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Chloride Secretion by Canine Tracheal Epithelium: III. Membrane Resistances and Electromotive Forces

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Summary. We used intracellular microelectrode techniques and equivalent electrical circuit analysis to examine the changes in individual membrane resistances and electromotive forces that accompany stimulation of Cl secretion across canine tracheal epithelium. Tissues were pretreated with indomethacin (10^{-6} M, mucosal solution) to reduce basal Cl secretion rate. Subsequent addition of epinephrine (10^{-6} M, submucosal solution) increased the rate of electrogenic Cl secretion as indicated by an increase in the short-circuit current (I_{sc}) and decrease in the transepithelial resistance (R_t). The reduction in R_t was due to decreases in both R_a and R_b (the resistances of the apical and basolateral cell membranes, respectively).

At the apical membrane, a nearly 10-fold decrease in R_a was accompanied by reversal of the electromotive force (E_a) from $+11 \pm 9$ mV to -31 ± 3 mV. Variations in Cl secretion rate induced by indomethacin and epinephrine disclosed a direct relation between R_a and E_a. In the presence of indomethacin R_a was high and E_a was consistent with the chemical potential difference for Na across the apical membrane (ca. $+60$ mV), reflecting the predominance of Na absorption across indomethacin-treated tissues. In the presence of epinephrine, R_a was low and E_a was consistent with the chemical potential difference for Cl across this barrier (-31 mV), reflecting the dominance of Cl secretion across epinephrine-treated tissues. These findings suggest that the conversion from absorption to secretion primarily involves a secretogogue-induced decrease in apical membrane resistance to Cl.

At the basolateral membrane, epinephrine decreased R_b threefold without markedly altering the electromotive force across this barrier (E_b). To the extent that R_b and E_b represent the resistance and chemical potential difference for K diffusion across the basolateral membrane, the inverse relation between R_b and I_{sc} suggests that stimulation is associated with increased basolateral membrane K permeability without marked changes in intracellular K activity.

Key words tracheal epithelium · Cl secretion · electrophysiology · equivalent electrical circuit · epinephrine

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Introduction

Canine tracheal epithelium is a member of a group of epithelial tissues involved in active, electrogenic Cl secretion (Frizzell, Field & Schultz, 1979; Welsh, Smith & Frizzell, 1982). We previously noted that several features of Cl-secreting epithelia resemble those of their Na-absorbing counterparts (e.g. amphibian skin and urinary bladder, mammalian colon, etc.). These include: 1) electrogenic nature of net ion transport, which is responsible for the spontaneous transepithelial electrical potential difference and short-circuit current, 2) variation of tissue conductance with transport rate, which has its basis in an ion-selective apical membrane conductance pathway (channel), 3) short-term regulation of transport rate by extracellular factors (e.g. hormones, neurohumoral agents) via regulation of apical membrane conductance to Cl or Na, and 4) coupling of metabolic energy conversion to ion transport via Na/K-ATPase located at the basolateral membrane.

For Na-absorbing epithelia, considerable insight into the mechanism of ion transport across the individual cell membranes has been obtained through the use of intracellular microelectrode techniques and analysis of equivalent electrical circuit-models of the epithelium (for example: Reuss & Finn, 1975; Boulpaep, 1976; Lewis, Eaton & Diamond, 1976; Frömter & Gebler, 1977; Schultz, Frizzell & Nellans, 1977). Thus, at each barrier to ion movement the ionic permeabilities are represented as electrical resistances, the chemical driving forces (or zero current potentials) as electromotive forces, and the flow of ions as an electrical current (Finkelstein & Mauro, 1963; Schultz, 1979). The purpose of our study was to

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evaluate an equivalent electrical circuit model for ion transport by canine tracheal epithelium and the effects of agents that alter Cl secretion rate.

List of Symbols

R_t	– transepithelial resistance
I_{sc}	– short-circuit current
ψ_t, ψ_a, ψ_b	– electrical potential difference across the epithelium, the apical cell membrane, and the basolateral cell membrane, respectively
R_a, R_b, R_p	– electrical resistance of the apical cell membrane, the basolateral cell membrane, and the paracellular pathway, respectively
E_a, E_b, E_t	– electromotive force across the apical cell membrane, the basolateral cell membrane, and the transcellular pathway, respectively
f_R	– fractional resistance of the apical membrane, $R_a/(R_a+R_b)$, calculated from the ratio of change in ψ_a to the change in ψ_t produced by a transepithelial constant-current pulse
α	– membrane resistance ratio, R_a/R_b
G_t, G_p	– electrical conductance ($1/R$) of the tissue and paracellular pathway, respectively

Materials and Methods

Theoretical Analysis

Figure 1 depicts the equivalent electrical circuit model which is similar to that used by others for Na-absorbing epithelia (*vide ante*). We designate I_{sc} as short-circuit current, ψ as electrical potential difference, R as electrical resistance, and E as electromotive force. The subscripts *a, b, p* and *t* designate apical, basolateral, paracellular and transepithelial parameters, respectively. The orientation of the E's shown in Fig. 1 was used in solving the equivalent circuit.

In a previous study, we found that secretogogue-induced changes in the electrical properties of canine tracheal epithelium displayed two sequential phases. Under open-circuit conditions, during the initial 0 to 20 sec of epinephrine action ψ_a and ψ_b depolarized and both the fractional apical membrane resistance f_R ($R_a/(R_a+R_b)$) and tissue resistance R_t were reduced. This

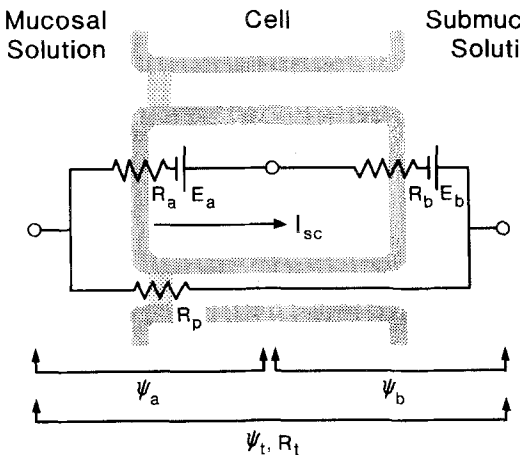


Fig. 1. An equivalent electrical circuit model of ion transport by canine tracheal epithelium. *See* text for details

suggested that the initial decrease in R_t resulted from a decrease in R_a, which was shown to be Cl-dependent. During the period 20 to 120 sec following the onset of epinephrine action ψ_a partially repolarized, ψ_b completely repolarized and f_R increased, despite the fact that R_t continued to fall. These findings suggested that a secondary decrease in R_t resulted from a decrease in R_b, causing f_R to rise (Welsh et al., 1982). After 120 sec a new steady-state obtains.

These time-dependent, secretogogue-induced, changes in R_a and R_b can be analyzed using Eqs. (1)–(4):

$$\frac{1}{R_t^o}=\frac{1}{R_a^o+R_b^o}+\frac{1}{R_P}, \tag{1}$$

$$\frac{1}{R_t^t}=\frac{1}{R_a^t+R_b^t}+\frac{1}{R_p}, \tag{2}$$

$$\alpha^o=\frac{R_a^o}{R_b^o}, \tag{3}$$

$$\alpha^t=\frac{R_a^t}{R_b^t}. \tag{4}$$

The superscripts *o* and *t* refer to measurements made at time zero, characterized by the steady state prior to the onset of stimulation, and at any time *t* after the onset of stimulation, respectively. The membrane resistance ratio α is determined from the changes in ψ_a and ψ_t that result from a transepithelial, constant-current pulse (Frömter, 1972).

These four simultaneous equations can be solved explicitly if two assumptions are made. First, we assume that R_p is constant throughout the secretory response. Evidence in support of this assumption is the finding that transepithelial fluxes of the nonelectrolyte, mannitol, which appear to be restricted to the paracellular pathway traversing this and other epithelia, are not altered by stimulation of secretion (Welsh & Widdicombe, 1980; Al-Bazzaz, Yadava & Westenfelder, 1981). Second, we assume that R_b is constant during the initial response to epinephrine, so that $R_b^o=R_b^t$ during the first 5 to 10 sec of stimulation; as we have pointed out, this assumption is made plausible by our previous observations of the time courses of f_R and R_t during epinephrine stimulation, and further evidence will be provided below.

By making frequent measurements of R_t and f_R during the first 5 to 10 sec of epinephrine action, Eqs. (1)–(4) can be solved for R_p. Once a value of R_p has been obtained, Eqs. (2) and (4) are used to solve for R_a and R_b.[1] The individual membrane resistances are then used to calculate the electromotive forces across the apical and basolateral membranes from Eqs. (5) and (6):

[1] In this analysis, the experimentally determined values of R_a and R_b represent "slope resistances," rather than "chord resistances." Thus, in Eqs. (5) through (8), we assume that the R_a and R_b are voltage-independent (ohmic) in the calculation of the electromotive forces. Any error introduced into the calculation of E_a and E_b by using "slope" rather than "chord" resistances will depend upon how far the point of measurement is from the true emf, because it is the "chord" resistance that directly relates the flow of an ion to its thermodynamic electrochemical driving force. Since, in tracheal epithelium the intracellular voltage ψ_a and ψ_b should be fairly close to the electrochemical gradient for Cl and K across the apical and basolateral membranes, respectively, the use of a "slope" resistance is unlikely to introduce a significant error into the calculation of emfs. This contrasts with the situation in Na-absorbing epithelia, where E_a may substantially differ from ψ_a.

$$\psi_a = E_a - (I_{sc}\ R_a), \quad (5)$$

$$\psi_b = E_b - (I_{sc}\ R_b). \quad (6)$$

This method of analysis of individual membrane resistances and electromotive forces is somewhat similar to that used for Na-absorbing epithelia by Reuss and Finn (1974), Lewis et al. (1976), and Frömter and Gebler (1977).

Since canine tracheal epithelium also displays amiloride-sensitive, electrogenic Na absorption under short-circuit conditions (Widdicombe & Welsh, 1980) we carried out the analysis described above during inhibition of Na absorption by amiloride. This procedure could not be performed in every experiment, since in many tissues the magnitude of active Na absorption and its associated electrical events do not provide a sufficient signal for accurate analysis. Calculation of the circuit parameters from the response to amiloride depends on the assumption that the initial effect of amiloride is blockade of apical membrane Na conductance. This appears reasonable in view of results obtained from similar analyses of a variety of Na-absorbing epithelia (Frömter & Gebler, 1977; Schultz et al., 1977; Cuthbert, Fanelli & Sciabine, 1979).

Technical Methods

Methods of tissue preparation, microelectrode fabrication, and measurement of transepithelial and transmembrane electrical properties have been previously described (Welsh et al., 1982). For this study, all measurements were made under short-circuit conditions. The epithelium was short-circuited using an automatic voltage clamp (University of Iowa, Bioengineering) that also provided constant-current pulses of sufficient magnitude to clamp ψ_t to ± 10 to 20 mV. Bipolar pulses of 25-msec duration were applied at 250-msec intervals with 25 msec between the positive and negative pulses. The command to pulse and the amplitude of the pulses were driven externally by a pulse generator via stimulus isolation units (W.P. Instruments Inc., New Haven, Conn.).

Indomethacin (10^{-6} M) was added to the mucosal solution of all tissues to minimize the baseline rate of Cl secretion (Al-Bazzaz et al., 1981; Smith et al., 1982). Secretion was stimulated by addition of epinephrine (10^{-6} M) to the submucosal solution. Amiloride was added to the mucosal solution at a final concentration of 10^{-4} M.

All values are presented as means ± SEM. Statistical significance was evaluated using a paired or unpaired *t*-test as indicated; $P<0.05$ was considered statistically significant.

Results

Response to Epinephrine

Figures 2–5 illustrate the time courses of I_{sc}, R_t, ψ_a, α and the equivalent circuit parameters in a single tissue during stimulation of Cl secretion with epinephrine. The records obtained using this tissue were chosen because of the large changes, particularly in R_a and E_a, induced by secretogogue. As will be discussed below, this appears to result from a relatively complete suppression of spontaneous Cl secretion by indomethacin so that the conversion from a relatively nonsecreting (Na-absorbing) state to a predominantly Cl-secreting state was most dramatic. It should be stressed, however, that all tissues yielded qualitatively-similar time courses of the equivalent circuit parameters in response to addition of secretogogue. All values were stable for several minutes prior to the onset of stimulation, so that time-zero represents the prestimulation steady-state.

Figure 2 shows the increase in I_{sc} and decrease in R_t produced by addition of epinephrine. A new steady-state level of transport was achieved within $1^1/_2$ to 3 min in all tissues, as previously observed (Welsh et al., 1982). Figure 3*A* shows the response of ψ_a (and ψ_b, since $\psi_t = 0$) to stimulation. This biphasic pattern, with an initial depolarization followed by partial repolarization, is similar to that observed under open-circuit conditions (Welsh et al., 1982). Figure 3*B* shows a biphasic response of the membrane resistance ratio to stimulation of secretion, with an initial rapid decrease and subsequent increase to a new steady-state value. The maximum decrease in R_a/R_b occurs at a time when R_t has decreased by less than 50% of its maximum change. As discussed above, (*see also* Welsh et al., 1982) the continued decrease in R_t accompanied by the secondary increase in R_a/R_b suggests that R_b decreases during the interval 20 to 120 sec.

Figure 4*A* and *B* show the time courses of R_a and R_b. R_p was calculated to be 595 Ω cm^2 for this tissue. The results indicate that there is a rapid, nearly 10-fold decrease in R_a which stabilizes 20 to 30 sec following the onset of stimulation. In contrast, R_b remains fairly stable for the first 15 to 20 sec following the onset of stimulation[2], and then decreases gradually to a steady-state value approximately 1/3 the initial value.

Figure 5*A* and *B* provide the time courses of the electromotive forces at the apical and basolateral membranes. During indomethacin treatment, E_a was +60 mV and fell to −25 mV following stimulation. This change in E_a is consistent with conversion from a predominance of Na absorption in the presence of indomethacin to Cl secretion with epinephrine. The value of +60 mV suggests that the electromotive force across the apical membrane of the indomethacin-treated tissue reflects the chemical potential difference for Na, whereas the shift of E_a to −25 mV with epinephrine stimulation provides the chemical potential difference for Cl in the secreting tissue. This will be discussed

[2] The stability of R_b during the first 20 sec of stimulation is consistent with, but not proof of, the assumption used to calculate R_p. The only other, but more unlikely explanation of these findings, is that R_b changed abruptly during the first 5- to 10-sec period, then remained constant for 20 sec, and then decreased.

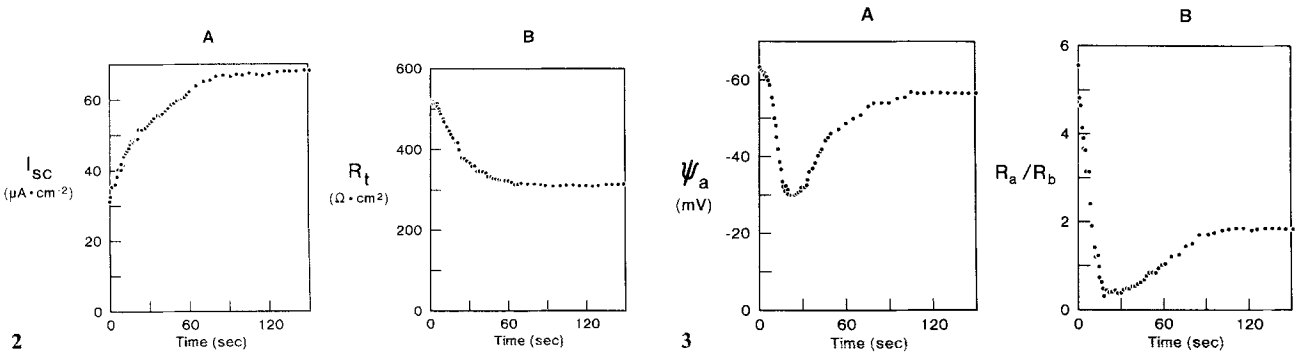


Fig. 2. Effect of epinephrine on the short-circuit current (I_{sc}) and transepithelial resistance (R_t). Values were obtained from one representative tissue. Time-zero indicates the onset of the response to epinephrine (10^{-6} M) added to the submucosal bathing solution

Fig. 3. Effect of epinephrine on the electrical potential difference across the apical cell membrane (ψ_a) and the membrane resistance ratio (R_a/R_b)

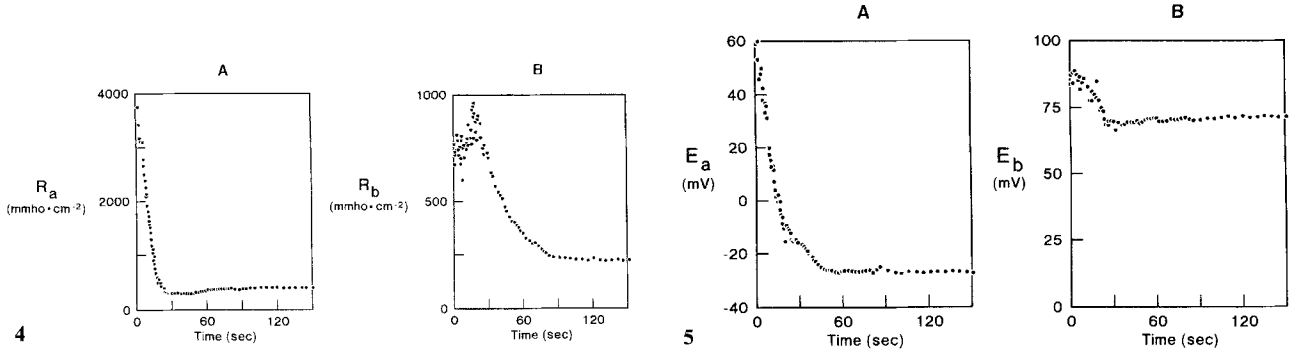


Fig. 4. Effect of epinephrine on the apical (R_a) and basolateral (R_b) membrane resistance

Fig. 5. Effect of epinephrine on the electromotive force at the apical (E_a) and basolateral (E_b) membrane

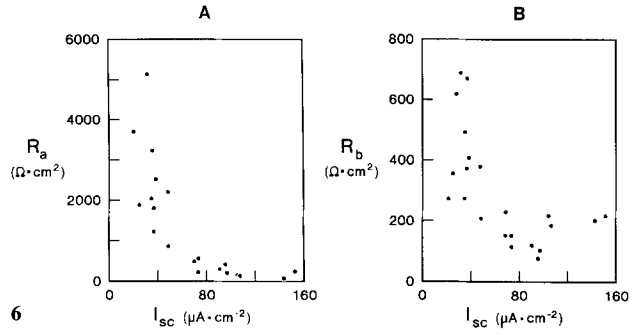
Table 1. Effect of epinephrine on electrical properties and equivalent circuit parameters[a]

	I_{sc} (μA cm^{-2})	R_t (Ω cm^2)	ψ_a (mV)	f_R	R_a (Ω cm^2)	R_b (Ω cm^2)	E_a (mV)	E_b (mV)
Control	35 ±3	546 ±69	−62 ±2	0.83 ±0.02	2268 ±290	430 ±50	+13 ±9	+77±2
Epinephrine	97*±9	249*±22	−53*±3	0.59*±0.06	274*± 50	157*±16	−30*±3	+69±3

[a] All values represent steady-state measurements. Indomethacin (10^{-6} M) was present in the mucosal solution during both conditions. $n=11$ tissues. $R_p=761+119$ Ω cm^2. *$P<0.05$.

below in more detail. In contrast to the marked alteration of E_a induced by secretogogue, E_b decreased slightly from +85 to +70 mV. The behavior of E_b following stimulation of secretion was somewhat variable; within the group of 11 tissues, E_b decreased somewhat in seven, increased somewhat in two, and in the remaining two tissues was essentially unchanged.

The compiled data for 11 tissues in which both transepithelial and intracellular electrical measurements were made during the transition from "control" (indomethacin-treated) to epinephrine-stimulated steady-state conditions are given in Table 1. The data represent the values calculated for the steady-state before and after stimulation, obtained from analysis of the intervening transient response, as above. The measurements in each tissue were made during a single cellular impalement that was stable for at least 2 min before and 2 min following the onset of stimulation. Values of the



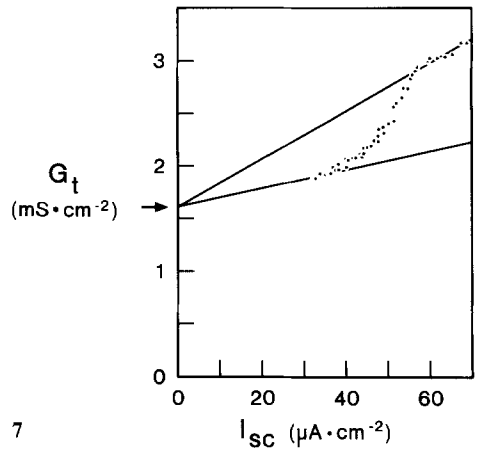


Fig. 6. Relation between membrane resistances and short-circuit current (I_{sc}). Values represent measurements made during the steady-state before and after stimulation of secretion. (*A*) Relation between apical membrane resistance (R_a) and I_{sc}. (*B*) Relation between basolateral membrane resistance (R_b) and I_{sc}

Fig. 7. Relation between transepithelial conductance (G_t) and short-circuit current (I_{sc}) following stimulation of secretion with epinephrine. Values taken from the data of Fig. 2

equivalent circuit parameters for these 11 tissues are qualitatively similar to the values for the single tissue given in Fig. 2–5. Addition of epinephrine consistently decreased both R_a and R_b. E_a also decreased consistently and significantly while the average value of E_b decreased to a small and variable extent, as discussed above.

Since calculation of the equivalent circuit parameters depends upon the assumption of a constant R_p, it is appropriate to consider the effect that an epinephrine-induced change in R_p would have on the steady-state values of membrane resistance and emf (i.e., Table 1). For example, if R_p were to increase or decrease by 25% with addition of epinephrine, the equivalent circuit parameters would be affected in the following way: a) the absolute value of R_a and R_b would be either overestimated by 11% (if R_p had increased by 25%) or underestimated by 19% (for a decrease in R_p) with no change in the membrane resistance ratio. Since, with stimulation of secretion, R_a underwent a nearly 10-fold decrease from the unstimulated value and R_b decreased three- to fourfold, an error in the estimate of the absolute value of 11–19% would be inconsequential; b) E_b would be 2% less or 4% greater with a 25% increase or decrease in R_p, respectively, well within experimental error; and c) E_a would be underestimated by 10% or overestimated by 23% with a 25% increase or 25% decrease in R_p, respectively. Thus, considering the magnitude of the changes we have observed with addition of secretagogue (Table 1), a change in R_p would minimally affect the equivalent circuit parameters, would not alter the direction of the changes, and would certainly not alter the interpretation of the data. Thus, these calculations serve to demonstrate the resiliency of the analysis.

To determine whether tissue-to-tissue variations in R_a and R_b were related to the ongoing rate of ion transport, we examined the relationship between I_{sc} and the individual membrane resistances. Values of R_a and R_b obtained during the steady-state before and after addition of epinephrine are plotted against the corresponding I_{sc} in Fig. 6*A* and *B*. Although there is some scatter in the data, an inverse relation of both R_a and R_b to I_{sc} is observed. There was no correlation between the calculated value of paracellular resistance and I_{sc}, R_a, or R_b (*not shown*).

A method frequently used for determination of cellular and paracellular resistances (or their inverses, the cellular [G_c] and paracellular [G_p] conductances) was introduced by Yonath and Civan (1971). Changes in I_{sc} and tissue conductance (G_t), induced by a maneuver which is thought to selectively alter cell membrane conductance, can be evaluated graphically, according to the relationship:

$$G_t = G_p + \frac{1}{E_t}(I_{sc}) \tag{7}$$

where E_t is the total, transepithelial electromotive force ($E_a + E_b$). A plot of G_t vs. I_{sc} yields a slope of ($1/E_t$) and intercept of G_p. In tracheal epithelium, the relation between I_{sc} and G_t obtained during the response to epinephrine was often sigmoidal. Figure 7 shows this relationship, taken from the

Table 2. Effect of amiloride on electrical properties and equivalent circuit parameters[a]

	I_{sc} ($\mu A\ cm^{-2}$)	R_t ($\Omega\ cm^2$)	Ψ_a (mV)	f_R	R_a ($\Omega\ cm^2$)	R_b ($\Omega\ cm^2$)	E_a (mV)	E_b (mV)
Control	63 ±9	371 ±53	−56 ±3	0.75 ±0.04	1568 ±293	478±88	+36±15	+82±5
Amiloride	46*±9	427*±75	−67*±2	0.86*±0.01	2797*±397	458±77	+53±20	+85±4

[a] Indomethacin (10^{-6} M) was present in mucosal solution during both conditions. $n=7$ tissues. $R_p=502\pm96\ \Omega\ cm^2$. *$P<0.05$.

data illustrated in Fig. 2. The curvilinearity reflects a combination of simultaneous changes in both cellular resistance and electromotive force and precludes an accurate estimate of G_p from the intercept, or of E_t from the slope of a straight line fitted to these data. In this Figure, the value of G_p ($1/R_p$) obtained through the use of Eqs. (1)–(4) is indicated by the arrow on the ordinate. The two straight lines are drawn to intercept this value of G_p with slopes of $1/E_t$, where E_t was calculated as the sum of E_a and E_b (taken from Fig. 5) during either the steady-state before addition of epinephrine (the lower line) or the steady-state following stimulation of secretion (the upper line). The data points obtained during the transition from one steady-state to the other are asymptotic to these lines as would be expected if the values obtained for G_p, E_a, and E_b from the analysis are correct. This relationship also provides independent support for the validity of our assumptions in obtaining a value of R_p from analysis of the transient information.

Response to Amiloride

Table 2 shows values of I_{sc}, R_t, ψ_a, f_R and the equivalent circuit parameters for seven tissues during the steady-state before and 10 to 15 sec following addition of amiloride (10^{-4} M) to the mucosal solution of indomethacin-treated tissues. Amiloride produced a rapid decrease in I_{sc}, increase in R_t, hyperpolarization of ψ_a, and increase in f_R; these effects of amiloride are similar to those observed in Na-absorbing epithelia (Frömter & Gebler, 1977; Schultz et al., 1977). Evaluation of the acute response to amiloride using equivalent circuit analysis, as above, yielded the circuit parameters, also given in Table 2. Amiloride increased R_a without altering R_b. Neither E_a nor E_b was significantly affected.

In four tissues we were able to obtain values for R_p from the transient responses to both amiloride and epinephrine. The values of R_p for individual tissues were 404 vs. 400, 610 vs. 594, 902 vs. 900 and 1200 vs. 850 $\Omega\ cm^2$, obtained from the responses to amiloride and epinephrine, respectively. The close agreement between these estimates of R_p provides independent support for the assumption that R_p is not affected by epinephrine, at least during the 2- to 3-min transition from one steady-state to the other.

Discussion

The Apical Cell Membrane: The Primary Phase of Stimulation

Stimulation of Cl secretion by addition of epinephrine to the submucosal bathing solution produced a rapid decrease in the apical membrane resistance. This decrease in R_a is due to an increase in apical membrane Cl permeability, as indicated by our earlier findings (Welsh et al., 1982). Replacement of Cl with sulfate or gluconate in the mucosal solution, or in both bathing media, prevents this depolarization of ψ_a and the decrease in relative apical membrane resistance elicited by secretogogue. The inverse relation between R_a and Cl secretion rate, as reflected by the I_{sc}, (Fig. 6*A*) is also consistent with secretogogue-induced increase in apical Cl permeability.

The finding that a large and rapid decrease in R_a is the first response to epinephrine (Fig. 4) suggests that the increase in apical membrane Cl permeability is the primary effect of cAMP-mediated secretogogue activity. This conclusion is consistent with the cellular model discussed previously for canine tracheal epithelium (Welsh et al., 1982) and other Cl-secreting tissues (Frizzell et al., 1979). Klyce & Wong (1977), using rabbit cornea, and Nagel & Reinach (1980), using frog cornea, have also concluded that epinephrine increases Cl secretion by decreasing R_a; Shorofsky, Field & Fozzard (1981) presented similar findings for canine treacheal epithelium.

Further insight into the transport properties of the apical membrane can be deduced from secretogogue-mediated changes in the electromotive force across this barrier E_a. As shown in Fig. 8, there is a wide range of values of E_a under "control" conditions (range +60 mV to −26 mV). However, in the steady-state following stimulation, the range

of E_a values was one-third that observed for the indomethacin-treated tissues. The variability of E_a during the "control" period, and the tendency toward more uniform values with stimulation, suggest that variation in E_a results from differences in the rates of Na absorption and Cl secretion among indomethacin-treated tissues. To more clearly examine the factors that determine E_a we depict, in Fig. 9, the apical membrane as an electrical circuit comprised of resistors and electromotive forces representing pathways for Na and Cl diffusion, as designated by the appropriate superscripts. As discussed by Finkelstein and Mauro (1963), while this circuit may not accurately represent the physical basis of transport at the apical membrane, it is useful for the purpose of illustrating factors that determine the value of E_a, which is given by Eq. (8):

$$E_a = \frac{R_a^{Cl} E_a^{Na} + R_a^{Na} E_a^{Cl}}{R_a^{Cl} + R_a^{Na}} \quad (8)$$

As a first approximation, we assume that secretogogues do not markedly alter cell Na and Cl concentrations or R_a^{Na} but only decrease R_a^{Cl}.[3] Examination of Eq. (8) reveals that as the resistance to Cl movement across the apical membrane becomes very large ($R_a^{Cl} >> R_a^{Na}$), as is the case during indomethacin treatment (Smith et al., 1982), the electromotive force associated with the chemical potential difference for Na, E_a^{Na}, becomes the dominant factor determining E_a. On the other hand, as R_a^{Cl} decreases and becomes much smaller than R_a^{Na}, as in the presence of epinephrine, E_a approaches the value of E_a^{Cl}, the chemical potential difference for Cl across the apical membrane.

This analysis explains the baseline variations in E_a values of indomethacin-treated tissues as well as the decrease in E_a with stimulation. Indomethacin suppresses spontaneous Cl secretion to a variable degree that is probably related to the dose utilized and the duration of the pretreatment. Three hours after indomethacin addition, net Cl flux across canine tracheal epithelium was abolished (Smith et al., 1982). However, the exposure to indomethacin was not this prolonged in the present studies so that complete inhibition of Cl secretion was probably obtained infrequently (e.g., Fig. 5). In most tissues, a reduced but significant rate of Cl secretion probably continued at the time when epinephrine was added so that the "control" value of E_a in most tissues is determined by *both* Na and Cl concentration differences across the apical membrane, having approximately equal permeabilities to both ions (i.e. $R_a^{Cl} \simeq R_a^{Na}$). It would be difficult to determine whether indomethacin had completely suppressed spontaneous Cl secretion in studies of this type since the baseline rate of Na absorption across this tissue is small and variable (Olver et al., 1975; Al-Bazzaz & Al-Awqati, 1979). Nevertheless, epinephrine elicits a large decrease in the resistance to Cl movement across the apical membrane (Fig. 4*A* and Table 1)

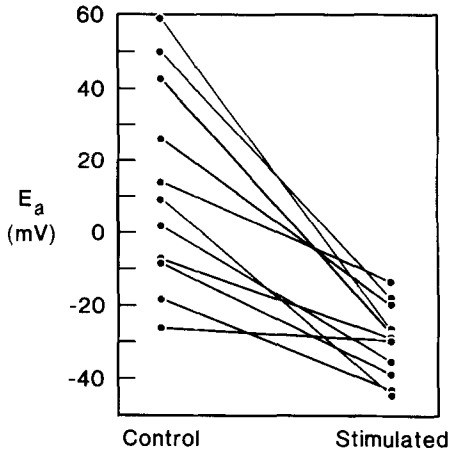


Fig. 8. Electromotive force at the apical membrane (E_a) under control and stimulated conditions. Control refers to the steady-state value obtained in the presence of indomethacin and stimulated to the steady-state value obtained after addition of epinephrine

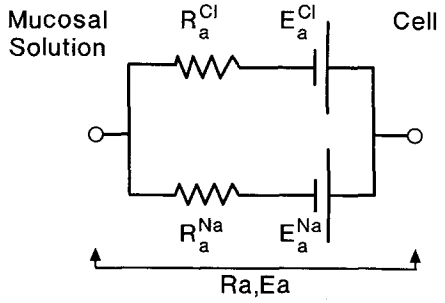


Fig. 9. Equivalent electrical circuit model of the apical membrane. *See text* for details

[3] It is reasonable to make these assumptions for the demonstration of this point. Measurements in another Cl-secreting epithelium, the isolated perfused shark rectal gland (Welsh et al., 1981), suggest that the intracellular Cl activity does not change significantly following the stimulation of secretion. There is no information about the relationship of intracellular Na activity to the transport rate in Cl-secreting epithelia. However, intracellular Na activity does not undergo large transport-related changes in Na-absorbing epithelia. Finally, there is no reason to suspect a change in R_a^{Na} during stimulation with epinephrine or inhibition of secretion with indomethacin, since these maneuvers produce only minimal changes in the net rate of Na absorption in tracheal epithelium (Al-Bazzaz & Cheng, 1979; Al-Bazzaz et al., 1981).

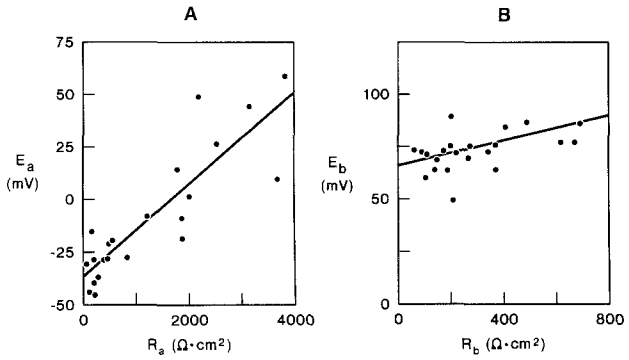


Fig. 10. Relation between membrane resistances and electromotive forces. Values represent measurements made during the steady-states before and after stimulation of secretion. (*A*) Relation between apical membrane electromotive force (E_a) and resistance (R_a). (*B*) Relation between basolateral membrane electromotive force (E_b) and resistance (R_b)

so that the chemical potential difference for Cl determines E_a during stimulation. If this reasoning is correct, we would expect a direct correlation between R_a and E_a. Figure 10*A* shows this relation for tissues during "control" conditions and during the steady-state following addition of epinephrine; E_a varies with R_a, as anticipated from the foregoing discussion.

The average value of E_a in the presence of epinephrine was -30 mV (Table 1) so that E_a^{Cl} is less negative than ψ_a and a net driving force of 23 mV favors Cl exit from cell to mucosal solution across the apical membrane. Using the Nernst equation, and assuming that $E_a = E_a^{Cl}$ during stimulation, we calculate a cell Cl activity of 30 mM, which exceeds the expected equilibrium cell Cl activity by a factor of 2.4. The conclusion that Cl is accumulated within secretory cells agrees with measurements of Cl activity made using Cl-selective, intracellular microelectrodes in two epithelia whose transport properties resemble Cl secretion by canine tracheal epithelium. In the isolated, perfused, shark rectal gland we found that cell Cl activity was 5 to 6 times the value expected for a passive distribution across the basolateral membrane (Welsh, Smith & Frizzell, 1981). In the frog corneal epithelium, Zadunaisky, Spring and Shindo (1979) measured a Cl activity of 30 mM; this value was approximately three times that predicted for a passive distribution across the apical cell membrane. Finally, Widdicombe, Basbaum and Highland (1981) reported a cell Cl concentration in isolated cells from canine trachea of 50 mM using ^{36}Cl distribution; this translates to a cell Cl activity of 37 mM, if intracellular and extracellular activity coefficients are equal.

The Basolateral Cell Membrane: The Secondary Phase of Stimulation

Approximately 20 sec after the onset of stimulation, when the decrease in R_a is virtually complete, the basolateral membrane resistance R_b begins to decrease. R_b fell more slowly than R_a, and reached a level that was approximately 1/3 its initial value. The finding that R_b was reduced by a factor of 2.7 while I_{sc} rises by a factor of 2.8 (Table 1) explains the repolarization of ψ_b that occurs during this secondary phase of stimulation. We previously found that ψ_b returned completely to its prestimulation value when similar experiments were conducted under open-circuit conditions (Welsh et al., 1982). These observations indicate that increased current flow across the basolateral membrane results almost entirely from a decrease in R_b with minimal changes in ψ_b and E_b (*see* Eq. (6) and Fig. 5*B*).

The correlation between transport rate and R_b is documented in Fig. 5*B*. An inverse relation between R_b and I_{sc} has been reported in other Cl-secreting, as well as Na-absorbing, epithelia. Nagel and Reinach (1980) observed decreases in both R_a and R_b during epinephrine stimulation of Cl secretion across frog cornea. In *Necturus* urinary bladder, Frömter and Gebler (1977) found that high rates of electrogenic Na absorption were associated with relatively low values of R_b and vice versa. Davis and Finn (1982) reported that inhibition of electrogenic Na absorption across toad urinary bladder by amiloride results in an increase in R_b. Finally, Gunter-Smith, Grasset and Schultz (1982) suggest that stimulation of rheogenic Na absorption in *Necturus* small intestine by mucosal addition of alanine or galactose is accompanied by an initial decrease in apical membrane resistance followed by a secondary decrease in basolateral membrane resistance.

The relation between R_b and E_b for all tissues is illustrated in Fig. 10*B*. Despite the fairly wide variations in R_b that are related to transport rate, E_b varies little, if at all. Evaluation of the factors that determine E_b is complicated since the basolateral membrane transport processes probably include passive ionic permeabilities in parallel with NaCl cotransport and the Na/K pump (Na/K-ATPase) (Widdicombe, Basbaum & Yee, 1979*a*).[4]

[4] The basolateral cell membrane appears to contain the Na/K pump, or Na/K ATPase, and NaCl cotransport processes (Welsh et al., 1982). The former is indicated by the inhibition of transport produced by the submucosal but not mucosal addition of ouabain (Widdicombe et al., 1979*b*) and by the autoradiographic localization of ouabain binding to the basolateral cell membrane (Widdicombe et al., 1979*a*). The latter by the requirement for submucosal solution Na, and inhibition by loop diuretics, of Cl secretion (Welsh, 1983).

Furthermore, the Na/K pump might function as a constant current source or constant voltage source so that its contribution to E_b, R_b or ψ_b is uncertain. Nevertheless, several possibilities can be ruled out from our present and prior findings.

The results of ion replacement studies (Welsh et al., 1982) indicate that the decrease in R_b that accompanies stimulation cannot be associated with a conductive pathway for Cl transport across the basolateral membrane. It also seems unlikely, as is the case for Na-absorbing epithelia, that a diffusional pathway for Na movement contributes to basolateral membrane conductance. This would compromise the efficiency of both the Na/K pump and NaCl cotransport processes. By exclusion, we tentatively conclude that basolateral membrane K conductance is the primary determinant of R_b, as it appears to be in Na-absorbing epithelia. In support of this conclusion, elevation of K concentration or addition of barium to the submucosal solution depolarizes the electrical potential difference across the basolateral membrane (Smith & Frizzell, 1982; Welsh, 1982). Thus, the close correspondence between R_b and I_{sc} raises the possibility that basolateral membrane K permeability varies directly with the rate of transepithelial ion transport.

The importance of a reduction in basolateral membrane resistance to K that parallels the increase in Cl secretion rate is twofold. First, an increase in NaCl entry across this barrier will lead to an increase in Na/K pump turnover. This, in turn, will increase K entry from the submucosal solution which, in the absence of net secretion, must be returned to the submucosal solution via K conductance pathways. Unless the driving force for diffusional K exit increases markedly, this can only be accommodated by an increase in basolateral membrane K permeability, which would account for the decrease in R_b that accompanies stimulation. The finding that E_b is not markedly affected by epinephrine supports this line of reasoning if E_b approximates the chemical potential difference for K across the basolateral membrane. Measurements of cell K activity using K-selective microelectrode techniques suggest close agreement between E_b and E_b^K, the equilibrium potential for K, both before and after stimulation of canine tracheal epithelium with epinephrine (Smith & Frizzell, 1982). Clearly, a mechanism whereby the K conductance of the basolateral membrane parallels Na/K pump turnover would serve to minimize changes in intracellular K activity that could compromise a variety of cellular functions. Schultz (1981) has recently summarized evidence favoring the existence of a similar regulatory mechanism in Na-absorbing epithelia.

The second point of significance is that an increase in basolateral K permeability may be required to maintain Cl secretion rate following a marked increase in apical membrane Cl permeability. This can be appreciated by examining the time courses of I_{sc} and ψ_a during stimulation (Figs. 2*A* and 3*A*). Following the addition of epinephrine, I_{sc} increases and ψ_a depolarizes, as ψ_a approaches E_a. Since the secretogogue-induced increase in apical membrane Cl permeability causes E_a to approach E_a^{Cl}, the equilibrium potential for Cl across this barrier, secretion would abate as ψ_a nears E_a^{Cl}. Indeed, if a secondary repolarization of ψ_b did not occur, little net secretion of Cl would result. However, repolarization of ψ_a, which results from the decrease in R_b, shifts ψ_a away from the E_a^{Cl}, thereby establishing a net driving force for Cl exit across the apical membrane. A similar decrease in R_b may assist in maintaining the driving force for Na entry across the apical membranes of Na-absorbing epithelia; however, the net driving force for Na entry is not markedly compromised by an increase in apical membrane Na permeability since the chemical potential difference for Na is always oriented from mucosal solution to cell. This is not true of Cl-secreting epithelia since Cl leaves the cell against its chemical potential difference. Therefore, the repolarization of ψ_a which results from a secondary decrease in R_b is critical in providing the electrical driving force for Cl exit across the apical membrane.

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