · tracheal epithelium · Cl permeability · epinephrine · equivalent circuit analysis

Introduction

The epithelium lining the canine trachea actively secretes Cl into the tracheal lumen. This secretion requires the presence of Na in the submucosal bathing solution (Al-Bazzaz & Al-Awqati, 1979; Marin & Zaremba, 1979), and it can be stimulated by epinephrine (Al-Bazzaz & Cheng, 1979; Davis, Marin, Yee & Nadel, 1979). The similarity between the Na dependence and furosemide sensitivity (Davis, Marin, Ueki & Nadel, 1977; Marin & Zaremba, 1979) of Cl secretion in this and other epithelial tissues has led to the proposal of a model for Cl secretion shown in Fig. 1 (Frizzell, Field & Schultz, 1979; Welsh, Smith & Frizzell, 1982). Two predictions that arise from this model are: (1) Cl must accumulate in the cell to a concentration higher than would be expected by passive distribution and it must remain above equilibrium during maximal rates of secretion; and (2) epinephrine and other secretagogues stimulate secretion by increasing the Cl permeability of the apical membrane. Consequently, the electrochemical gradient driving Cl efflux across the apical membrane should decrease in the presence of epinephrine (assuming no other major effects of the hormone).

We previously demonstrated that the apical membrane's permeability to Cl is increased by epinephrine (Shorofsky, Field & Fozzard, 1983). We now show that a^i_{Cl} falls in the presence of epinephrine. These measurements allow us to estimate the extent to which Cl efflux could be increased via simple diffusion, when the apical membrane Cl permeability is increased by epinephrine.

Materials and Methods

Tissue Preparation, Perfusion System, and Pharmacologic Agents

Tracheal mucosa was isolated from adult mongrel dogs and mounted, mucosal surface up, in a perfusion chamber as previously described (Shorofsky et al., 1983). Both surfaces of the mucosa were continuously superfused in a nonrecirculating manner with HCO_3-free Ringer solution gassed with 100% O_2 at 37°C for at least 1 hr before microelectrode impalements were begun. The bathing solution contained (in mM): 138 NaCl, 5 KCl, 2 $CaCl_2$, 1.5 NaH_2PO_4, 1.1 $MgCl_2$, 5 N-2-Hydroxyethylpiperazine-N′-2-ethanesulfonic acid (HEPES) at 7.4. Indomethacin (to 10^{-6} M; Sigma, St. Louis, Mo.) was added to both bathing solutions at least 1 hr prior to microelectrode impalements in order to decrease the spontaneous rate of Cl secretion (Al-Bazzaz, Yadava & Westenfelder, 1981; Welsh et al., 1982). In some experiments epinephrine (to 2×10^{-5} M) was added to the submucosal bathing solution along with sodium meta-bisulfite (to 2×10^{-5} M) to prevent its oxidation.

Electrical and Chloride Activity Measurements

The electrical apparatus and techniques for determining the transepithelial potential (V_{sm}; mucosal reference), transepithelial re-

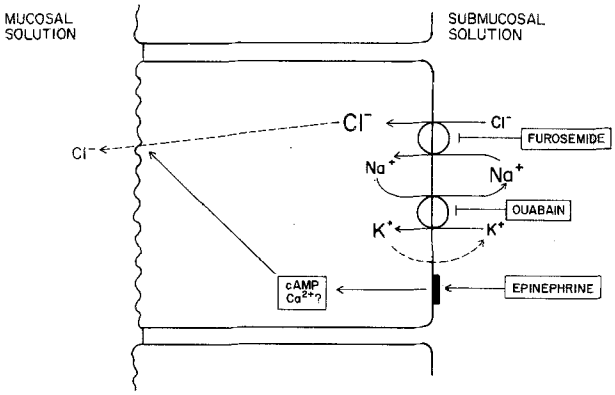

Fig. 1. A cellular model for Cl^- secretion. Cl^- enters the cell across the basolateral membrane, coupled to Na^+ entry. Furosemide is postulated to block this process. Due to the Na^+ gradient, Cl^- accumulates in the cell above electrochemical equilibrium; it is then transported across the apical membrane by simple diffusion. The Na^+ entering with Cl^- is recycled across the basolateral membrane by the Na^+/K^+ exchange pump. K^+ entering in exchange for Na^+ diffuses back across the basolateral membrane. Epinephrine stimulates Cl^- secretion by increasing the apical membrane's permeability to Cl^-

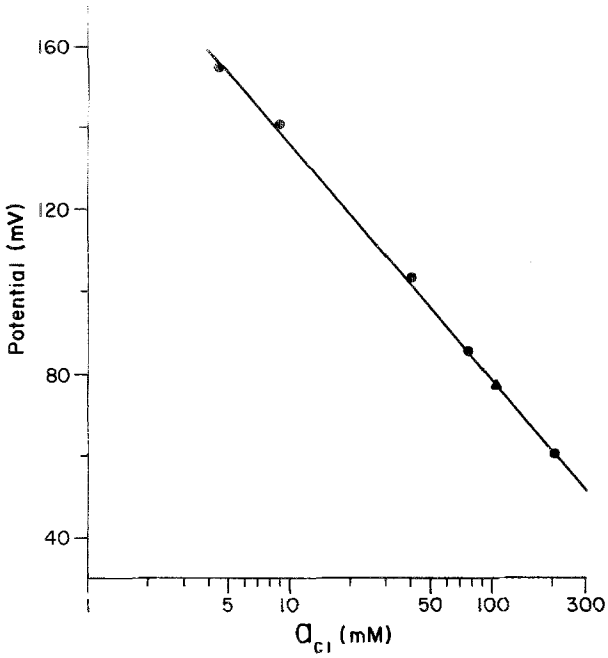

Fig. 2. A typical calibration curve of a Cl-sensitive microelectrode (dots). The slope was −57.7 mV/10-fold change in Cl activity. The triangle represents the chloride activity measured in the Ringer's bathing solution and corresponds to an a_{Cl} of 107.5 mM

sistance (R_t), and intracellular potential (V_{cm} or V_{cs} when referenced to the mucosal or submucosal solution, respectively) have been described previously (Shorofsky et al., 1983). The short-circuit current (I_{sc}) was calculated by dividing V_{sm} by R_t. The potential from the ion-selective microelectrode (V_{Cl}) was measured by a high input impedance electrometer (Model FD223, W.P. Instruments, New Haven, Conn.).

The ion-selective microelectrodes (ISM) were manufactured by the method of Walker (1971). Glass blanks, identical to those used for the conventional 3 M KCl electrodes, were pulled from 1.2 mm OD fiber-filled capillary tubes (W.P. Instruments) using a microelectrode puller (Model PD5, Narishige, Tokyo, Japan, or David Kopf Instruments, Tujunga, Cal.). The electrode tips were then dipped into a solution containing either γ-methacryloxypropyltrimethoxysilane or hexamethyldisilizane in 1-chloronaphthalene (3 and 10% vol/vol, respectively) for 30 sec, and then baked at 120°C for 1 hr. After the electrodes had cooled, the Cl-sensitive ion exchange resin 477913 (Corning, Medfield, Mass.) was introduced into the electrode tip by suction. A drop of resin was then placed as far down the shank of the electrode as possible. After trapped air bubbles were removed, the electrodes were back-filled with 0.3 M KCl.

The response of the Cl-sensitive microelectrodes to simple salt solutions can be described by the following equation:

$$V_{Cl} = E_o + S \ln (a_{Cl} + \Sigma k_{Cl-j} a_j) \quad (1)$$

where E_o and S are empirically determined constants, a_{Cl} and a_j are the activities of Cl and the interfering anions, respectively, and k_{Cl-j} is the selectivity coefficient of the electrode for Cl over interfering anion j (Thomas, 1978). The Cl-sensitive electrodes were calibrated at 37°C in KCl solutions of 5, 10, 50, 100, and 300 mM both before and after their use. Since these solutions contained no interfering anions, E_o and S could be determined from a graph of V_{Cl} *versus* log a_{Cl} (Fig. 2). The activity coefficients for the calibrating solutions were calculated according to the equations and tables of Pitzer and Mayorga (1973). The slopes of the electrodes (S) used in these experiments averaged 52.5 ± 1.6 mV per 10-fold change in a_{Cl}, which compares favorably to the 61.5 mV predicted for an "ideal" electrode at 37°C. Some of the electrodes used were selected for their "low" slopes so as to enlarge the signal seen on impaling a cell. The agar bridges used to ground the mucosal bathing solution were also used to ground the calibration chamber.

Impalements with the ISMs were performed in the manner previously described for the conventional, voltage-sensing microelectrodes (Shorofsky et al., 1983), requiring: (1) an abrupt negative deflection that reaches a plateau value; (2) maintenance of the plateau value (±10%) for at least 15 sec; (3) an abrupt change in potential upon withdrawal of the electrode from the cell to a potential no greater than 5 mV from the value obtained prior to impaling the cell. Since the ISMs generally had slow response times, the voltage divider ratio could not be used as a criterion for defining "successful" impalements.

If we assume there is no significant interference from anions other than Cl either inside or outside the cell and E_o is not altered upon impaling the cell, then intracellular chloride activity (a^i_{Cl}) is calculated from the following equation:

$$\Delta V_{Cl} = V_{cm} + S \ln [(a^i_{Cl})/(a^m_{Cl})]$$

where ΔV_{Cl} is the change in potential seen by the ISM upon entering the cell, V_{cm} is the potential difference between the cell and the mucosal bathing solution (mucosal reference) determined with conventional microelectrodes, S is the slope of the calibration curve and a^i_{Cl} and a^m_{Cl} are the activities of chloride in

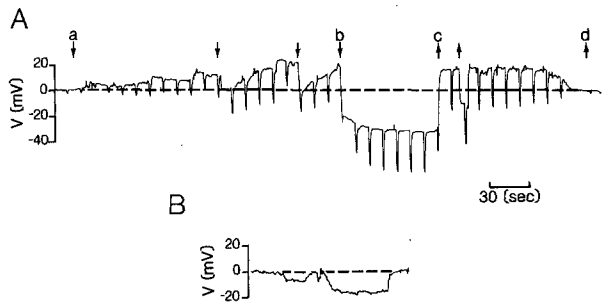

Fig. 3. Sample intracellular impalements with Cl-sensitive microelectrodes. (*A*): The downward arrows indicate advancement at the electrode into the tissue and the upward arrows indicate withdrawal of the electrode from the tissue. *See* text for further explanation

the cell and mucosal bathing solution, respectively. Because it was not possible to impale the tissue with both types of electrodes simultaneously, experiments were performed by averaging multiple punctures with each type of electrode to determine ΔV_{Cl} and V_{cm}. Since V_{cs}, the potential between the cell and the submucosal bathing solution (submucosal reference), is less affected than V_{cm} by variations in the spontaneous behavior of these tissues (Welsh et al., 1982; Shorofsky et al., 1983), V_{cs} was assumed to be constant with time and V_{cm} was calculated as the sum of V_{sm}, measured at the same time as ΔV_{Cl}, and V_{cs}, measured at another time.

All results reported are means ± SEM. Paired or unpaired *t* tests were performed where appropriate.

Results

Sample intracellular recordings with Cl-sensitive microelectrodes are shown in Fig. 3. In the control tissue (Fig. 3*A*), impalement occurred after the electrode had passed through another cell (point *b*). The impalement was lost spontaneously, approximately 1 min later (point *c*). After attempts to reimpale the cell were unsuccessful, the electrode was withdrawn cleanly into the mucosal bathing solution (point *d*) and the potential measured by the electrode returned to the value recorded prior to the impalement attempt (point *a*). This electrode had a reasonably fast response time as evidenced by its ability to respond to the passage of transepithelial current pulses.

An intracellular recording with Cl-sensitive microelectrodes in an epinephrine-stimulated tissue is shown in Fig. 3*B*. In contrast to the recording shown in *A*, in this recording the first cell encountered was impaled. The response time of the electrode used in this impalement was much slower than the response time of the electrode used in Fig. 3*A*, as evidenced by the almost complete absence of voltage deflections during the transepithelial current pulses and the slower potential deflections seen upon entering and exiting the cell.[1] This greatly lengthened response time is most likely due to the fact that these Cl-sensitive microelectrodes had extremely high input resistances (10^{10} to 10^{11} MΩ).

Table 1. Effect of epinephrine on intracellular chloride

	V_{sm} (mV)	V_{cm} (mV)	V_{cs} (mV)	I_{sc} (μA/cm²)	a^i_{Cl} (mM)	$V_m - E_{Cl}$ (mV)
Control	13.5	−49.1	−62.6	18.1	47.2	27.0
± SEM	±2.2	±2.7	±2.5	±1.6	±1.9	±1.8
Epinephrine	31.4[a]	−43.5	−74.9[b]	160.[a]	32.2[a]	10.9[a]
± SEM	±3.9	±2.1	±4.8	±16.	±2.7	±1.9

$V_m - E_{Cl}$ = the driving force for Cl exit from the cell via the apical membrane.
[a] $P < 0.01$; [b] $P < 0.05$; $n = 6$ tissues. I_{sc} calculated as described in text.

The magnitude of the potential deflections observed upon entering the cell, together with the knowledge of the slope of that particular electrode's response to changes in Cl concentration, reflects the electrochemical gradient driving Cl out of the cell via the apical membrane (i.e., the difference between the Cl equilibrium potential, as defined by the Nernst equation, and the membrane potential). The two electrodes used in Fig. 3*A* and *B* had the exact same slope in response to changes in Cl concentration, so it is obvious from a visual comparison of the two recordings that the force driving Cl exit from the cell diminished upon stimulation of secretion with epinephrine.

The results of the experiments using Cl-sensitive microelectrodes are given in Table 1. In the control tissues (calculated $I_{sc} = 18.1 \pm 1.6$ μA/cm²), a^i_{Cl} was 47.2 ± 1.9 mM. This value is 2.8 times what would be expected if Cl were passively distributed across the apical membrane. In the epinephrine-stimulated tissues (calculated $I_{sc} = 160 \pm 16$ μA/cm²), a^i_{Cl} was 32.2 ± 2.7 mM. This is significantly lower than a^i_{Cl} in control tissues ($P < 0.01$) although still greater (by 1.5 times) than the value predicted for passive distribution across the apical membrane. Cl activity measured in the mucosal bathing solution was 107.2 ± 1.2 mM, which compares well with the calculated value of 112 mM (assuming an activity coefficient of 0.75).

If Cl exits the apical surface of the cell by passive diffusion down its electrochemical gradient,

[1] It must be remembered that under epinephrine-stimulated conditions, a smaller change in potential should be recorded by the intracellular electrode when compared to that seen in control tissues. This is due to a shift in the dominance of the cellular resistance from R_a to R_b. The lack of response of the Cl electrodes, however, is less than would be expected and is reflective of their slow response times.

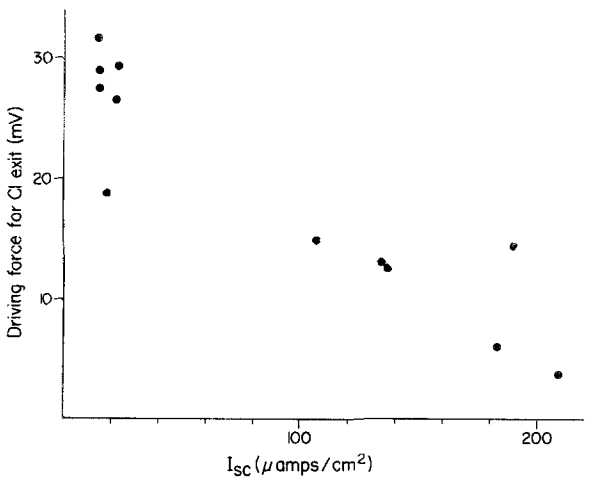

Fig. 4. A comparison of the electrochemical gradient for Cl to leave the cell across the apical membrane *vs.* the calculated short-circuit current

then the driving force for its efflux is the difference between the Cl electrochemical equilibrium potential and V_{cm} ($V_{cm} - E_{Cl}$). This difference in the control tissues was 27.0 ± 1.8 mV and, in the epinephrine-treated tissues, 10.9 ± 1.9 mV. Values from individual experiments can be seen in Fig. 4. Although the experimental procedure involved only minimal and maximal rates of secretion and the data tend to be grouped about the two extremes, an inverse relation between the calculated I_{sc} and the driving force for Cl across the apical membrane is apparent.[2]

Discussion

Distribution of Cl

The secretion model illustrated in Fig. 1 requires that Cl be accumulated within the cell to a level above its electrochemical equilibrium for the apical membrane so that it can exit from the cell passively. Our results support this hypothesis. Gradients similar to ours have been measured in short-circuited canine trachea (Welsh, 1982), rabbit trachea (Duffey & Cloutier, 1980), and in two other Cl-secreting epithelia, the bullfrog cornea (Zadunaisky, Spring & Shindo, 1979) and the dog fish rectal gland (Welsh, Smith & Frizzell, 1981). Our measured value for a^i_{Cl} is also similar to that obtained by chemical analysis of the intracellular fluid in isolated, canine tracheal epithelial cells (Widdicombe, Basraum & Highland, 1981).

As required by the model, a^i_{Cl} remained above electrochemical equilibrium for the apical membrane after the tissue was stimulated by epinephrine, although the driving force for Cl exit across this membrane decreased from 27.8 to 10.9 mV. The inverse relation between the driving force for Cl exit across the apical membrane and the calculated I_{sc}, which largely reflects the rate of active Cl secretion, indicates that the ease with which Cl leaves the cell via the apical membrane is increased by epinephrine. Thus the present results lead to the same general conclusion we arrived at earlier from observing the effects of changing mucosal medium ion concentrations on cellular potential differences (Shorofsky et al., 1983).

The above discussion is predicated on the assumption that the a^i_{Cl} measurements obtained in this study are accurate. Errors in these measurements could occur by damage to the cell during the impalement or response of the ISM to anions other than Cl. To minimize cell damage, both the conventional and ion-selective microelectrodes were constructed from the same batch of blank capillary tubes. Since the KCl-filled microelectrodes yielded stable, intracellular recordings like those previously reported (Welsh et al., 1982; Shorofsky et al., 1983), it is unlikely that the Cl-selective microelectrodes caused any impalement damage.

Another possible source of error is a response of the Cl-sensitive microelectrode to anions other than Cl. The Cl ion-exchanger resin used in these studies (Corning 477913) has been shown to have good selectivity for Cl when compared to various anions such as bicarbonate, methane sulfonate, acetate, or propionate (k_{Cl-j} was 0.004, 0.21, 0.22, and 0.4, respectively; Baumgarten, 1981). This does not exclude the possibility that the ISM responds to other unidentified organic intracellular anions. When Baumgarten and Fozzard (1981) measured a^i_{Cl} in mammalian cardiac cells bathed in Cl-free Tyrodes, they concluded that 4.8 mM Cl-equivalents was the maximal interference from intracellular anions. Spring and Kimura (1978) obtained similar results with Cl-free perfusion of *Necturus* proximal tubule cells (5–6 mM interference). The ion-exchange resin used in our study was reported by Baumgarten (1981) to be more selective for Cl over organic anions than the resin used by Baumgarten and Fozzard (1981) and by Spring and Kimura (1978). Even if we overestimated a^i_{Cl} by as

[2] It is possible to fit these points with a linear regression line with a significant correlation coefficient. This may be incorrect however, since the points tend to be grouped at the two extremes. Therefore we limit our conclusion to the inverse relationship but not its linearity.

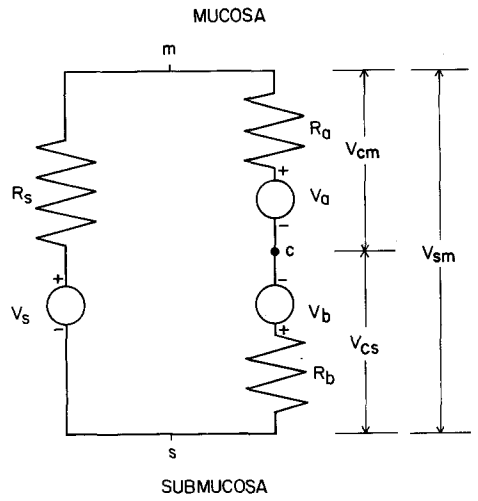

Fig. 5. The lumped, Thevenin equivalent circuit model for the tissue; V_a and R_a, V_b and R_b, and V_s and R_s represent the batteries and resistances of the apical membrane, basolateral membrane, and shunt pathway, respectively. V_{sm} is the transepithelial potential (mucosal reference). V_{cm} and V_{cs} are the cell potentials referenced to the mucosal and serosal bathing solutions, respectively

much as 5–6 mM, our main conclusions would not be altered.

Is the Permeability Change Sufficient for Passive Efflux?

We previously showed that epinephrine increases the Cl permeability of the apical membrane (Shorofsky et al., 1983). Our present results demonstrate that epinephrine also decreases the driving force for Cl to exit the cell via this membrane. In light of this decrease in Cl gradient, it is necessary to ask whether an increase in simple diffusive permeability to Cl can account for the shifts in cellular potentials caused by epinephrine in the open-circuited state (which correspond to the increase in Cl secretion seen under short-circuited conditions) or whether a more complex efflux process (e.g., carrier-mediated efflux, single file, diffusion, etc.) needs to be postulated. The measurements of a^i_{Cl} also enable us to calculate values for the equivalent circuit parameters before and after epinephrine, employing different assumptions (*see below*) than those invoked by Welsh et al. (1983) for their calculations. A quantitative analysis of the data presented in this paper, together with that published previously (Shorofsky et al., 1983), was performed as outlined in the Appendix, and values were derived for each parameter of the lumped-equivalent circuit model shown in Fig. 5. These values are given in Table 2. The increase in P_{Cl}, in combination with the lowered Cl gradient, is sufficient to account for the sixfold increase in Cl efflux measured experimentally by Al-Bazzaz et al. (1981).[3]

This analysis is based on three assumptions: first, that the intracellular ionic activities do not change during the time required to measure the shifts in potentials caused by altering the ionic composition of the mucosal bathing solution (*see* Shorofsky et al., 1983); second, that V_b and R_b (*see* Figure 5) do not change during the same time interval described above; and third, that ion movements across the apical membrane can be described by the Goldman-Hodgkin-Katz (GHK) constant field equation at least over the range of potentials between the measured trans-apical potential and the current reversal potential for the apical membrane (ranging from about −40 to 0 mV).

The first assumption of constant intracellular ion activities is based in part on obtaining the measurements in less than 20 sec in all instances. The magnitudes of the potentials generated by the changes in medium ion concentrations indicate that the most any ion concentration could have changed during our measurements was 12%, a fraction too small to alter significantly the results in Table 2.[4]

The assumption that the ion movements across the apical membrane can be described by the GHK equation has not yet been independently verified. There is evidence that the characteristics of Na channels in certain Na-transporting epithelia do fit the GHK equation at least over the ranges in potentials with which we are concerned (Fuchs, Larsen & Lindemann, 1977; Palmer, Edelman & Lindemann, 1980; Thompson, Suzuki & Schultz, 1982; Turnheim, Thompson & Schultz, 1983). To date,

[3] Chloride fluxes have been measured under epinephrine-stimulated conditions (Al-Bazzaz & Cheng, 1979). However, the controls for that study were not indomethacin-treated. Therefore it is more appropriate to compare the results of our experiments in which indomethacin was employed, to those of Al-Bazzaz et al. (1981), where PGE was used to stimulate Cl secretion in tissues pretreated with indomethacin.

[4] If the intracellular ion activities are altered when the ionic composition of the mucosal bathing solution is changed, then our measurements would underestimate the true changes in potential that occur and the analysis would underestimate the change in V_a (ΔV_a). This error would be greatest when the conductance of the apical membrane for an ion was maximal, i.e., the Cl conductance of the apical membrane under epinephrine-stimulated conditions. Under these conditions the analysis calculates a ΔV_a at 23.3 mV for a decrease of mucosal Cl concentration from 144 to 53 mM (maximal expected ΔV_a is 26.6 mV). If Cl were the only permeable ion, this ΔV_a would mean that the a^i_{Cl} decreased from 32.2 to 28.4 mM, a 12% decrease. Since there is also a Na and K permeability in the apical membrane under these conditions, the true decrease in a^i_{Cl} must be less than 4 mM.

Table 2. Thevenin equivalent circuit parameters

	R_a	R_b	R_s	V_a	V_b
	($\Omega \cdot cm^2$)			(mV)	(mV)
Control	2317	767	1140	11.3	76.7
Epinephrine-stimulated	254	510	738	15.7	96.7

For control tissues $P_{Na}/P_K = 0.7$, $P_{Cl}/P_K = 2.0$, $(a^i_{Na}) = 10$ mM; for epinephrine-stimulated tissues $P_{Na}/P_K = 0.7$, $P_{Cl}/P_K = 8.0$, and $(a^i_{Na}) = 20$ mM. (*See* text for further explanation.)

however, there is no similar evidence concerning Cl channels in epithelia.

It is interesting to note that the GHK assumption yields values for R_a before and after epinephrine (Table 2) that are virtually identical to those derived by Welsh et al. (1983) employing a different set of assumptions.[5] The analysis suggests that epinephrine, to account for the increase in Cl secretion, need only increase the relative Cl permeability (P_{Cl}/P_K) from 2.0 to 8.0 while increasing the overall conductance of the apical membrane about ninefold. A greater increase in P_{Cl}/P_K may have occurred but would have little additional effect on the equivalent circuit parameters.

The analysis also predicts that epinephrine decreases the resistance of the basolateral membrane by 257 $\Omega \cdot cm^2$, which is again similar to the change calculated by Welsh et al. (1983). This decrease has been attributed to an increase in the K permeability of the basolateral membrane (Shorofsky et al., 1983; Welsh et al., 1983). The 33% increase is interesting and may have important implications regarding the stoichiometry of the Na-Cl coupled influx process, including whether or not K also participates in the cotransport. The analysis further suggests that epinephrine might decrease the resistance of the shunt pathway about 35%, a result that differs from the analysis of Welsh et al. (1983), who assumed no change in shunt conductance. This decrease in R_s is consistent with recent reports that certain effectors of cellular transport processes also alter the characteristics of the "shunt" pathway (Duffey, Hainau, Ho & Bentzel, 1981; Krasney, DiBona & Frizzell, 1983).

Lastly, the analysis predicts that the intracellular Na activity (a^i_{Na}) is around 10 mM in the unstimulated tissue and that it rises to about 20 mM when Cl secretion is stimulated with epinephrine. The control value is similar to those measured in other epithelial tissues. (Lee & Armstrong, 1972; Palmer & Civan, 1977; Zeuthen, 1978; Armstrong, Garcia-Diaz, O'Doherty & O'Regan, 1979; Reuss & Weinman, 1979; Thompson et al., 1982). If Cl influx across the basolateral membrane is coupled to Na influx, then it is not surprising that stimulation of secretion would cause intracellular Na to rise. The increase in intracellular Na will be limited, of course, by the associated increase in the activity of the Na-K pump. Possibly, epinephrine may also more directly stimulate the pump, as appears to be the case in cardiac tissue (Wasserstrom, Schwartz & Fozzard, 1982). Our preliminary results with Na-selective microelectrodes (two experiments) indicate that intracellular Na increases from 11 mM before to 17.5 mM after addition of epinephrine.

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[5] Welsh et al. (1983) calculated equivalent circuit parameters before and after epinephrine on the basis of the following assumptions: (1) shunt conductance remains constant and (2) R_b is constant during the first 5–10 sec of stimulation with epinephrine and R_a is constant during the subsequent two minutes, during which time R_b changes gradually. They also assumed that slope and chord conductances are indistinguishable since the electrochemical gradients for Cl and K did not differ very much from the measured intracellular potentials. The present analysis which assumes adherence to the GHK equation, does not, however, invoke the above assumptions.

References

Al-Bazzaz, F.J., Al-Awqati, R. 1979. Interaction between Na^+ and Cl^- transport in canine tracheal mucosa. *J. Appl. Physiol.* **46:**111–119

Al-Bazzaz, F.J., Cheng, E. 1979. Effect of catecholamines on ion transport in dog tracheal epithelium. *J. Appl. Physiol.* **47:**397–403

Al-Bazzaz, F.J., Yadava, V.P., Westenfelder, C. 1981. Modification of Na and Cl transport in canine tracheal mucosa by Prostaglandins. *Am. J. Physiol.* **240:***F*101–*F*105

Armstrong, W. McD., Garcia-Diaz, J.F., O'Doherty, J., O'Regan, M.G. 1979. Transmucosal Na^+ electrochemical potential difference and solute accumulation in epithelial cells small intestine. *Fed. Proc.* **38:**2722–2728

Baumgarten, C.M. 1981. An improved liquid ion exchanger for chloride ion-selective microelectrodes. *Am. J. Physiol.* **241:***C*258–*C*263

Baumgarten, C.M., Fozzard, H.A. 1981. Intracellular chloride activity in mammalian ventricular muscle. *Am. J. Physiol.* **241:***C*121–*C*129

Davis, B., Marin, M.G., Ueki, I., Nadel, J.A. 1977. Effect of furosemide on chloride ion transport and electrical properties of canine tracheal epithelium. *Clin. Res.* **25:**132 (*abstr.*)

Davis, B., Marin, M.G., Yee, J.E., Nadel, J.A. 1979. Effect of terbutaline on movement of Cl^- and Na^+ across the trachea of the dog *in vitro*. *Am. Rev. Respir. Dis.* **120:**547–552

Duffey, M.D., Cloutier, M.M. 1980. Intracellular chloride activities and active Cl secretion by rat trachea. *Physiologist* **23:**62

Duffey, M.D., Hainau, B., Ho, S., Bentzel, C.J. 1981. Regulation of epithelial tight junction permeability by cyclic AMP. *Nature (London)* **294:**451–453

Frizzell, R.A., Field, M., Schultz, S.G. 1979. Sodium-coupled chloride transport by epithelial tissues. *Am. J. Physiol.* **236:***F*1–*F*8

Fuchs, W., Hviid Larsen, E., Lindemann, B. 1977. Current-voltage curve of sodium channels and concentration dependence of sodium permeability in frog skin. *J. Physiol. (London)* **267:**137–166

Helman, S.I., Thompson, D.M. 1982. Interpretation and use of electrical equivalent circuits in studies of epithelial tissues. *Am. J. Physiol.* **243:***F*519–*F*531

Hodgkin, A.L., Huxley, A.F. 1952. A quantitative description of membrane current and its application to conduction and excitation in nerve. *J. Physiol. (London)* **117:**500–544

Krasny, E.J., Jr., Madara, J., DiBona, A.I., Frizzell, R.A. 1983. Cyclic AMP regulates tight-junctional permselectivity in flounder intestine. *Fed. Proc.* **42:**1100

Lee, C.O., Armstrong, W. McD. 1971. Activities of sodium and potassium in epithelial cells of small intestines. *Science* **175:**1261–1264

Marin, M.G., Zaremba, M.M. 1979. Interdependence of Na^+ and Cl^- transport in dog tracheal epithelium. *J. Appl. Physiol.* **47(3):**598–603

Palmer, L.G., Civan, M.M. 1977. Distribution of Na^+, K^+, and Cl^- between nucleus and cytoplasm of *Chironomus* salivary gland cells. *J. Membrane Biol.* **33:**41–61

Palmer, L.G., Edelman, I.S., Lindemann, B. 1980. Current-voltage analysis of apical sodium transport in toad urinary bladder: Effects of inhibitors of transport and metabolism. *J. Membrane Biol.* **57:**59–71

Pitzer, K.S., Mayorga, G. 1973. Thermodynamics of electrolytes: II. Activity and osmotic coefficients for strong electrolytes with one or both ions univalent. *J. Phys. Chem.* **77(19):**2300–2308

Reuss, L., Weinman, S.A. 1979. Intracellular ionic activities and transmembrane electrochemical potential differences in gallbladder epithelium. *J. Membrane Biol.* **49:**345–362

Shorofsky, S.R., Field, M., Fozzard, H.A. 1983. Electrophysiology of Cl secretion in canine trachea. *J. Membrane Biol.* **72:**105–115

Smith, P.L., Frizzell, R.A. 1984. Chloride secretion by canine tracheal epithelium: IV. Basolateral membrane K permeability parallels secretion rate. *J. Membrane Biol.* **77:**187–200

Spring, K.R., Kimura, G. 1978. Chloride resorbtion by renal proximal tubules of *Necturus*. *J. Membrane Biol.* **38:**233–254

Thomas, R.C. 1978. Ion-Sensitive Intracellular Microelectrodes: How to Make and Use Them. Academic Press, New York

Thompson, S.M., Suzuki, Y., Schultz, S.G. 1982. The electrophysiology of rabbit descending colon: I. Instantaneous transepithelial current-voltage relations and the current-voltage relations of the Na-entry mechanism. *J. Membrane Biol.* **66:**41–54

Turnheim, K., Thompson, S.M., Schultz, S.G. 1983. Relation between intracellular sodium and active sodium transport in rabbit colon: Current-voltage relations of the apical sodium entry mechanism in the presence of varying luminal sodium concentrations. *J. Membrane Biol.* **76:**299–309

Walker, J.L., Jr. 1971. Ion specific liquid ion exchanger microelectrodes. *Anal. Chem.* **43:**89–92

Wasserstrom, J.A., Schwartz, D.J., Fozzard, H.A. 1982. Catecholamine effects on intracellular sodium activity and tension in dog heart. *Am. J. Physiol.* **243:***H*670–*H*675

Welsh, M.J. 1982. Intracellular chloride activity in canine tracheal epithelium. *Physiologist* **25(14):**334

Welsh, M.J., Smith, P.L., Frizzell, R.A. 1981. Intracellular Cl activities in the isolated, perfused sharks rectal gland. *Clin. Res.* **29:**480*A*

Welsh, M.J., Smith, P.L., Frizzell, R.A. 1982. Chloride secretion by canine tracheal epithelium: II. The cellular electrical potential profile. *J. Membrane Biol.* **70:**227–238

Welsh, M.J., Smith, P.L., Frizzell, R.A. 1983. Chloride secretion by canine tracheal epithelium: III. Membrane resistances and electromotive forces. *J. Membrane Biol.* **71:**209–218

Widdicombe, J.H., Basbaum, C.B., Highland, E. 1981. Ion contents and other properties of isolated cells from dog tracheal epithelium. *Am. J. Physiol.* **241:***C*184–*C*192

Zadunaisky, J.A., Spring, K.R., Shindo, T. 1979. Intracellular chloride activity in the corneal epithelium. *Fed. Proc.* **38:**1059 (*abstr.*)

Zeuthen, T. 1978. Intracellular gradients of ion activities in the epithelial cells of the *Necturus* gallbladder recorded with ion-selective microelectrodes. *J. Membrane Biol.* **39:**185–218

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Appendix

This analysis is an extension of the one we published previously (Shorofsky et al., 1983) and is used to analyze the shifts in cellular potentials that resulted from alterations in the ionic composition of the mucosal bathing solutions. To do this we have added to our prior data the measurements of intracellular Cl activities reported in this paper. The assumptions employed are those discussed in the text and only those derivations not described previously (Shorofsky et al., 1983) will be presented here.

It is worth noting that we cannot simply use our calculated I_{sc}'s to estimate the Cl flux across the apical membrane. The net Cl flux across the apical membrane is unlikely to be the same in the open-circuited as in the short-circuited tissue, since short circuiting abolishes the transepithelial potential and alters the electrical gradients across the cell membranes.

The current through a membrane may be represented in two ways. The first is that the membrane conforms to the Goldman-Hodgkin-Katz regime, so that the relation between the current carried by an ion (I_a^i) and the transapical potential (V_{cm}) is

$$I_a^i = -\left(\frac{P_i z_i^2 F^2 V_{cm}}{RT}\right)\left[\frac{a_i^m - a_i^c \exp(z_i F V_{cm}/RT)}{1 - \exp(z_i F V_{cm}/RT)}\right] \quad \text{(A1)}$$

where a_i^m and a_i^c are the activities of the ion (i) in the mucosal bathing solution and cell interior, respectively, P_i is the permeability coefficient of the apical membrane to ion i (assumed to be a constant during the solution change experiments), z_i is the valence of i, and R, T and F have their usual meanings. The slope of the I–V relation at a given potential is the "slope" conductance (G_a^i; *see* Helman & Thompson, 1982, for a discussion of "slope" and "chord" conductances) and is obtained by differentiating Eq. (A1), i.e.

$$\frac{\partial I_a^i}{\partial V_{cm}} = G_a^i = -\left(\frac{P_i z_i^2 F^2}{RT}\right) \left[\frac{(a_i^m - a_i^c e^K(1 + K)(1 - e^K) + Ke^K(a_c^m - a_i^c e^K)}{(1 - e^K)^2}\right] \quad \text{(A2)}$$

where $K = \frac{Z_i F V_{cm}}{RT}$.

If the apical membrane is assumed to be a linear resister about the operating potential, the inverse of the "slope" conductance given in the above equation is the membrane resistance.

The second method for representing the current through a membrane was proposed by Hodgkin and Huxley (1952). In their formalization, the I–V relation is given by

$$I_a^i = g_a^i(E_i - V_{cm}) \quad \text{(A3)}$$

where g_a^i is the "chord" conductance for the apical membrane for i, and E_i is the reversal potential for i, which can be obtained from the Nernst equation

$$E_i = \frac{RT}{z_i F} \ln \frac{a_i^m}{a_i^c}. \quad \text{(A4)}$$

If a_i^m and a_i^c are constant, the two methods of representing membrane current can be combined [equate Eqs. (A1) and (A3)] to yield

$$g_a^i = \left(\frac{P_i z_i^3 F^3 V_{cm}}{R^2 T^2}\right) \left[\frac{a_i^m - a_i^c e^K}{(1 - e^K) \ln\left(\frac{a_i^m}{a_i^c e^K}\right)}\right]. \quad \text{(A5)}$$

By rearranging Eqs. (A2) and (A5), equations can be derived for the ratios g_a^i/P_K and G_a^i/P_K for each of the three ions (Na, K, and Cl). Since these conductances are in parallel and thus add linearly

$$G_a/P_K = \Sigma G_a^i/P_K \text{ and } g_a/P_K = \Sigma g_a^i/P_K$$

where G_a and g_a are the total "slope" and "chord" conductances for the apical membrane, respectively. Using these equations along with Eqs. (A1) through (A5), relations for the ratio of "slope" to "chord" conductances (G_a/g_a), the ratios between each individual ionic conductance and the total membrane conductance (i.e., g_a^i/g_a and G_a^i/G_a) and finally the ratio between the current through the apical membrane and that membrane conductance (I_a/g_a or I_a/G_a) can be derived.

From the Thevenin equivalent circuit it then follows that:

$$V_a = -V_{cm} - \frac{I_a}{G_a} \quad \text{(A6)}$$

$$R_a/R_s = \frac{I_a}{G_a V_{cm}} \quad \text{(A7)}$$

$$R_a/R_b = \beta/(1 - \beta) \quad \text{(A8)}$$

$$V_b = V_{cs} + V_{cm}(R_b/R_s) \quad \text{(A9)}$$

where V_{cm}, V_{cs}, V_{sm} and β are all measured parameters. These equations, together with those presented in our previous paper (Shorofsky et al., 1983), are then solved simultaneously using the data from both sets of experiments to yield values for I_a/g_a, V_a, V_b, R_a, R_b, R_s, P_{Cl}/P_K, P_{Na}/P_K and the intracellular Na activity (a_{Na}^i).

The only unknown value needed in this analysis is the intracellular K activity. We used a value of 81 mM for both the control and epinephrine-stimulated tissues (Smith & Frizzell, 1984).