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Chloride Secretion by Canine Tracheal Epithelium: IV. Basolateral Membrane K Permeability Parallels Secretion Rate

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Summary. We evaluated the K conductance properties of the basolateral membranes of the surface cells of canine tracheal epithelium using microelectrode techniques. Studies were conducted under basal conditions (indomethacin, 10^{-6}M, mucosal solution) and after stimulation of electrogenic Cl secretion with epinephrine (10^{-6}M, serosal solution). Elevated serosal solution [K] depolarized the electrical potential differences across the apical (ψ_a) and basolateral (ψ_b) membranes in both the presence and absence of epinephrine. Serosal barium (0.5 mM) also depolarized ψ_a and ψ_b and selectively increased basolateral membrane resistance threefold. We also used K-selective microelectrodes to determine cell K activity (a_c^K) and the driving force for K transport across the limiting membranes under basal and stimulated conditions. Stimulation of Cl secretion was not associated with significant changes in ψ_b or a_c^K so that the driving force for K exit from cell to serosal solution (*ca.* 20 mV) was not altered. There was close agreement between the basolateral membrane electromotive force (E_b) determined from prior studies (M.J. Welsh, P.L. Smith and R.A. Frizzell, *J. Membrane Biol.* **71**:209–218, 1983) and the chemical potential difference for K across this barrier (E_b^K) in the presence and absence of epinephrine. These findings support the notion that the basolateral membrane is characterized by a high conductance to K under both secreting and nonsecreting conditions and indicate that the decrease in basolateral membrane resistance that accompanies stimulation of Cl secretion results from an increase in its K conductance. This obviates changes in a_c^K that would otherwise accompany increased Na/K pump activity and, by hyperpolarizing ψ_a, establishes the electrical driving force for Cl secretion across the apical membrane.

Key Words tracheal epithelium · chloride secretion · cell potassium activity · potassium conductance · epinephrine

Introduction

Canine tracheal epithelium absorbs Na and secretes Cl under short-circuit conditions. The rate of active Cl secretion can be enhanced by a variety of secretory stimuli, including epinephrine (Al-Bazzaz & Cheng, 1979). Results of microelectrode studies (Shorofsky, Field & Fozzard, 1983; Welsh, Smith & Frizzell, 1982, 1983) indicate that epinephrine elicits a primary decrease in the resistance of the apical membrane (R_a), and a secondary decrease in basolateral membrane resistance (R_b). The decrease in R_a requires the presence of Cl in the bathing media and therefore reflects a secretagogue-induced increase in apical membrane Cl conductance (Welsh et al., 1982); the nature of the conductance pathway(s) responsible for the decrease in R_b were not firmly established. Nevertheless, it was clear that this secondary decrease in R_b altered the cellular electrical potential profile in a manner that enhanced the driving force for diffusional Cl exit across the apical membrane during secretion (Welsh et al., 1983).

The present study explores the conductance properties of the basolateral membrane under both basal and stimulated conditions. We used ion-replacement studies, serosal Ba and K-selective microelectrode techniques to characterize the ionic basis of the decrease in R_b that accompanies Cl secretion. A preliminary account of these findings has been presented (Smith & Frizzell, 1982).

Materials and Methods

Techniques for obtaining the posterior membranous portion of canine tracheal epithelium, free of submucosal smooth muscle layers, have been described elsewhere (Smith, Welsh, Stoff & Frizzell, 1982). Tissues were bathed in a Ringer's solution of the following composition (mmol/liter): Na, 143; K, 5.4; Ca, 1.2; Mg, 1.2; Cl, 124; HCO_3, 25; HPO_4, 2.4; H_2PO_4, 0.6; glucose, 10; pH 7.4 at 37 °C gassed with 5% CO_2 in O_2. Studies were conducted under two conditions: a) after pretreatment with indomethacin (10^{-6} M, mucosal solution) to suppress spontaneous Cl secretion and b) after addition of epinephrine (10^{-6} M, serosal solution), to maximally stimulate active Cl secretion. Thus, the control or basal state is that obtained after about 1 hr of indomethacin treatment. These tissues remain fully responsive to exogenous secretogogues (Al-Bazzaz, Yadava & Westenfelder, 1981; Smith et al., 1982).

Transepithelial Flux Measurements

For transepithelial K and Cl flux determinations, four tissue segments from a single animal were mounted in Ussing-type

Table 1. Effects of epinephrine and barium on K and Cl fluxes across canine tracheal epithelium

Condition	J^K_{ms}	J^K_{sm}	J^K_{net}	J^{Cl}_{ms}	J^{Cl}_{sm}	J^{Cl}_{net}	I_{sc}	G_t
Indomethacin	0.15±0.02	0.14±0.02	0.01±0.01	3.5±0.5	3.2±0.4	0.3±0.2	0.97±0.16	4.3±0.6
Epinephrine	0.16±0.02	0.16±0.02	0.00±0.01	3.6±0.4	5.6±0.5*	−2.0±0.3*	3.4 ±0.3*	5.6±0.5*
Barium	0.14±0.01	0.38±0.04**	−0.24±0.05**	4.1±0.4**	5.1±0.4**	−1.0±0.3**	2.2 ±0.2**	4.9±0.4**

Values in μeq/cm² hr except G_t in mS/cm² represent mean±SEM of eight paried determinations. Indomethacin (10^{-6} M, mucosal solution); epinephrine (10^{-6}M, serosal solution); barium (0.5 mM, serosal solution). Sequential flux periods were 40 min in total duration with an initial 20-min equilibration period. K fluxes in the presence of barium were calculated from the last sample obtained during this period, when J^K_{sm} has not yet reached steady state (*see* text and Fig. 1). (*) Significant difference from indomethacin value, (**) from epinephrine value; $P<0.05$.

chambers (1.25 cm² aperture). Transepithelial electrical potential difference (ψ_t), transepithelial conductance (G_t) and short-circuit current (I_{sc}) were measured as previously described (Smith et al., 1982). Unidirectional fluxes from mucosa (*m*) -to-serosa (*s*) and *s*-to-*m* were measured on paired tissues (resistances differing by less than 25%) under short-circuit conditions. Indomethacin (10^{-6} M) was added to the mucosal solution approximately 1 hr after the tissues were mounted *in vitro*, and tracer quantities of ^{42}K and ^{36}Cl were added to one of the bathing media 1 hr thereafter. Samples were taken from the initially unlabeled side at 10-min intervals beginning 1 hr after isotope addition. During each flux period, values from three consecutive samples under steady-state conditions were averaged for calculation of unidirectional K or Cl fluxes. Additional details are given in the legend to Table 1. In all experiments, K and Cl fluxes were determined simultaneously, ^{42}K and ^{36}Cl were counted immediately thereafter and ^{36}Cl again after one week.

Intracellular Electrical Potentials

The preparation and use of conventional microelectrodes to record the electrical potential differences across the apical (ψ_a) and basolateral (ψ_b) membranes of tracheal surface epithelial cells have been described in detail (Welsh et al., 1982). In experiments where the effects of an agent or ion replacement on the electrical potential profile were evaluated, we recorded 4 to 5 values of ψ_a both prior to and following the experimental perturbation and always attempted to retain impalements during the transition in conditions, usually with success. Most experiments were performed with tissues at their spontaneous ψ_t (open-circuit conditions); periodically, ψ_t was clamped at zero to record I_{sc}. Changes in ψ_a and ψ_t resulting from passage of constant-current pulses across the epithelium were used to calculate transepithelial resistance (R_t) and the fractional apical membrane resistance (f_R) as follows:

$$\frac{\Delta\psi_a}{\Delta\psi_t}=f_R=\frac{R_a}{R_a+R_b}$$

where R_a and R_b are the resistances of the apical and basolateral membranes, respectively.

Fabrication of K-selective microelectrodes followed the approach described by Palmer and Civan (1975, 1977) using pipettes pulled exactly as for conventional microelectrodes (tip diameter 0.05 to 0.1 μm; Welsh et al., 1982). Briefly, the pipette barrel was exposed to the vapors of methyltrichlorosilane for 2 min and cured in an oven for 1 hr at 120 °C. After cooling, the pipette tip was filled with approximately 0.02 ml of Corning liquid ion-exchanger (No. 477317, Dow Corning Corp.) using a fine needle. After removing excess exchanger, the barrel was back-filled with 0.5 M KCl.

The electrical measuring circuit consisted of the microelectrode in contact with a Ag/AgCl junction which was connected to a high input impedance electrometer (Model F23-B, W.P. Instruments, New Haven, Conn.). This was referenced to a calomel electrode filled with saturated KCl. The total potential (E_t) measured by these electrodes is:

$$E_t=E_o+S\ln(a^K+K_{K,Na}\,a^{Na})$$

where E_o is the standard electrode potential, a is the activity of K or Na as indicated by the superscripts and $K_{K,Na}$ is the selectivity for Na over K (Palmer & Civan, 1977). Electrodes were calibrated before and after each experiment using standard KCl solutions of 100, 50 and 10 mM. Ion activities of the solutions were calculated as described by Fujimoto and Kubota (1976). For the electrodes used in our studies, plots of E_t versus ln a^K yielded straight lines. The slope for each electrode, obtained during calibration at room temperature (22 °C) was multiplied by (310/295) to obtain a value appropriate for the temperature at which the experiments were conducted. The average slope S for all electrodes averaged 26.4±0.1 mV (SEM) at 37 °C in good agreement with the ideal value (RT/zF)=26.7 mV. The selectivity coefficient $K_{K,Na}$ calculated from the difference in E_t in pure KCl solutions versus pure NaCl solutions or Ringer's solution having the same K activity was 0.024±0.003 (range, 0.014 to 0.039). Their resistances were 10^{10} to 10^{11} Ω.

The criteria for successful impalement with either conventional or K-selective microelectrodes were those described by Welsh et al. (1982). In the case of K-selective electrodes, the change in E_t (ΔE_t) resulting from impalement across the apical membranes of surface epithelial cells was employed to calculate the intracellular K activity (a^K_c), as follows:

$$\Delta E_t=S\ln[a^K_c/(a^K_m+K_{K,Na}\,a^{Na}_m)]+\psi_a \qquad (1)$$

where the subscripts *c* and *m* refer to the intracellular and mucosal compartments, respectively. This approach assumes that the contribution of cell Na to ΔE_t is negligible (Palmer & Civan 1977). At least 4 values of ψ_a were obtained both before and after at least 4 determinations of ΔE_t. These were averaged for calculation of a^K_c using Eq. (1).

Results are expressed as mean±SEM based on the number of tissues studied. Statistical significance was evaluated using paired *t*-tests; values of $P<0.05$ were considered significant.

Results

Transepithelial K and Cl Fluxes

Unidirectional and net fluxes of K and Cl across short-circuited canine tracheal epithelium are given

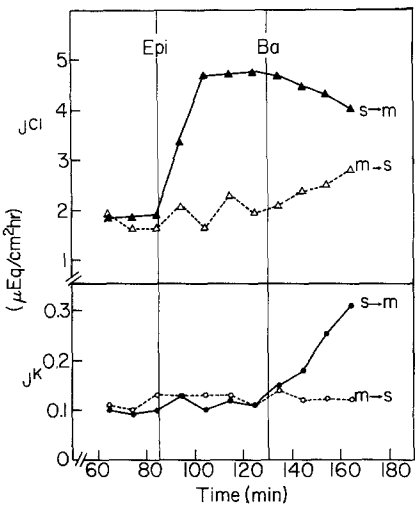

Fig. 1. Effect of epinephrine (10^{-6} M, serosal solution) and barium (0.5 mM, serosal solution) unidirectional Cl and K fluxes under short-circuit conditions. Data from a representative experiment are illustrated; average results are presented in Table 1. Tissues were pretreated with indomethacin (10^{-6} M mucosal solution) for 2 hr prior to flux determination. Abscissa indicates time after isotope addition

in Table 1. Figure 1 presents the time-course of K and Cl fluxes from a representative experiment. Under basal conditions (10^{-6} M indomethacin, mucosal solution) the bidirectional fluxes of either K or Cl did not differ from one another; that is, no significant net flux of either ion was observed. At the same time, I_{sc} averaged approximately 1 μeq/cm² hr. Results of prior flux studies in either the absence (Olver, Davis, Marin & Nadel, 1975; Al-Bazzaz & Al-Awqati, 1979) or presence of indomethacin (Al-Bazzaz et al., 1981; Smith et al., 1982) suggest that the undetermined net ion flux, reflected by the I_{sc}, can be attributed to net Na transport from mucosa to serosa.

Addition of epinephrine (10^{-6} M) to the serosal bathing solution increased I_{sc} and G_t and elicited electrogenic Cl secretion (Table 1, Fig. 1). The increase in I_{sc} with epinephrine did not differ from the corresponding rate of net Cl transport from serosa to mucosa, in agreement with the report of Al-Bazzaz and Cheng (1979). Epinephrine had no significant effect on the bidirectional K fluxes; net K flux remained at zero. The absence of net K transport across this tissue is consistent with the conclusion that the I_{sc} can be completely attributed to the sum of the opposing net fluxes of Na and Cl. This is true, regardless of variations in I_{sc} induced by suppression or stimulation of Cl secretion rate (Olver et al., 1975; Al-Bazzaz & Al-Awqati, 1979; Al-Bazzaz et al., 1981).

We also determined the effects of 0.5 mM Ba, added to the serosal solution alone. Barium reduced I_{sc} and G_t; this could be largely attributed to a 50% inhibition of electrogenic Cl secretion. At the same time, serosa-to-mucosa K flux more than doubled, resulting in K secretion. It is obvious from Fig. 1 that the values of J^{K}_{sm} and J^{K}_{net} in the presence of Ba, presented in Table 1, were not obtained during steady-state movement of K from serosa to mucosa, although the I_{sc} remained fairly stable throughout this period. The K fluxes presented in Table 1 are those obtained from samples taken at the completion of the flux period in the presence of Ba (Fig. 1). Therefore, while it is clear that serosal Ba elicits net K secretion, the ultimate magnitude of this effect cannot be discerned from the findings presented here.

Cellular Electrical Potential Profile: Effects of Elevated $[K]_s$

The results of recent studies by Shorofsky et al. (1983) revealed a small but significant depolarization of ψ_a (*ca.* 4 mV) when the [K] bathing the mucosal surface of tracheal epithelium was elevated from 5 to 50 mM. In the present study, the effects of serosal [K] were examined. The electrical potential profile, R_t and f_R were determined at the normal extracellular K concentration of 5.4 mM and after raising serosal [K] to 75 mM; K replaced Na. Several values of ψ_a with $[K]_m = [K]_s = 5.4$ mM were obtained before and after $[K]_s$ was elevated; these did not differ and were averaged. All ψ_a values were recorded at times when the values of ψ_t and R_t were time-independent to assure optimal changes in the ionic composition of the solution bathing the basolateral membrane.

The effects of Na replacement *per se* were evaluated using two indomethacin-treated tissues: ψ_b was determined before and after replacing 70 mM Na in both bathing media by tetraethylammonium (TMA). In the presence of Na- and TMA-Ringer's, ψ_b averaged 62 ± 2 and 60 ± 1 mV, respectively (10 impalements in each of two experiments under each condition). The absence of an effect of 50% Na replacement of ψ_b indicates that the effects of replacing serosal Na by K can be attributed to the elevated $[K]_s$.

Figure 2 illustrates an experimental record in which a cellular impalement was maintained during the increase in $[K]_s$ from 5.4 to 75 mM. Increasing $[K]_s$ depolarized ψ_t and ψ_a. The rapidity with which changes in the composition of the serosal solution can alter the electrical potential profile of the surface cells is compromised by the 200 to 400 μm thick collagen layer of the submucosa. Sev-

Table 2. Effects of elevated serosal K concentrations on electrical properties

$[K]_m/[K]_s$(mM)		ψ_t	ψ_a	ψ_b	R_t	f_R
A. Indomethacin (10^{-6} M)						
5.4/5.4	(5, 23)	14±3	−48±4	61±2	261±35	0.77±0.06
5.4/75	(5, 21)	2±2*	− 7±2*	9±2*	165±29*	0.69±0.08
B. Indomethacin (10^{-6} M) + Epinephrine (10^{-6}M)						
5.4/5.4	(4, 15)	27±4	−32±1	59±4	140±11	0.46±0.04
5.4/75	(4, 17)	−3±3*	−12±2*	9±2*	93±11*	0.57±0.05*

Numbers in parentheses are the number of animals and total number of punctures, respectively. ψ_t, ψ_a and ψ_b are in mV and R_t in Ω cm^2. (*) $P<0.05$.

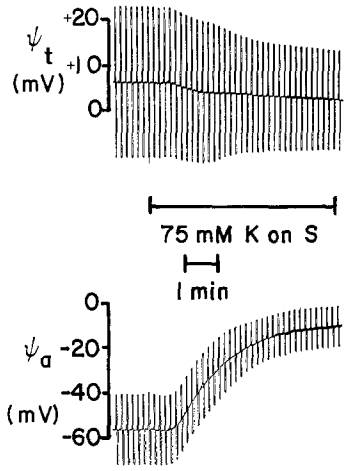

Fig. 2. Changes in ψ_t and ψ_a produced by increasing $[K]_s$ from 5.4 to 75 mM. Deflections in ψ_t and ψ_a are due to the passage of bipolar, constant-current pluses across the epithelium

eral minutes were required for ψ_t, ψ_a and R_t to obtain stable values.

A summary of the steady-state changes in electrical properties of control tissues resulting from increased $[K]_s$ is given in Table 2A. Elevated $[K]_s$ depolarized ψ_b by 52 mV and ψ_a by 41 mV. The depolarization of ψ_a by serosal K can be explained by passive electrical coupling of ψ_a to the primary change in ψ_b via current flow through the paracellular pathway: the change in ψ_a was always less than that in ψ_b. These findings suggest that the basolateral membranes of control tissues are characterized by a significant K conductance.[1]

Table 2B presents the results of experiments in which $[K]_s$ bathing epinephrine-treated tissues was elevated. Addition of epinephrine to the serosal solution increased ψ_t, depolarized ψ_a, and decreased R_t and f_R. All of these changes are consistent with epinephrine-induced active Cl secretion, as previously noted (Welsh et al., 1982; Shorofsky et al., 1983). When $[K]_s$ bathing epinephrine-treated tissues was increased from 5.4 to 75 mM, ψ_t and R_t decreased, f_R increased and ψ_a and ψ_b depolarized. The epinephrine-induced reduction in f_R, which results from an increase in apical membrane Cl conductance (Welsh et al., 1982), enables us to detect the increase in f_R induced by elevated $[K]_s$. These findings are similar to those obtained from the indomethacin-treated tissues, suggesting that the surface cells of canine trachea are endowed with a high basolateral membrane K conductance under both basal and secreting conditions.[2] They parallel the findings of Welsh (1983).

Cellular Electrical Potential Profile: Effects of Serosal Ba under Open-Circuit Conditions

We evaluated the effects of Ba (0.5 mM, serosal solution) on the electrical properties of the surface cells with tissues at their spontaneous ψ_t. Figure 3 is an experimental record in which a cellular impalement was maintained during the response to serosal Ba. Barium depolarized ψ_t and ψ_a, in-

[1] With a significant basolateral membrane K conductance, elevation of $[K]_s$ would not only be expected to depolarize ψ_b but also reduce R_b and thus increase the fractional apical membrane resistance, f_R. However, the f_R of indomethacin-treated tissues is high because the Cl conductance of the apical membrane is suppressed (Smith et al., 1982; Welsh et al., 1982). This elevated value of f_R may compromise our ability to detect a further increase in fractional resistance. The effect of elevated $[K]_s$ on epinephrine-treated tissues (below) supports this notion.

[2] While a decrease in R_t of control and stimulated tissues with elevated $[K]_s$ is consistent with basolateral membrane K conductance, this effect is rather large, particularly in indomethacin-treated tissues. Sample calculations based on previously determined equivalent circuit parameters (Welsh et al., 1983) suggest that an 80% decrease in basolateral membrane resistance, R_b, would decrease R_t by only 4%, due to the high R_a of indomethacin-treated tissues. Part of the observed decrease in R_t presumably results from higher paracellular mobility of K than Na, but even if the *entire* paracellular conductance were assigned to cations, this effect would decrease R_t by only 17%. Thus it is difficult to account for the 37% reduction of R_t of indomethacin-treated tissues by elevated $[K]_s$. It is possible that an increase in apical membrane conductance accompanies the marked depolarization of ψ_a (i.e. voltage-dependence) induced by elevated $[K]_s$, by an unknown mechanism.

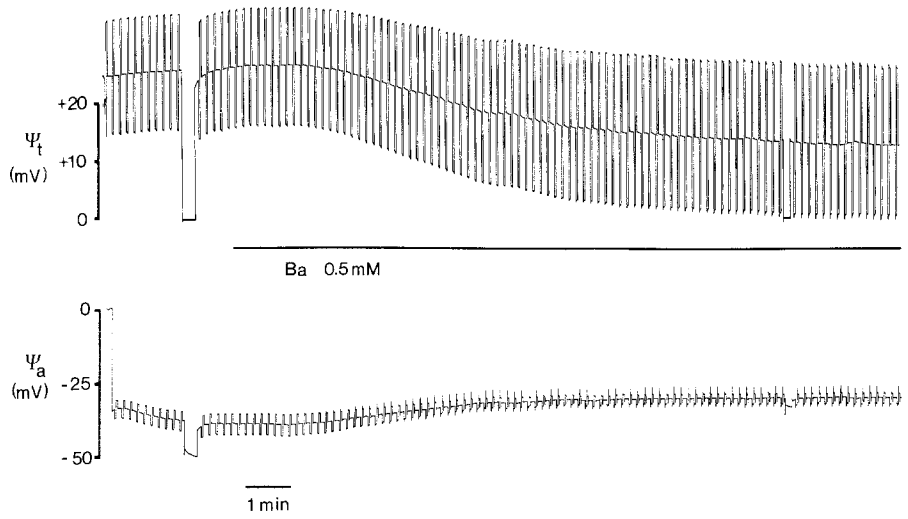

Fig. 3. Changes in ψ_t and ψ_a of an epinephrine-treated tissue produced by addition of Ba (0.5 mM) to the serosal solution. Current pulses are interrupted during short-circuiting, $\psi_t = 0$

Table 3. Effects of Ba on electrical properties under open-circuit conditions

Condition		ψ_t	ψ_a	ψ_b	I_{sc}	R_t	f_R
A. Indomethacin	(6, 29)	14±3	−45±4	58±2	43± 9	310±59	0.76±0.07
+Ba	(6, 22)	8±3*	−30±3*	38±4*	22± 6*	351±76	0.56±0.11*
B. Indomethacin +Epinephrine	(3, 20)	32±3	−32±1	64±2	184±30	130±11	0.45±0.06
Indomethacin +Epinephrine +Ba	(3, 11)	17±2*	−26±2*	43±4*	89±12*	188±18*	0.22±0.08*

Numbers in parentheses are the number of animals and number of punctures, respectively. ψ_t, ψ_a and ψ_b are in mV, I_{sc} in μA/cm² and R_t in Ω cm². I_{sc} was recorded by briefly short-circuiting the tissue as illustrated in Fig. 3. Indomethacin and epinephrine concentrations were 10^{-6}M; Ba, 0.5 mM. (*) $P<0.05$.

creased R_t and reduced $\Delta\psi_a/\Delta\psi_t$ in response to current pulsing.

The results presented in Table 1, as well as those of other studies (Smith et al., 1982) show that indomethacin is capable of selectively suppressing spontaneous Cl secretion, so that Na absorption accounts for much of the I_{sc} remaining after prolonged (*ca.* 1 hr) indomethacin treatment. This absorptive process is amiloride-sensitive (Welsh et al., 1983) and resembles that of Na-transporting epithelia in which electrogenic Na absorption can be inhibited by serosal Ba (Ramsay, Gallagher, Shoemaker & Sachs, 1976; Nagel, 1979). Table 3A summarizes the results obtained from six experiments in which the effects of adding Ba to the serosal solution of indomethacin-treated tissues were examined. The reduction in I_{sc} suggests that Na absorption across canine tracheal epithelium is Ba-sensitive. Serosal Ba also depolarized ψ_a and ψ_b. The smaller depolarization of ψ_a (relative to that of ψ_b) can be attributed to passive electrical coupling of ψ_a to a primary change in ψ_b. The reduction in f_R is consistent with an increase in R_b due to a Ba-induced decrease in basolateral membrane K conductance, which will be discussed in detail on pages 195 and 196.

Table 3B summarizes the effects of Ba on epinephrine-treated tissues. As discussed earlier (*see also* results of Table 1), the increase in I_{sc} with epinephrine reflects its stimulation of active Cl secretion. The large Ba-sensitive I_{sc} and ψ_t (relative to those observed in the indomethacin-treated tissues) indicates that Ba inhibits Cl secretion. Serosal Ba increased R_t and decreased f_R which, again, suggest an increase in basolateral membrane resistance due to blockage of basolateral K conductance, as in the control tissues.

Membrane Resistances and Electromotive Forces: Effects of Serosal Ba under Short-Circuit Conditions

In these experiments, the effects of serosal Ba on the electrical properties of short-circuited tissues provided an estimate for the conductance of the paracellular pathway (G_p) according to the method of Yonath and Civan (1971). Accordingly, changes

Table 4. Effects of Ba on membrane resistances and electromotive forces under short-circuit conditions

Condition	ψ_t	ψ_a	ψ_b	I_{sc}	α	R_a	R_b	E_a	E_b
Indomethacin	19±2	−44±2	62±2	55±9	6.0±1.8	1,280±260	280± 65	19±12	80±5
+Ba	14±2*	−32±3*	46±3*	38±6*	1.8±0.5*	1,260±270	820±100*	12±11	78±8

Results from seven experiments. Indomethacin (10^{-6} M, mucosal solution) and Ba (0.5 mM, serosal solution). ψ_t, ψ_a, ψ_b, E_a and E_b are in mV, I_{sc} in μA/cm², R_a and R_b in Ω cm². R_p in these tissues averaged 490±95 Ω cm². *See* text for further details. (*) $P<0.05$.

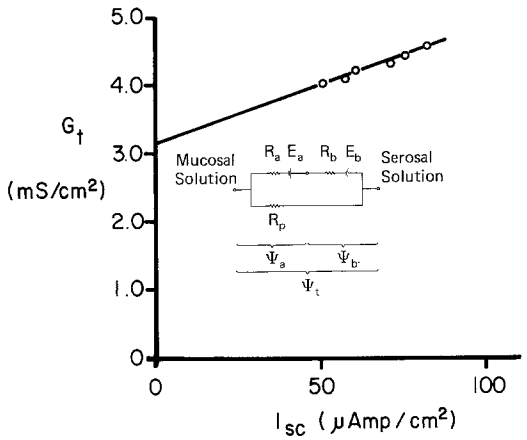

Fig. 4. Relation between I_{sc} and G_t during the action of Ba, added to the serosal solution (0.5 mM). Results of a representative experiment are shown. Insert: Equivalent electrical circuit model for canine tracheal epithelium. *See* text or Welsh et al. (1983) for symbol designations

in I_{sc} and transepithelial conductance (G_t) induced by an agent that is thought to specifically alter transcellular conductance are plotted. If G_p and transepithelial electromotive force (E_t) are minimally affected, a linear relation between G_t and I_{sc} is obtained:

$$G_t = \left(\frac{1}{E_t}\right) I_{sc} + G_p. \tag{2}$$

Results from an experiment of this type are shown in Fig. 4; G_p ($1/R_p$) is the intercept on the ordinate.

For each tissue studied, the value of R_p obtained in this manner was used to evaluate the individual membrane resistances, R_a and R_b, using equations that describe the equivalent electrical circuit model (also shown in Fig. 4) previously applied to this tissue (Welsh et al., 1983):

$$\frac{1}{R_t} = \frac{1}{R_a + R_b} + \frac{1}{R_p} \tag{3}$$

$$\alpha = \frac{R_a}{R_b} = \frac{1}{(1/f_R) - 1}. \tag{4}$$

During a single cell impalement, values of R_t and α were determined before Ba was added to the serosal solution. A value of R_p was then obtained from the time-course of G_t and I_{sc} in response to Ba addition, and then additional values of R_t and α were obtained in the presence of Ba. This approach enabled us to calculate R_a and R_b using Eqs. (3) and (4) for each tissue in the presence and absence of Ba. Linearity of the relation between G_t and I_{sc} suggested that Ba had no acute effect on R_p. The electromotive forces at the apical (E_a) and basolateral (E_b) membranes (*see* Fig. 4 and Welsh et al., 1983) in the presence and absence of Ba were obtained from the following relations:

$$\psi_a = E_a - I_{sc} \cdot R_a \tag{5}$$

$$\psi_b = E_b - I_{sc} \cdot R_b. \tag{6}$$

The results of this analysis are presented in Table 4. In these seven tissues, the paracellular pathway resistance R_p, averaged 490±95 Ω cm², in good agreement with values obtained in other studies (Welsh et al., 1983) from the actions of epinephrine (760 Ω cm²) and amiloride (500 Ω cm²). Similarly, R_a and R_b agree favorably with values obtained previously in indomethacin-treated tissues (1900 Ω cm² and 450 Ω cm², respectively), and the values of E_a and E_b given in Table 4, are in reasonable agreement with our previous estimates (26 and 80 mV, respectively; Welsh et al., 1983).

Table 4 also provides values of R_a, R_b, E_a and E_b in the presence of serosal Ba. None of the parameters were affected except for R_b, which increased threefold. The finding that 0.5 mM Ba did not alter E_a or E_b lends independent support to the implicit conclusion offered by linearity of the Yonath-Civan plot; namely that Ba does not affect E_t ($E_a + E_b$). These results indicate that serosal Ba elevates basolateral membrane resistance,[3] and the

[3] As discussed by Boulpaep and Sackin (1980) the evaluation of epithelial cell membrane resistances by techniques relying on changes in f_R can be complicated by a "distributed resistance" along the paracellular pathway. In electrically leaky epithelia, if the lateral intercellular space resistance is a significant component of R_p, then externally driven current flow across the basolateral membrane is nonuniform and f_R is underestimated. However, it should be emphasized that an underestimate of f_R (or α) in the present analysis would only serve to minimize the calculated effect of Ba on R_b. If we assume that α (Table 4) is underestimated by a factor of three, the increase in R_b with serosal Ba would be fourfold rather than threefold. At the same time, R_a would be altered by only 20%. Clearly, this would not affect our conclusion that Ba selectively increases R_b.

Table 5. Intracellular K activities in the presence or absence of epinephrine

Condition		ψ_t	ψ_a	ΔE_t	ψ_b	I_{sc}	R_t	f_R	a_c^K
A. *Indomethacin*:									
Conventional electrode	(9, 36)	16±2	−44±2		60±1	75±10	223±10	0.77±0.04	
K-selective electrode	(9, 46)			17±2				0.76±0.04	80±5
B. *Indomethacin*:									
Conventional electrode	(7, 30)	17±3	−44±2		61±2	73±13	234±19	0.75±0.04	
K-selective electrode	(7, 36)			18±2				0.74±0.04	83±6
Indomethacin + Epinephrine:									
Conventional electrode	(7, 32)	23±4*	−34±3*		58±2	130±25*	162±16*	0.58±0.04*	
K-selective electrode	(7, 34)			23±3*				0.59±0.06*	69±6

Results are means±SEM. Numbers in parentheses are the number of animals and number of punctures, respectively. Tissues were open-circuited throughout except for brief periods of short-circuiting to obtain I_{sc} values. Indomethacin and epinephrine concentrations were 10^{-6} M. ψ_t, ψ_a, ψ_b, and ΔE_t are in mV, I_{sc} in μA/cm², R_t in Ω cm² and a_c^K in mM. (*) $P<0.05$.

fact that 0.5 mM Ba did not alter R_a, R_p, E_a or E_b argues for specificity of its action; we will address this issue in greater detail on page 195.

Intracellular K Activities

Typical recordings obtained with K-selective microelectrodes are illustrated in Fig. 5. Successful impalements were of two types that are similar to those obtained previously with conventional (voltage-sensing) microelectrodes (Welsh et al., 1982). The first pattern (Fig. 5, left) is characterized by an abrupt positive deflection which attains stable values of ΔE_t and f_R almost immediately. In the second type (Fig. 5, right) the abrupt positive deflection decreased to a stable positive value during the initial 30 to 60 sec. This secondary decrease in voltage was generally accompanied by an increase in the deflections due to current pulsing (i.e. an increase in fractional apical membrane resistance) and has been interpreted as sealing of the membrane around the microelectrode tip (Welsh et al., 1982). As was true of the values of ψ_a and f_R obtained with conventional microelectrodes, there was no systematic difference in the values of ΔE_t and f_R obtained from these two patterns using K-selective electrodes. In addition, the values of f_R obtained with conventional microelectrodes were in excellent agreement with those determined using K-selective microelectrodes in every tissue studied (*see below* and Table 5). This provides sound justification for separate steady-state measurements of ψ_a and ΔE_t to obtain the cell K activity, as outlined in Materials and Methods.

A summary of the results obtained with K-selective microelectrodes in indomethacin-treated tissues is presented in Table 5A. The values of ΔE_t and ψ_a together with Eq. (1) yielded an average

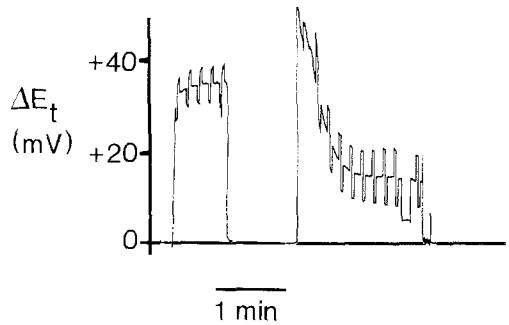

Fig. 5. Representative cellular impalements with K-selective liquid ion exchanger microelectrodes. Deflections in ΔE_t due to current pulses; interrupted briefly during short-circuiting of tissue, right tracing only

intracellular K activity (a_c^K) of 80±5 mM under control conditions. Quantitatively similar values have been obtained in other epithelial cells (Lee & Armstrong, 1972; Kimura & Fujimoto, 1977; DeLong & Civan, 1978; Lewis, Wills & Eaton, 1978; Reuss & Weinman, 1979; Fujimoto, Kazuyot & Kubota, 1980). If we assume an activity coefficient for intracellular K of 0.76, the intracellular K concentration calculated from our data is 107 mM. This is lower than the values of 163 and 142 mM obtained by Widdicombe, Basbaum and Highland (1981) and Cotton and Gatzy (1982) from chemical analysis of isolated tracheal cells, a difference of 25 to 33%. Discrepancies between cell K concentrations estimated using K-selective microelectrode techniques and chemical analysis have been reported for a variety of tissues including frog oocytes (Palmer, Century & Civan, 1978), *Amphiuma* small intestine (White, 1976), frog urinary bladder (Kimura & Fujimoto, 1977), toad urinary bladder (DeLong & Civan, 1980) and rat distal tubule (Khuri, Agulian & Kalloghlian, 1972).

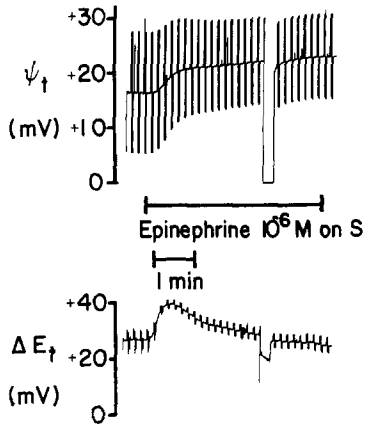

Fig. 6. Recordings of ψ_t and ΔE_t in response to epinephrine (10^{-6} M) added to the serosal solution. The period when $\psi_t = 0$ represents short-circuiting of the tissue

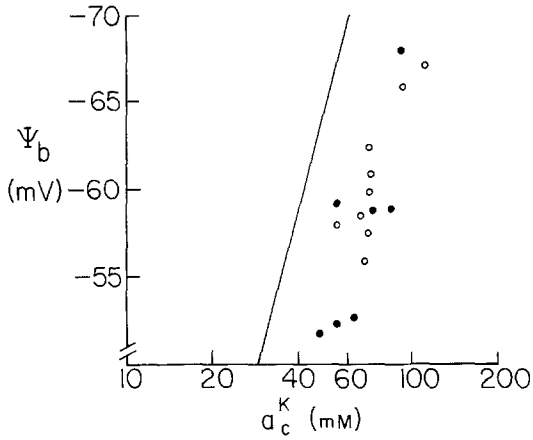

Fig. 7. Relation between intracellular potassium activity, a_c^K and ψ_b, determined from punctures with conventional and K-selective microelectrodes. Each point plots the average values of a_c^K and ψ_b obtained from each tissue in the presence (●) or absence (○) of epinephrine. The solid line is the relation predicted for an equilibrium distribution of cell K at an extracellular K activity of 4.1 mM, according to the Nernst equation

This probably represents compartmentation of cell K in organelles inaccessible to the electrode (Civan, 1980).[4] Nevertheless, the value of a_c^K obtained for tracheal surface cells seems to provide a reasonable estimate of the chemical potential difference for K across the basolateral membrane, as will be discussed below.

Figure 6 illustrates the time-courses of ψ_t and ΔE_t in response to epinephrine. The increase in ψ_t and decrease in R_t ($\Delta\psi_t$ in response to constant-current pulses) provide evidence of epinephrine-induced active Cl secretion. The time-course of ΔE_t during the response to epinephrine closely resembles that of ψ_a reported previously (Welsh et al., 1982): biphasic changes in both ψ_a and $\Delta\psi_a$ (in response to constant-current pulses). The fact that the transient response of ΔE_t to epinephrine mimics that previously reported for ψ_a provides additional evidence that the recordings obtained with K-selective microelectrodes are, indeed, intracellular recordings. This is also supported by the fact that ΔE_t was not zero during short-circuiting of either control or stimulated tissues (*see* Figs. 5 and 6), as would be expected if the electrode had passed through the epithelium.

Table 5B presents results from conventional and K-selective microelectrode measurements where both control and epinephrine values were obtained in the same tissues. While ΔE_t became more positive with epinephrine, this was largely the result of a similar change in ψ_a (*see* Eq. 1), so that the value of a_c^K calculated from data obtained in the presence of epinephrine did not differ significantly from the control value. Figure 7 shows the relation between ψ_b and a_c^K for all tissues studied. Clearly, a_c^K exceeds, by approximately 20 mM, the values predicted for an equilibrium distribution of K across the basolateral membranes, given by the line in Fig. 7.

Discussion

In this section we discuss the K transport properties of the apical and basolateral membranes of the surface cells of canine tracheal epithelium, the driving forces for K transport across these barriers and the effects thereon, of epinephrine. We will focus on the parallel relation of basolateral membrane K permeability to Cl secretion rate and the importance of changes in K permeability to the overall secretory process.

Potassium Transport at the Apical Membrane

The results of prior studies of canine tracheal epithelium imply that the K permeability of the apical membrane is small. The short-circuit current across this tissue can be attributed to net transport of Na and Cl under a variety of conditions leaving little leeway for an electrogenic K secretory process (Al-Bazzaz & Al-Awqati, 1979).

[4] While a fraction of cell K may be compartmentalized or immobilized, it is also possible that the techniques involved in chemical analysis underestimate cell water due to dehydration of tissue samples (Widdicombe et al., 1981). This would lead to an overestimate of intracellular concentrations, particularly K.

Results of the recent study of Shorofsky et al. (1983) provide a more direct indication of low apical membrane K permeability. Elevation of mucosal solution K concentration from 5 to 50 mM depolarized ψ_a by approximately 4 mV under control (untreated) conditions and by about 1 mV after epinephrine treatment. Using these values, they calculate an ion-dependent partial potential ratio, which represents the change in ψ_a observed with increased $[K]_m$ relative to that which would be expected for exclusive permeability of the apical membrane to K. The value of 0.18 for K was less than those obtained for Na (0.28) or Cl (0.58) under control (untreated) conditions and was even lower in the presence of epinephrine, due to increased apical membrane Cl conductance.

The results of transepithelial K fluxes determined under short-circuit conditions are also consistent with a low apical K permeability. Under basal conditions, the electrochemical potential difference for K across the apical or basolateral membranes of short-circuited tissues is approximately 20 mV, favoring K exit.[5] This is similar in magnitude to the driving force for Cl exit across the apical membranes of tissues in which Cl secretion is stimulated with epinephrine (*ca.* 20 mV, Welsh et al., 1983). Despite these similar driving forces, we were unable to detect a significant rate of net K transport across canine tracheal epithelium under basal or stimulated conditions. However, net K secretion resulted from addition of Ba to the serosal solution of epinephrine-treated tissues. Net K exit across the apical membrane with serosal Ba probably traverses the conductance pathways described by Shorofsky et al. (1983). However, the absence of net K secretion across control or stimulated tissues suggests that the predominant conductance pathway for exit of cell K lies at the basolateral membrane.

The Cl and K fluxes presented in Table 1 are also consistent with our prior analysis of indomethacin- and epinephrine-treated tissues (Welsh et al., 1983). Decreased apical Cl conductance with indomethacin caused the electromotive force across the apical membrane (E_a) to approach E_a^{Na}, the chemical potential difference for Na across this barrier. This is consistent with the complete suppression of Cl secretion, due to reduced apical Cl conductance (Welsh et al., 1982 and results of Table 1). Conversely, when Cl secretion is stimulated by epinephrine and apical Cl conductance increased, E_a approached E_a^{Cl}, the chemical potential difference for Cl. This, again, implies that the conductance of the apical membrane to K is normally minimal, relative to its Na and Cl conductances.

Potassium Transport Across the Basolateral Membrane: K Conductance Properties

Several observations suggest that the basolateral membrane, in contrast to its apical counterpart, is endowed with a relatively high K permeability: (i) Increased $[K]_s$ elicited a marked depolarization of ψ_b and decreased the relative resistance of the basolateral membrane ($1-f_R$). (ii) Serosal Ba depolarized ψ_b and increased basolateral membrane resistance. (iii) Reductions in serosal solution Na or Cl concentrations did not influence ψ_b or increase relative basolateral membrane resistance, consistent with an absence of significant conductive pathways for Na or Cl (page 189 and Welsh et al., 1982). All of these findings apply to both indomethacin-treated tissues, where Cl secretion rate is depressed, as well as to epinephrine-stimulated tissues, where Cl secretion rate is maximal (Smith et al., 1982). Thus, a high K conductance at the basolateral membranes is evident regardless of the existing Cl secretion rate.

The effect of 0.5 mM Ba was selective for R_b. Table 4 shows that R_a, E_a and E_b were unaffected. Despite a threefold increase in R_b with Ba, the overall electromotive force (emf) across the basolateral membrane, E_b, was unchanged. This observation provides additional support for our conclusion that the only significant conductance present in the basolateral membrane under normal conditions is that for K. Even after Ba has blocked a fraction of basolateral K channels sufficient to elicit a threefold increase in R_b, E_b has *not* shifted to values that would be expected for the chemical potential differences of other ions (e.g. Na or Cl), but remains at the chemical potential difference of K across this barrier, E_b^K.

The Ba-induced increase in basolateral membrane resistance causes ψ_b to shift away from the chemical potential difference for K (E_b^K) toward the chemical potential differences of other permeant ions. Electrical coupling between the opposing membranes permits ionic gradients or emfs present at the apical border (E_a) to influence ψ_b, and this effect increases as R_b increases (Boulpaep,

[5] As will be shown below, the net driving force for K exit across the basolateral membranes is approximately 20 mV under open-circuit conditions. In the presence of indomethacin, short-circuiting has little effect on ψ_b since R_a/R_b is high (compare data of Tables 3 and 4; also Welsh et al., 1982, 1983). If this is also true of a_c^K, then the driving force for K exit is approximately 20 mV under short-circuit conditions as well.

1971; Schultz, 1974; Miller & Steinberg, 1977). Thus, Ba blockade of the basolateral K conductance of tracheal surface cells causes ψ_b to shift away from E_b^K (depolarize) toward E_a. As discussed above and elsewhere (Welsh et al., 1983), E_a is the combined result of the chemical potential differences for Na and Cl across the apical membrane, weighted by their relative conductances (or transference numbers). Under control conditions the values of E_a and E_b determined from equivalent circuit analysis (Table 4) were 19 and 80 mV, respectively, so that ψ_b (62 mV) was closer to E_b. Barium blockade of serosal K channels shifted ψ_b toward E_a; ψ_b depolarized to 46 mV.

Role of Basolateral K Conductance in Cl Secretion

Barium blockade of the basolateral K conductance pathway (channel) not only depolarized ψ_b, but via electrical coupling also depolarized ψ_a and inhibited Cl secretion (Tables 1 and 3). Under short-circuit conditions in the presence of epinephrine, the chemical potential difference for Cl across the apical membrane (E_a^{Cl}) was approximately -30 mV whereas ψ_a averaged -53 mV (Welsh et al., 1983). Therefore, a net driving force favors Cl exit across the apical membrane since ψ_a exceeds E_a^{Cl} (i.e. $E_a^{Cl}-\psi_a=23$ mV). However, if the electrical driving force for Cl exit is reduced so that ψ_a approaches E_a^{Cl}, as is the case with elevated serosal K or Ba, then the driving force for Cl exit is compromised and Cl secretion is inhibited. This is evident from the effect of Ba on net Cl flux (Table 1) or the effect of Ba or increased $[K]_s$ on the I_{sc} of secreting tissues (Tables 2B and 3B). This conclusion also applies to the Ba-induced inhibition of Na absorption across indomethacin-treated tissues (Table 3A) since part of the driving force for Na entry across the apical membrane is electrical (Welsh et al., 1983). However, since ψ_a normally lies much closer to E_a^{Cl} (-30 mV) than to E_a^{Na} ($+60$ mV), depolarization of ψ_a is expected to compromise net Cl exit far more than it reduces net Na entry.

Variation in Basolateral Membrane K Permeability with Secretion Rate

The basolateral transport mechanisms thought to be involved in active Cl secretion by canine tracheal epithelium (Welsh et al., 1982, 1983) are similar to those proposed for other Cl-secreting epithelia (Frizzell, Field & Schultz 1979). Chloride enters the secretory cell from the serosal solution coupled to the entry of Na, which, in turn, is extruded across the basolateral membrane by the Na/K pump. The presence of Na/K-ATPase at the basolateral membranes was demonstrated by ouabain binding and autoradiography (Widdicombe, Basbaum & Yee, 1979). Evidence for the role of this enzyme in Na absorption and Cl secretion is similar to that in other tissues: transport is inhibited by addition of ouabain to, or removal of K from, the serosal solution alone (Westenfelder, Earnest & Al-Bazzaz, 1980; Widdicombe & Welsh, 1980).

The increase in Na/K pump activity that parallels stimulation of Cl secretion would lead to increased uptake and cellular accumulation of K from the submucosa. There is, however, no K secretion during stimulation (Table 1) so that all of the additional K taken up is returned, via basolateral membrane K channels, to the serosal solution. If this were accomplished by increasing the driving force for K exit alone, the two- to fourfold increase in transport rate elicited by epinephrine would require that the driving force for K exit ($E_b^K-\psi_b$) increase to a similar extent. Epinephrine did not significantly affect ψ_b (Tables 2, 3 and 5), so that E_b^K would increase from approximately 80 mV to 100–140 mV, resulting in cell K activities of 170 to 750 mM, depending on the secretory rate. An increase in basolateral membrane K permeability, in parallel with the stimulation of Cl secretion, obviates this problem.

Figure 8 provides another representation of this line of reasoning. Shown here are: (i) the electromotive force across the basolateral membrane (E_b) determined from equivalent circuit analysis (Welsh et al., 1983), (ii) the chemical potential difference for K across the basolateral membrane (E_b^K) calculated from our a_c^K measurements (Table 5) and the extracellular K activity of 4.1 mM using the Nernst equation, (iii) the electrical potential difference across this barrier (ψ_b), also taken from Table 5, and (iv) the net driving force for K exit ($E_b^K-\psi_b$). Values for both control (indomethacin-treated) and stimulated (epinephrine-treated) tissues are provided.

Several conclusions emerge from these findings: (i) The electromotive force across the basolateral membranes (E_b) is largely, if not solely, determined by the chemical potential difference for K across this barrier (E_b^K). This is in agreement with the results of ion-replacement studies and the effects of serosal Ba which indicate that the only detectable conductance traversing the basolateral membrane is for K. (ii) The chemical potential dif-

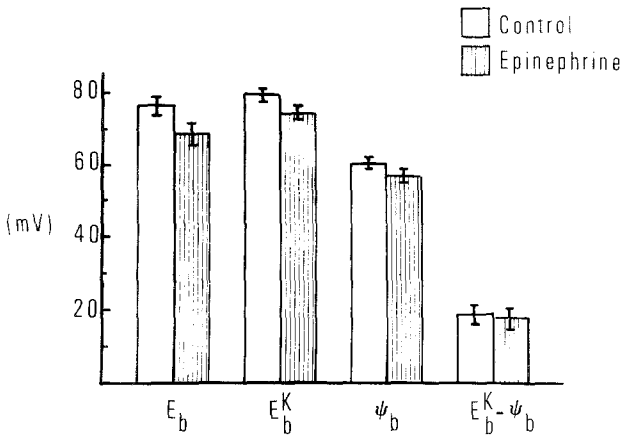

Fig. 8. Values of the electromotive force (E_b), chemical potential difference for K (E_b^K), electrical potential difference (ψ_b) and net driving force for K exit ($E_b^K - \psi_b$) across the basolateral membranes of control (open bars) and epinephrine-treated (hatched bars) tissues. Values of E_b^K and ψ_b from data of Table 5, E_b from Welsh et al. (1983)

ference for K (E_b^K) is not altered by stimulation. The presence of epinephrine did not significantly affect a_c^K and the identity between E_b and E_b^K persists. This implies that the basolateral membrane remains K selective during stimulation and, again, is consistent with the results of our studies with Ba and ion-replacement. (iii) The electrical potential difference across the basolateral membrane ψ_b is not affected by epinephrine in the steady state (Tables 2, 3 and 5 and Welsh et al., 1982). This results from the fact that the basolateral membrane K conductance increases with stimulation (*see below*). Finally, (iv) the net driving force for K exit across the basolateral membrane ($E_b^K - \psi_b$) is approximately 20 mV and is not altered with stimulation, despite the two- to fourfold increase in transport rate. The only conclusion compatible with these findings is that the K permeability of the basolateral membrane varies in parallel with the rate of Cl secretion, and with Na/K pump activity. The results of prior studies (Welsh et al., 1983) showed that epinephrine elicited a 2.8-fold increase in I_{sc} while decreasing basolateral membrane resistance 2.7-fold. The present findings indicate that this decrease in R_b reflects an increase in basolateral membrane K permeability.

A direct relation between Na/K pump activity and basolateral membrane conductance also emerges from studies of Na-transporting epithelia. Helman, Nagel and Fisher (1979) reported that ouabain led to a decrease in R_b in frog skin. Similar findings were obtained by Davis and Finn (1982) who used amiloride to inhibit Na transport across toad urinary bladder. Conversely, increased rates of Na transport led to decreased R_b in frog skin (Helman & Fisher, 1977), *Necturus* urinary bladder (Higgins, Gebler & Fromter, 1977; Schultz, 1981) and *Necturus* small intestine stimulated by sugars or amino acids (Gunter-Smith, Grasset & Schultz, 1982). Schultz (1981) reviewed this subject for absorptive epithelia and suggested that the increase in basolateral membrane conductance that parallels Na/K pump activity could be explained by an increase in K permeability. Our results indicate that this conclusion also applies to secretory epithelia.

The factor(s) that underlie the K permeability change in canine tracheal epithelium are unknown at present. Certainly, a prime candidate is an increase in intracellular Ca activity which is an effective stimulus for active Cl secretion (Al-Bazzaz & Jayaram, 1981; Smith et al., 1982). Calcium-activiated K conductances have been described in nerve (Meech, 1978), cardiac muscle (Caroni & Carafoli, 1982), and red cell membranes (Lew & Ferreira, 1976). An increase in cell Ca in tracheal surface cells could result from the presence of Na/Ca exchange in the basolateral or internal membranes. Secretagogue-induced entry of NaCl from the serosal solution could raise cell Na and compromise Na/Ca exchange so that cytosolic Ca rises. Alternatively, secretagogues may directly elicit Ca release, via cAMP, from intracellular stores (Smith et al., 1982), or cAMP may directly activate basolateral K channels. The coupling between transport rate and K conductance, whatever its nature, must be fairly stoichiometric because of the precise correlation between I_{sc} and R_b cited earlier.

Finally, the increase in basolateral membrane K permeability that accompanies Cl secretion serves two important functions: First, it prevents large changes in cell composition that could impair cellular processes, several of which require optimal K concentrations for activity (Civan, 1978). The osmotic effects of increased cell K (plus anion) in the absence of a permeability change could lead to cell rupture. Second, as discussed previously (Welsh et al., 1983), increased basolateral K conductance minimizes the depolarization of ψ_a that results from secretagogue-induced increases in apical membrane Cl permeability. The importance of basolateral K channels in maintaining ψ_a is apparent from the effect of serosal Ba which inhibits Cl secretion by depolarizing ψ_b and, via electrical coupling, ψ_a (Table 3). The same conclusion applies to elevated serosal [K]. As ψ_a approaches the equilibrium potential for Cl across the apical mem-

brane (E_a^{Cl}, approximately -30 mV) Cl secretion is reduced. Indeed, this occurs transiently in response to epinephrine whose initial action is to increase apical Cl conductance (Welsh et al., 1982). However the secondary elevation of basolateral K permeability hyperpolarizes ψ_a and thereby maintains the driving force for diffusional Cl exit.

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