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Basolateral Membrane Potassium Conductance is Independent of Sodium Pump Activity and Membrane Voltage in Canine Tracheal Epithelium

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Summary. When secretagogues stimulate Cl secretion in canine tracheal epithelium, apical membrane Cl conductance (G_a^{Cl}) increases, and then basolateral membrane K conductance (G_b^K) increases. Conversely, inhibition of G_a^{Cl} results in a secondary decrease in G_b^K. The coordination of the two membrane conductances and regulation of G_b^K is critical for maintaining constant intracellular ion concentrations and transepithelial Cl secretion. The purpose of this study was to test two hypotheses about the regulation of G_b^K. First, we asked whether G_b^K is directly linked to the activity of the Na,K-ATPase. We found that pump activity could be dissociated from K conductance. Inhibition of the Na pump with ouabain, in nonsecreting tissues led to an increase in G_b. Elevation of the bathing solution K concentration produced a similar effect. Addition of ouabain to secreting tissues did not appear to alter G_b. These results indicate that G_b^K does not directly parallel Na pump activity. Second, we asked whether changes in G_b^K are voltage dependent. We prevented secretagogue-induced depolarization of the electrical potential difference across the basolateral membrane Ψ_b by clamping Ψ_b at its resting value during stimulation of Cl secretion with epinephrine. Despite maintaining Ψ_b constant, the typical changes in transepithelial resistance and the ratio of membrane resistances persisted. This observation indicates that depolarization is not required for the secretagogue-induced increase in G_b^K. In addition we examined the effect of depolarizing and hyperpolarizing Ψ_b by passing transepithelial current in secreting and nonsecreting epithelia. Despite depolarizing and hyperpolarizing Ψ_b within the physiologic range, we observed no significant changes in transepithelial resistance or the ratio of membrane resistance that would suggest a change in G_b^K. This observation indicates that changes in Ψ_b are not sufficient to alter G_b^K. Thus, G_b^K appears to be regulated by factors other than membrane voltage, or direct coupling to the Na pump.

Key Words electrophysiology · ouabain · membrane permeabilities · potassium conductance · chloride conductance

Introduction

In the study of epithelial ion transport, considerable attention has been paid to the relation between conductive apical membrane transport processes and the rate of transepithelial transport. However, it has recently become apparent, that in many epithelia, there is also a direct relation between the rate of transepithelial transport and the basolateral membrane K conductance. Thus it appears that there is a coupling or coordination between the two individual membrane conductances, such that both membrane conductances parallel the rate of transepithelial transport. This coupling, and regulation of the two conductances, serves to maintain the functional integrity of the cell in the face of changes in the rate of transepithelial transport (Schultz, 1981).

Canine tracheal epithelium, a Cl-secreting epithelium, displays this relation between the membrane conductances. Tracheal epithelium secretes Cl in response to a variety of secretagogues (Olver et al., 1975; Al-Bazzaz & Al-Awqati, 1979; Widdicombe & Welsh, 1980). Following addition of secretagogues, apical membrane Cl conductance (G_a^{Cl}) increases, and then basolateral membrane K conductance (G_b^K) increases (Welsh, Smith & Frizzell, 1982, 1983; Shorofsky, Field & Fozzard, 1983; Welsh, 1983*b,c*). Thus, under steady-state secreting conditions, both G_a^{Cl} and G_b^K are greater than during nonsecreting conditions. The opposite sequence occurs following inhibition of Cl secretion with mucosal anthracene-9-carboxylic acid (9-AC): G_a^{Cl} decreases, and then G_b decreases (Welsh, 1983*a*). Thus, following inhibition of secretion with 9-AC, both G_a^{Cl} and G_b (presumably G_b^K) are less than during secreting conditions.

The direct relation between these conductances has at least two significant consequences: first, large alterations of intracellular ion concentrations are prevented (Welsh, 1983*f*; Smith & Frizzell, 1984) and second, large, sustained changes in membrane voltage are prevented (Welsh et al., 1983; Welsh, 1983*c*).

The mechanisms that regulate G_b^K, thereby

coupling the apical and basolateral membranes are unknown. The aim of this work was to test two hypotheses about the regulation of G_b^K. The first hypothesis is that G_b^K is directly linked to the activity of the Na,K-ATPase. This possibility has been proposed by a number of investigators (*see* Schultz, 1981 for a review) and stems from the observation that G_b^K varies directly with transepithelial transport rate and thus Na-pump activity. The second hypothesis is that the changes in G_b^K might be voltage dependent. In tracheal epithelium, secretagogues increase apical Cl conductance resulting in cell depolarization, then G_b^K increases. The sequential changes in basolateral membrane voltage and G_b^K in tracheal epithelium, as well as in a variety of Na-absorbing epithelia, are consistent with this hypothesis. These observations, plus the finding of a voltage-dependent K conductance in epithelia as well as a variety of excitable cells (Garcia-Diaz, Nagel & Essig, 1983; Muruyama, Gallacher & Petersen, 1983) raised the possibility that the basolateral membrane voltage might regulate or trigger the changes in G_b^K.

List of Symbols

Ψ_t, Ψ_a, Ψ_b	The electrical potential difference across the epithelium, the apical membrane, and the basolateral membrane, respectively, in millivolts.
I_{sc}	The short-circuit current, i.e., the current required to clamp Ψ_t to zero, in $\mu A \cdot cm^{-2}$.
G	Electrical slope conductance, in $mS \cdot cm^{-2}$.
R	Electrical slope resistance, in $\Omega \cdot cm^2$.
f_R	Fractional resistance of the apical membrane, $R_a/(R_a + R_b)$.
a, b, p, t	As subscripts, refer to apical, basolateral, paracellular, and transepithelial, respectively.
Cl, Na, K	As superscripts refer to the respective ions.

Materials and Methods

The methods of preparing the posterior membranous portion of canine tracheal epithelium were similar to those previously described (Welsh & Widdicombe, 1980). The bathing solution contained (in mM): 118.9 NaCl, 20.4 $NaHCO_3$, 2.4 K_2HPO_4, 0.6 KH_2PO_4, 1.2 $CaCl_2$, 1.2 $MgCl_2$, and 10 glucose, unless otherwise noted. The solution was maintained at 37°C and bubbled with 95% O_2 and 5% CO_2 (pH 7.4). Sodium and potassium concentrations were measured with a flame photometer (Instrumentation Lab, Inc., Boston, Mass.).

For measurement of transepithelial electrical properties and radioisotope fluxes, tissues were mounted in Ussing chambers with 1.5 cm^2 surface area (Jim's Instruments, Inc., Coralville, Iowa). All studies were performed under short-circuit conditions; the transepithelial electrical potential difference (Ψ_t) (referenced to the mucosal solution) was automatically clamped to zero by automatic voltage-current clamps (University of Iowa, Bioengineering). Transepithelial resistance (R_t) and transepithelial conductance (G_t) were calculated from the change in current required to clamp Ψ_t to ± 10 mV (pulses delivered by a pulse generator built into the voltage-current clamp, duration 1 sec, period 60 sec).

Unidirectional and calculated net transepithelial fluxes of ^{36}Cl were measured in paired tissues from the same dog. Seven μCi of ^{36}Cl were added to the appropriate side of the tissue, 45 min were allowed for isotope fluxes to reach a steady state, and then three samples of both bathing solutions were taken at 20-min intervals during each control and experimental period. Isotope flux measurements were made during the steady state at least 30 min after an experimental intervention.

The methods for measuring transepithelial and transmembrane electrical properties, construction of microelectrodes, and performance of cellular impalements have been previously described (Welsh et al., 1982). The electrical potential difference across the apical cell membrane (Ψ_a) was referenced to the mucosal bathing solution. Bipolar, square-wave pulses sufficient to clamp Ψ_t to ± 10 to 20 mV were intermittently passed across the epithelium in order to measure R_t and the fractional resistance of the apical membrane (f_R):

$$f_R = \frac{\Delta\Psi_a}{\Delta\Psi_t} = \frac{R_a}{R_a + R_b} \quad (1)$$

where $\Delta\Psi_a$ and $\Delta\Psi_t$ refer to the change in Ψ_a and Ψ_t induced by the current pulse and R_a and R_b refer to the apical and basolateral membrane resistance, respectively.

The following chemicals were used: Epinephrine (Elkin-Sinn Inc., N.J.), and indomethacin, barium chloride, and ouabain (all from Sigma Chemical, St. Louis, Mo.). Furosemide was a generous gift of Hoechst Pharmaceuticals, Somerville, N.J. Amiloride was a generous gift of Merck, Sharp and Dohme Research Laboratories, West Point, Pa.

The goal of some of these studies was to determine whether a specific maneuver increases membrane conductance. Therefore, it was first necessary to minimize the conductance of the two cell membranes. For the majority of the studies that follow, amiloride (10^{-4} M) and indomethacin (10^{-6} M) were present in the mucosal bathing solution. Amiloride inhibits Na absorption in tracheal epithelium (Widdicombe & Welsh, 1980) by inhibiting an apical Na conductance (Welsh et al., 1983). Indomethacin inhibits prostaglandin production, decreases intracellular cAMP levels, and thereby minimizes the rate of Cl secretion (Al-Bazzaz, Yadava & Westenfelder, 1981; Smith et al., 1982; Welsh et al., 1982). Neither maneuver interferes with the subsequent response to secretagogues. Although indomethacin minimizes the rate of Cl secretion it does not always completely abolish it (Al-Bazzaz et al., 1981; Smith et al., 1982; Welsh et al., 1983). However, for the sake of brevity and clarity, indomethacin-treated tissues will be referred to as "nonsecreting tissues."

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

Effect of Ouabain

To test the hypothesis that G_b^K (and G_a^{Cl}, if the two membrane conductances are coupled) is directly

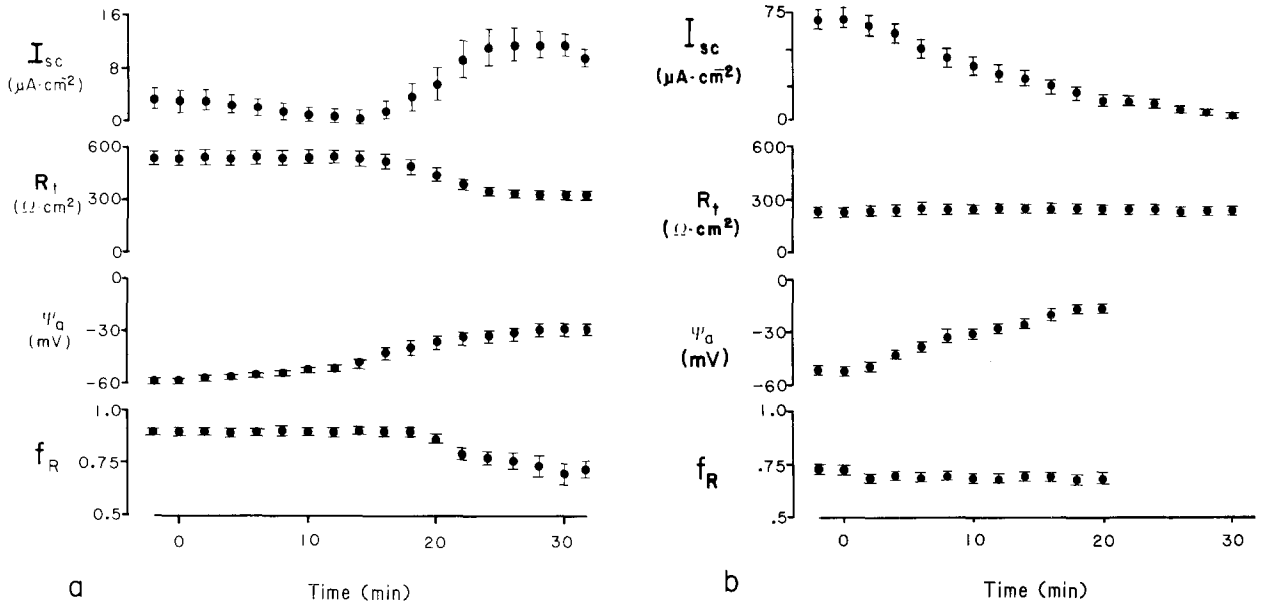

Fig. 1. Effect of ouabain on transepithelial and intracellular electrical properties. *a*) Nonsecreting tissues. The rate of transepithelial transport was minimized by the addition of indomethacin (10^{-6} M, mucosal solution) and amiloride (10^{-4} M, mucosal solution). Ouabain (10^{-4} M, submucosal solution) was added at time zero. Values represent the mean ± SEM of five tissues. In three tissues, one cellular impalement was maintained throughout the duration of the experiment; in the other two tissues, one cellular impalement could not be maintained for the entire duration, therefore a second impalement was obtained. *b*) Secreting tissues. Epinephrine (10^{-6} M, submucosal solution) was present throughout to stimulate Cl secretion. Ouabain was added at time zero. Values represent mean ± SEM of six tissues. Figure 1*b* is taken from Welsh (1983*b*) with permission

linked to Na-pump activity, we added ouabain to the submucosal solution. Ouabain inhibits the basolateral membrane Na,K-ATPase which provides the energy for transepithelial Cl secretion (Al-Bazzaz & Al-Awqati, 1979; Widdicombe, Basbaum & Yee, 1979; Widdicombe, Ueki et al., 1979). Figure 1*a* shows that when the rate of transepithelial transport is minimal (indomethacin and amiloride in the mucosal solution) addition of ouabain produces a biphasic response. I_{sc} and Ψ_a first decrease slightly. Then, approximately 15 min following addition, I_{sc} increases transiently, Ψ_a depolarizes more rapidly, and R_t and f_R decrease. Following the transient increase, I_{sc} decreases to zero over the course of approximately 10 min while R_t remains stable.

The fall in R_t and f_R in nonsecreting tissues most likely results from an increase in both G_a and G_b. Three observations provide support for this statement. First, R_t and f_R decrease to values similar to those found in secreting tissues (epinephrine added to the submucosal solution, Fig. 1*b*) in which both G_a and G_b are greater than during nonsecreting conditions (Welsh et al., 1983; Shorofsky et al., 1983). Second, if one assumes that paracellular pathway conductance (G_p) remains relatively constant over the time frame of the experiment, then the decrease in R_t and f_R after ouabain can not be explained by an increase in only one membrane conductance; both G_a and G_b must increase.[1] Third, I_{sc} transiently increases following addition of ouabain (Fig. 1*a*), a response consistent with an increase in G_a and G_b. Under nonsecreting conditions, both Cl and K are accumulated intracellularly at activities greater than predicted for electrochemical equilibrium (Welsh,

[1] For these calculations I used an equivalent electrical circuit model of the epithelium (Welsh et al., 1983) in which the cellular pathway is represented as an apical (R_a) and basolateral (R_b) membrane electrical resistance in series. The paracellular pathway is represented as an electrical resistance (R_p) in parallel with the cellular pathway. If two of the three resistances remain constant following addition of ouabain, then values for each of the circuit resistances can be obtained from the measured values of R_t and f_R obtained before and after addition of ouabain.

Let us assume that ouabain does not substantially alter R_p. This assumption, is supported by the observations that ouabain does not alter either R_t or f_R in secreting tissues (Fig. 1*b*), ouabain does not alter the transepithelial flux of Na in the passive direction, the submucosal to mucosa flux (Al-Bazzaz & Al-Awqati, 1979; Widdicombe et al., 1979), nor does it alter transepithelial fluxes of mannitol, a marker of paracellular permeability (Welsh & Widdicombe, 1980; Welsh, 1983*a*). If it is assumed that either R_b or R_a is constant following addition of ouabain, then calculation of the three resistances yields negative numbers. Thus, the assumption that a change in R_a alone or a change in R_b alone accounts for the results must be incorrect. Therefore, if R_p is relatively constant, both R_a and R_b must decrease following addition of ouabain.

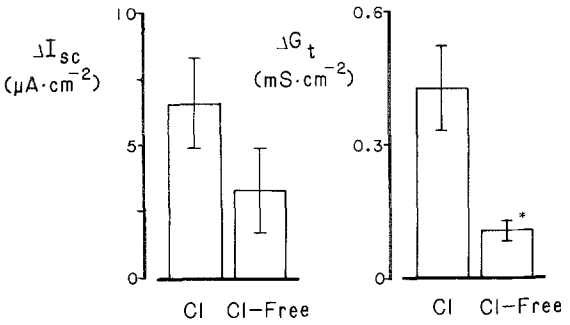

Fig. 2. Effect of Cl-free media on the electrical response to ouabain. Values represent the change in I_{sc} (ΔI_{sc}) and the change in G_t (ΔG_t) following the addition of ouabain. Indomethacin and amiloride were present in the mucosal bathing solution throughout. ΔI_{sc} represents the maximum increase in I_{sc} following addition of ouabain. ΔG_t was taken at the time of the maximum change in I_{sc}. Values represent mean ± SEM of six pairs of tissues; six in normal Ringer's "Cl," and six in "Cl-free" gluconate Ringer's for 45 min. $^*P < 0.05$ by paired t-test

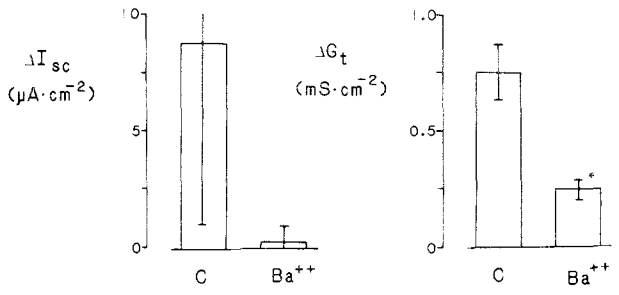

Fig. 3. Effect of barium on the electrical response to ouabain. ΔI_{sc} refers to the maximum change in I_{sc} following the addition of ouabain. ΔG_t was taken at the same time as ΔI_{sc}. Indomethacin and amiloride were present throughout. Values represent mean ± SEM of six pairs of tissues; one tissue was a control, "C"; and one tissue received Ba (2×10^{-3} M, submucosal solution), "Ba." $^*P < 0.05$ *vs.* "C" by paired t-test

1983*e*,*f*; Smith & Frizzell, 1984). An increase in G_a^{Cl} and G_b^K would allow the passive dissipation of these gradients, resulting in a transient increase in current.[2] Under these experimental conditions there are no other reasonable alternatives to account for both the increased conductances and increased current.

Because we were not able to directly measure G_b^K and G_a^{Cl} following addition of ouabain, we felt it would be useful to perform three other experiments to support the conclusion that G_b^K and G_a^{Cl} increased, following addition of ouabain. First, Cl was removed from the bathing solution. Figure 2 shows that when ouabain was added to tissues bathed in Cl-free, gluconate Ringer's, the change in G_t (ΔG_t) was smaller than that observed in Cl Ringer's. This observation suggests that the ouabain-induced changes are partially dependent on Cl.

Second, we examined the effect of Ba (2×10^{-3} M, submucosal solution) on the response to ouabain in nonsecreting tissues. Submucosal Ba inhibits G_b^K in tracheal epithelium (Welsh, 1983*b*). The results are shown in Fig. 3. Six pairs of tissues were studied; one tissue served as a control and one received Ba. Addition of Ba had minimal effects, tending to decrease I_{sc} and G_t slightly. Thirty minutes later ouabain was added. Submucosal Ba significantly diminished the increase in G_t produced by ouabain. The magnitude of the ouabain-induced change in I_{sc} also tended to decrease in Ba-treated tissues. These observations are consistent with a ouabain-induced increase in G_b^K that is inhibited by submucosal Ba.

Finally, we examined the possibility that HCO_3 might contribute to the increase in I_{sc} and G_t. HCO_3 is the only other ion with a substantial concentration in the bathing solution. HCO_3 was replaced by Cl, the tissues were bubbled with 100% O_2, and amiloride and indomethacin were added to the mucosal solution. Following addition of ouabain, I_{sc} increased transiently from 12 ± 4 to 25 ± 6 $\mu A \cdot cm^{-2}$ ($n = 4$ tissues) and G_t increased from 1.15 ± 0.26 to 1.99 ± 0.52 $mS \cdot cm^{-2}$. These changes are similar to those found in HCO_3-containing Ringer's, indicating that the ouabain-induced changes in G_t and I_{sc} are not dependent on HCO_3.

Comparison of Fig. 1*a* and 1*b* shows that the response to ouabain is strikingly different depending upon the secretory state of the tissue and thus the baseline magnitude of the membrane conductances. When membrane conductances are increased, due to the presence of secretagogues (Fig. 1*b*), ouabain does not alter R_t or f_R (Welsh, 1983*b*). When membrane conductances are minimal (Fig. 1*a*), ouabain increases conductances.

Effect of Increased K Concentrations

In light of the finding that addition of ouabain increased G_b^K when it was low, I sought another maneuver that would inhibit transepithelial transport and possibly produce effects similar to those of oua-

[2] Calculation of the flow of charge associated with the increase in I_{sc} after ouabain can be estimated from the area under the curve in Fig. 1*a*. If the thickness of the epithelium is estimated at 50 μm, then the flow of charge is approximately 16 meq $liter^{-1}$ of cell volume or 8 meq · $liter^{-1}$ of KCl. This calculation represents a reasonable amount of KCl compared to total intracellular concentrations.

bain. Therefore, I increased the bathing solution K concentration to depolarize the cell; the basolateral membrane of tracheal epithelium is predominantly K conductive and increasing the K concentration inhibits transepithelial transport (Welsh, 1983*b*,*c*; Smith & Frizzell, 1984). The K concentration was progressively increased in exchange for tetramethylammonium while the Na concentration was maintained constant at 80 mM. K was increased symmetrically in both bathing solutions to prevent the generation of paracellular diffusion potentials. Transepithelial transport was minimized with mucosal indomethacin and amiloride. As shown in Fig. 4, with progressive increases in K concentration we observed an electrical pattern somewhat similar to that observed following addition of ouabain. As K concentration increased from 5 to 30 mM, I_{sc} and Ψ_a decreased, while f_R increased and R_t was little changed. Then, as the K concentration increased above 30 mM, R_t and f_R decreased, I_{sc} increased and Ψ_a tended to depolarize to a greater degree.

This pattern indicates that increased K concentrations alter membrane ionic permeabilities; the results cannot be explained solely by changes in membrane conductance due to the increase in K concentration. First, consider the basolateral membrane. Under nonsecreting conditions the basolateral membrane is K conductive, thus, given constant ionic permeabilities and increasing K concentrations, G^K would be expected to increase slightly, producing a slight decrease in R_t, increase in f_R, depolarization of Ψ_a, and, as a result, a decrease in I_{sc}. These are precisely the results observed as the K concentration began to increase. However, in contrast to the changes predicted for constant ionic permeabilities, at higher K concentrations f_R decreases, I_{sc} increases, and the magnitude of depolarization of Ψ_a is greater with increasing increments in the K concentration.[3] Next, consider the apical membrane. The decrease in f_R and more rapid decrease in R_t observed at higher K concentrations indicate an increase in G_a.[4] Thus, the changes observed in Fig. 2 are best explained by an increase in both G_a and G_b. The conductance changes also explain the small increase in I_{sc}, which might result from the flow of Cl, and K, down their electrochemical gradients.

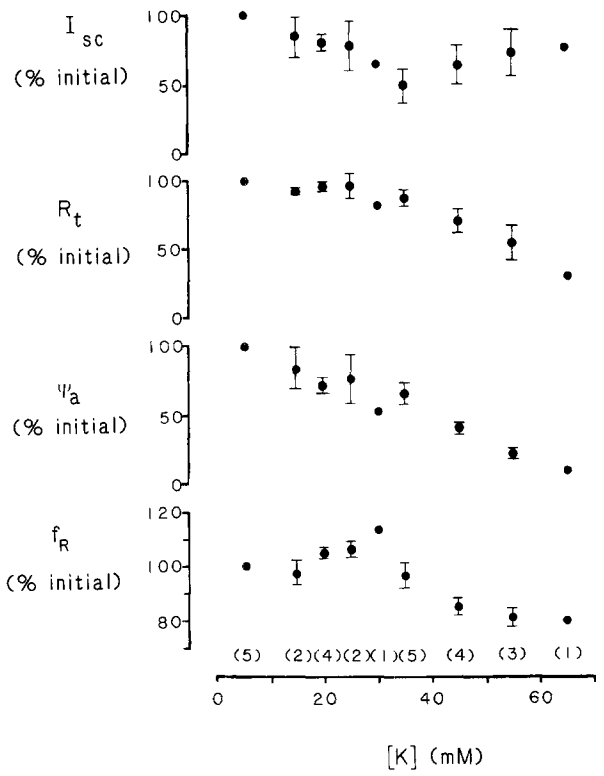

Fig. 4. Effect of increasing K concentrations on the electrical properties of nonsecreting tissues. Indomethacin and amiloride were present throughout to minimize the rate of transepithelial transport. Values represent the mean ± SEM of five tissues. Two to five cellular impalements were made in each tissue at several different concentrations. The numbers in parentheses represent the number of tissues studied at each K concentration. The initial values for the five tissues were: I_{sc}, 6.3 ± 2.7 $\mu A \cdot cm^{-2}$; R_t, 644 ± 93 $\Omega \cdot cm^2$; Ψ_a, −60 ± 7 mV; f_R, 0.86 ± 0.04

To provide further evidence that an elevation of bathing solution K concentration increased G_a^{Cl}, we examined the effect on unidirectional Cl fluxes. Table 1 shows the electrical properties and unidirectional Cl fluxes of seven pairs of tissues in which the rate of transepithelial transport was minimal. When the bathing solution was replaced by one containing 100 mM K (exchanged for Na), G_t increased and both unidirectional Cl fluxes increased. The net flux of Cl was not significantly different from zero. These changes are consistent with an increased cellular Cl flux. However, to provide further evidence that the increase in unidirectional Cl fluxes occurred through a cellular pathway, furosemide (10^{-3} M) was added to the submucosal solution; furosemide inhibits electrically neutral Cl transport at the basolateral membrane (Welsh, 1983*d*). Following addition of furosemide both unidirectional Cl fluxes decreased. This contrasts with the effect of furosemide in nonsecreting tissues bathed in media of normal K content; under those conditions there

[3] With a constant K permeability, the magnitude of the depolarization produced by fixed increments in the K concentration would be expected to diminish at higher bathing solution K concentrations.

[4] These changes could also occur if G_p increased and G_b decreased. However, this explanation seems less likely since it would require that the conductance of a K-permeable membrane would decrease when the K concentration bathing that membrane increases.

Table 1. Effect of increased K concentration on transepithelial Cl fluxes[a]

	I_{sc} ($\mu A \cdot cm^{-2}$)	G_t ($mS \cdot cm^{-2}$)	J^{Cl}		
			ms	*sm*	Net
			($\mu eq \cdot cm^{-2} \cdot hr^{-1}$)		
Control	18.9	1.39	1.37	2.31	0.94
	±2.6	±0.30	±0.23	±0.51	±0.44
100 mM K	2.2*	7.57*	4.08*	4.61*	0.53
	±2.2	±1.06	±0.74	±0.89	±0.70
100 mM K and furosemide	3.2 ±1.3	5.09* ±0.88	2.59* ±0.46	2.63* ±0.57	0.04 ±0.34

[a] Tissues studied under three conditions: first, with normal Ringer's solution; second, with 100 mM K Ringer's (K substituted for Na in both bathing solutions); and third, with 100 mM K Ringer's plus furosemide (10^{-3}, submucosal solution). Indomethacin (10^{-6} M) and amiloride (10^{-4} M) were present throughout. n = seven tissue pairs. * $P < 0.05$ compared to the preceding period.

Table 2. Effect of depolarization and hyperpolarization of Ψ_b on transepithelial resistance and fractional resistance of the apical membrane in nonsecreting tissues[a]

	Ψ_b (mV)	Ψ_t (mV)	Ψ_a (mV)	R_t ($\Omega \cdot cm^2$)	f_R
Baseline	+63 ±5	+9 ±4	−54 ±4	165 ±36	0.68 ±0.09
Depolarization	+56* ±11	−30* ±3	−87* ±12	170 ±37	0.70 ±0.09
Hyperpolarization	+79* ±4	+53* ±4	−26* ±3	161 ±35	0.66 ±0.09

[a] Indomethacin (10^{-6} M, mucosal solution) was present throughout. Values were taken after passing current for 2 min. Baseline measurements were repeated after each maneuver to be sure that there was no change in membrane voltages or relative resistances. n = five tissues. * $P < 0.05$ compared to the baseline period.

is minimal if any effect (Welsh, 1983*d*). The failure of furosemide to decrease Cl fluxes back to control values may represent an incomplete inhibition of the basolateral Cl transport process (Welsh, 1983*d*; Widdicombe, Nathanson & Highland, 1983). Thus, these results provide further evidence that an increase in G_a^{Cl} is induced by the elevation of bathing solution K concentration.

Is G_b^K Voltage Dependent?

While the results presented so far clearly indicate that G_b^K can be dissociated from pump activity and transepithelial transport rate, they would be consistent with an effect of voltage on G_b^K. That is, both addition of ouabain and elevation of bathing solution K depolarize Ψ_b and increase G_b. To directly examine the possibility of a voltage-dependent ion conductance,[5] we voltage clamped Ψ_b during the response to the secretagogue, epinephrine (10^{-6} M, submucosal solution). Ψ_b was continuously measured with an intracellular microelectrode under open-circuit conditions. Then, following addition of epinephrine, sufficient transepithelial current was passed to maintain Ψ_b constant.

Figure 5*a* and 5*b* show representative examples of the electrical response to epinephrine while Ψ_b was maintained constant at its initial value. Despite clamping Ψ_b, the decrease in R_t and the biphasic changes in f_R, Ψ_a, Ψ_t and I_t (the clamping current) were not prevented. These biphasic changes in f_R and Ψ_a, and monophasic changes in R_t, are nearly identical to those observed under open circuit (*see* Fig. 8, Welsh et al., 1982) and short-circuit conditions (*see* Figs. 3 and 4, Welsh et al., 1983). Previous analysis indicates that the biphasic changes result from an initial increase in G_a^{Cl} followed by a secondary increase in G_b^K (Welsh et al., 1983; Welsh, 1983*c*; Smith & Frizzell, 1984).

These results provide direct evidence that a depolarization of Ψ_b is not *required* for the fall in G_b^K that accompanies secretion. To determine if a change in Ψ_b is *sufficient* to alter G_b^K, we passed a constant transepithelial current across the tissues, in order to hyperpolarize and depolarize Ψ_b. Sufficient current was passed to alter Ψ_t by approximately ±40 mV. Table 2 shows the results obtained in nonsecreting tissues. Neither depolarization nor hyperpolarization of Ψ_b resulted in significant changes in R_t or f_R. Likewise, depolarization and hyperpolarization of Ψ_b in secreting epithelia (Table 3) produced only minimal changes in R_t and f_R. The changes in Ψ_b under both conditions are of similar magnitude to those observed with changes in the rate of transepithelial transport. The small changes in R_t and f_R can be readily explained by both Goldman-type rectification (Schultz, 1980) at the apical and basolateral membranes and possible changes in cellular ion concentrations. Certainly, the results do not support voltage-dependent changes in G_b^K (or G_a^{Cl}), when Ψ_b is altered within the physiologic range.

[5] The term "voltage dependent" is being used in its most general sense. The author does not mean to imply any specific molecular mechanism such as an ion-gated channel.

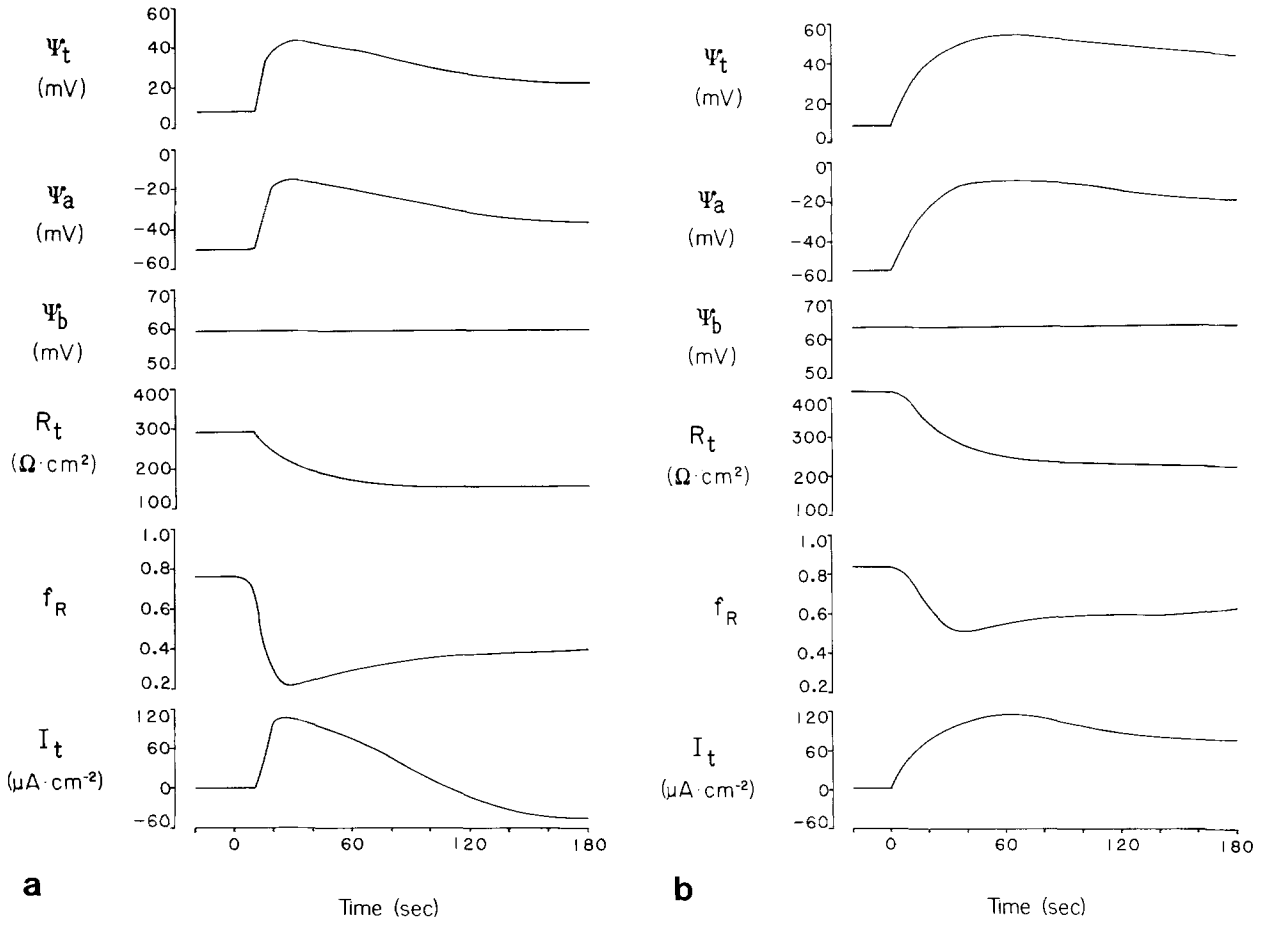

Fig. 5. Effect of voltage clamping Ψ_b on the response to epinephrine. Indomethacin (10^{-6} M, mucosal solution) was present throughout. The onset of the response to epinephrine (10^{-6} M, submucosal solution) is indicated by time zero. Ψ_b was maintained at its initial value by the passage of transepithelial current, I_t. *a* and *b* represent results obtained in two representative tissues

Discussion

Basolateral K Conductance is Independent of Na-Pump Activity

In a previous study in canine tracheal epithelium (Welsh et al., 1983), this author speculated that the direct relation between basolateral K permeability and the rate of transepithelial transport might result from coupling of the Na-K pump and the K conductance. Work in a variety of Na-absorbing epithelia has also revealed parallel changes in pump rate and G_b^K (Higgins, Gebler & Fromter, 1977; Davis & Finn, 1982; Gunter-Smith, Grasset & Schultz, 1982; Thomas et al., 1983). This direct relation has led several authors to suggest the possibility of an integral, possibly structural, link between the pump and G_b^K (Blum & Hoffman, 1971; Higgins et al., 1977; Schultz, 1981).

Table 3. Effect of depolarization and hyperpolarization of Ψ_b on transepithelial resistance and fractional resistance of the apical membrane in secreting tissues[a]

	Ψ_b (mV)	Ψ_t (mV)	Ψ_a (mV)	R_t ($\Omega \cdot cm^2$)	f_R
Baseline	+61	+18	−43	129	0.49
	±3	±4	±4	±17	±0.06
Depolarization	+42*	−25*	−67*	128	0.42
	±3	±5	±6	±17	±0.08
Hyperpolarization	+86*	+64*	−22*	137	0.57
	±5	±8	±3	±18	±0.05

[a] Epinephrine (10^{-6} M, submucosal solution) was present throughout. Values were taken after passing current for 2 min. Baseline measurements were repeated after each maneuver to be sure that there was no change in membrane voltages or relative resistances. n = five tissues. * $P < 0.05$ compared to the baseline period.

However, the results of this study clearly show that control of membrane conductances is independent of the activity of the Na-K pump or transepithelial transport in tracheal epithelium. The most direct evidence in this regard comes from the observation that ouabain, which inhibits the Na-K pump, increased G_b^K (and G_a^{Cl}) in nontransporting tissues. Thus, transepithelial transport (and pump activity) was dissociated from G_b^K. This finding suggests that changes in activity of the Na-K pump do not directly regulate cellular conductance.

The conclusion that pump activity and G_b^K are not coupled is supported by several other observations. First, submucosal furosemide inhibits transepithelial Cl secretion, and presumably indirectly decreases the activity of the Na-K pump, without altering G_b (Welsh, 1983*d*). Second, elevation of bathing solution K concentration, a maneuver that inhibits net transport (Welsh, 1983*c*) (and probably pump activity), increased G_t and decreased f_R, suggesting an increase in both membrane conductances (Fig. 2). Third, when added to maximally secreting tissues, ouabain does not alter G_t or f_R (Fig. 1*b*), suggesting that a decrease in pump activity does not mediate a decrease in G_b^K.[6] The marked contrast between the effect of ouabain in nonsecreting (Fig. 1*a*) and secreting (Fig. 1*b*) tissues clearly indicates that the activity of the Na-K pump does not control or directly govern membrane conductances. In support of this conclusion is the observation of DeWeer and Geduldig (1978) that in squid axon, direct inhibition of the Na pump with cardiotonic steroids does not alter membrane K permeability.

Basolateral K Conductance is not Regulated by Membrane Voltage

Several observations suggested that G_b^K might be regulated by membrane voltage Ψ_b. First, the sequential changes in Ψ_b and G_b^K that occur with addition of secretagogues or the Cl transport inhibitor, 9-AC, are consistent with a voltage effect. Second, results of the first part of this study (Fig. 1*a* and Fig. 4) are also consistent with the notion that a depolarization of Ψ_b might trigger a decrease in G_b^K. Finally, voltage-dependent K conductances have been observed in other epithelial cells (Garcia-Diaz et al., 1983; Maruyama et al., 1983).

However, the results of this study demonstrate that basolateral membrane voltage is not the primary factor regulating G_b^K. First, prevention of the secretagogue-induced changes in Ψ_b did not abolish the typical changes in R_t or f_R, indicating that secretagogues still increased G_b^K. Second, neither hyperpolarization nor depolarization of Ψ_b produced the changes in R_t or f_R expected for a voltage-dependent G_b^K. Thus, although it cannot be absolutely said that the basolateral K conductance is not voltage dependent, it seems clear that voltage does not play the major role in the physiologic range.

Regulation of Basolateral K Conductance

In addition to testing our two original hypotheses, the results of this study provide some insight into factors that may be involved in the control of G_b^K. For example, it seems unlikely that changes in Na, K or Cl concentrations are directly involved. First, K and Cl activities measured with ion-selective intracellular microelectrodes under short-circuit conditions do not change with stimulation of secretion (Welsh, 1983*e*,*f*). Second, while ouabain will likely increase intracellular Na activity, elevation of the bathing solution K concentration probably does not have a similar effect. Furthermore, decreasing the bathing solution Na concentration, which might decrease intracellular Na, does not increase G_t nor I_{sc} (*unpublished observation*). Third, two maneuvers which would likely have opposite effects on intracellular K, both increased the cellular conductances: ouabain probably decreases intracellular K concentration while an increase in the bathing solution K concentration probably increases the cellular K concentration.

Consideration of the results of this study in light of previous results in tracheal epithelium, indicate that under a wide variety of experimental conditions, G_a^{Cl} and G_b^K are directly related. When one membrane conductance increases, the other increases, and vice versa. This coupling of the membrane permeabilities is important for the maintenance of relatively constant intracellular ion concentrations and a negative intracellular voltage in the face of changes in the rate of transepithelial ion transport (Schultz, 1981). Although the cellular mechanisms involved are as yet unknown, the results of these studies would be consistent with regulation by intracellular Ca, intracellular pH, and/or cell volume.

[6] In contrast, in a number of Na-absorbing epithelia, inhibition of the Na-K pump with ouabain results in a decrease in G_b^K (Helman, Nagel & Fisher, 1979). The explanation for the difference is not clear, but it is possible that the decrease in Na-absorbing epithelia is a secondary event, possibly resulting from an effect of Ca on G_b^K (Schultz, 1981).

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