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Electrophysiology of Cl Secretion in Canine Trachea

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Summary. Conventional microelectrode techniques were employed to determine the mechanism of Cl secretion by canine tracheal epithelium. Epinephrine, a potent stimulator of Cl secretion in these cells, hyperpolarized both the transepithelial potential (20 to 38.9 mV) and the potential across the basolateral membrane (−63.9 to −68.2 mV) and depolarized the potential across the apical membrane (−43.9 to −29.3 mV). Epinephrine also caused a decrease in the transepithelial resistance and ratio of apical to apical+basolateral membrane resistances (777 to 379 $\Omega\,cm^2$ and 0.71 to 0.35, respectively) though the change in the latter was biphasic, first decreasing then slightly increasing.

Ionic substitutions, either Na, K or Cl, in the mucosal bathing solutions were found to cause changes in the resistances and potentials measured. In the presence of epinephrine, the changes produced by the Na and K substitutions decreased, while those produced by altering the mucosal Cl concentration increased. A model was designed to analyze the results from these experiments. When used in conjunction with the Goldman-Hodgkin-Katz equation, the results from this model indicate that epinephrine caused a large increase in the Cl permeability of the apical membrane of the cell.

From these results we conclude that the increase in Cl secretion caused by epinephrine is accompanied by at least two cellular events: the primary event is an increase in the Cl conductance of the apical membrane; the second event is either an increase in the conductance of the basolateral membrane (probably due to an increase in K permeability) or an increase in shunt conductance or a combination of both.

Key words Cl secretion · tracheal epithelium · membrane resistances · membrane potentials · ion permeabilities · Cl permeability · epinephrine

Introduction

Canine tracheal epithelia actively transport Cl from the submucosal to the luminal surface (Oliver, Davis, Marin & Nadel, 1975; Al-Bazzaz & Al-Awqati, 1979). This secretion can be increased by addition of a β-adrenergic agonist (Al-Bazzaz & Cheng, 1979; Davis, Marin, Yee & Nadel, 1979), cAMP (Al-Bazzaz, Yadava & Westenfelder, 1981), prostaglandin E_2 and $F_{2\alpha}$ (Al-Bazzaz et al., 1981) or Ca-ionophore A23187 (Al-Bazzaz & Jayaram, 1981). It can be inhibited by adding furosemide (Davis, Marin, Ueki & Nadel, 1977; Marin & Zaremba, 1979) or ouabain (Al-Bazzaz & Al-Awqati, 1979) to or removing Na from (Al-Bazzaz & Al-Awqati, 1979; Marin & Zaremba, 1979) the submucosal bathing solution.

The consistency with which Na dependence and the inhibitory effects of furosemide have been noted in a variety of Cl-secreting epithelia led to postulation of the model for Cl-secretion shown in Fig. 1 (Frizzell, Field & Schultz, 1979; Welsh, Smith & Frizzell, 1982). Its features are: (1) Cl entry across the basolateral membrane is coupled to Na entry. This is the postulated site of inhibition by furosemide. (2) As a consequence of the Na gradient, Cl accumulates in the cell above its electrochemical equilibrium, with transport across the apical membrane into the lumen as a consequence of simple diffusion.

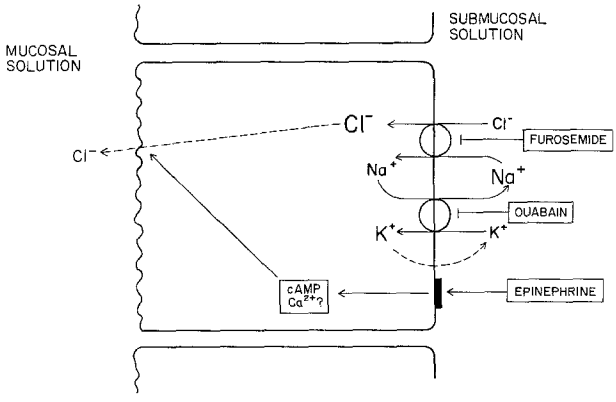

Fig. 1. Cellular model for Cl secretion. *See* text for explanation

(3) The Na entering with Cl is recycled across the basolateral membrane by the Na/K exchange pump. (4) K entering in exchange for Na diffuses back across the basolateral membrane; this K permeability increases as the Na/K pump rate increases. (5) Secretagogues (i.e., in trachea, epinephrine) stimulated Cl secretion by increasing the apical membrane's permeability to Cl.

We wished to investigate the appropriateness of the above model to Cl secretion by canine trachea. Using microelectrode techniques, we were able to determine the effects of epinephrine on cell potentials and cell membrane resistances and on apical membrane ionic permeabilities.

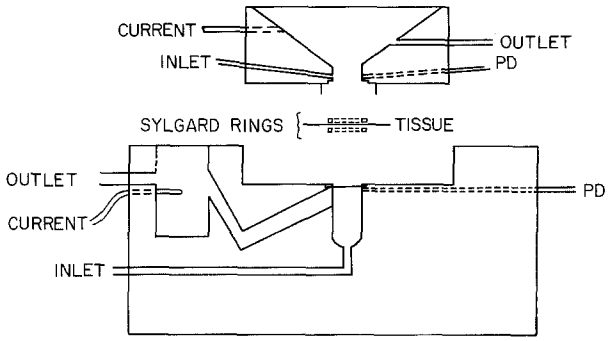

Fig. 2. Schematic representation of tissue chamber. Tissue is held in place by pins and mounted between two flexible Sylgard 184 rings. Both chambers are perfused separately and continuously. Location of current and PD bridges are as indicated. The area of exposed tissue is 0.83 cm^2. The Figure is not drawn to scale

Materials and Methods

Tracheal mucosa was isolated as previously described (Al-Bazzaz & Al-Awqati, 1979). Adult mongrel dogs, weighing more than 15 kg, were anesthetized with intravenous sodium pentobarbital (25 mg/kg). The trachea was removed from the animal in 3- to 4-cm-long segments. Each segment was opened anteriorly, pinned on a paraffin plate and bathed with standard Ringer's (for composition, *see below*), which was continuously oxygenated. The epithelium overlaying the cartilage-free portion of the posterior trachea was dissected free from all the underlying muscle and most of the underlying dense connective tissue.

The preparation was then mounted, mucosal surface up, in a Lucite® chamber (Fig. 2). To minimize damage, Sylgard 184 (Dow Corning) rings were placed between the tissue and the Lucite. The serosal surface of the tissue was supported with a nylon mesh (Small Parts, Inc., Miami, Fla.). Both bathing solutions were maintained at 37 °C and continuously changed by gravity superfusion (nonrecirculating) from oxygenated reservoirs at a flow rate of approximately 3 ml/min (about one "chamber volume" every 6 sec for the upper chamber and every 15 sec for the lower chamber). The preparation was allowed to equilibrate in the chamber for 1 to 2 hr prior to microelectrode impalements.

Solutions and Drug Procedures

The Ringer's solution used for these experiments was HCO_3- and CO_2-free; it had the following composition (in mM): 138 NaCl, 5 KCl, 2 $CaCl_2$, 1.5 NaH_2PO_4, 1.1 $MgCl_2$, 5 Hepes (N-20-hydroxyeithylpiperazine-N'-2-ethane sulfonic acid), and 5 glucose, and was titrated to pH 7.4 with NaOH. All solutions were gassed with 100% O_2. In the ion substitution experiments, both K and Na were replaced by Tris (hydroxymethyl) aminomethane (TRIS) while Cl was replaced by methane sulfonate.

Indomethacin (Sigma, St. Louis, Mo.), 1×10^{-6} M, was added to both bathing solutions at least 1 hr before impaling with microelectrodes. Indomethacin decreases the spontaneous rate of Cl secretion found in untreated tissues (Al-Bazzaz et al., 1981) but does not decrease the magnitude of Cl secretion following a maximally stimulating dose of epinephrine (Welsh et al., 1982). We observed that indomethacin significantly decreased the variability of the short-circuit current (I_{sc}) under basal conditions. When epinephrine (Sigma, St. Louis, Mo.) was used, it was added to the submucosal bathing reservoir to 2×10^{-5} M concentration. Sodium meta-bisulfite (2×10^{-5} M) was added with the epinephrine to prevent its spontaneous oxidation.

Electrical Measurements

Transepithelial PD was measured through 3 M KCl agar bridges and Ag/AgCl pellets with a high input impedence voltage follower constructed from a AD521 instrumentation amplifier (Analogue Devices). The potential of the microelectrode was monitored with a high impedence Picometric emitter-follower (Instrument Laboratories, Inc.) via a Ag/AgCl half-cell. Both potentials were observed on an oscilloscope (Tektronix 5113) and recorded by a chart recorder (Gould 220). Constant current pulses were generated by a modified pulse generator (Tektronix 160 series) and passed across the tissue through Ag/AgCl pellets and 3 M KCl bridges. The electrode configuration provided a reasonably uniform electric field in that V_{sm} varied by no more than 1.6 mV over the surface of the tissue when 250 μ amp/cm^2 were passed across the tissue, resulting in a mean change in V_{sm} of 85 mV. At flow rates used in these experiments no significant leakage from the KCl bridges could be detected by flame photometry (maximum recorded change in three experiments was 2%). The effect of K leakage was undoubtedly minimized by our use of a nonrecirculating flow system.

Microelectrodes were pulled from 1.2 mm o.d. fiber-filled capillary tubes (W-P Instruments, New Haven, Conn.) with a modified two-stage vertical puller (David Kopf Instruments). Immediately prior to use they were filled with 3 M KCl and had resistances of 25 to 50 MΩ in Ringer's solution. Tip potentials were less than 10 mV.

Transepithelial resistance (R_t) was calculated from the transepithelial voltage deflections (ΔV_{sm}) in response to biphasic, one-second, transepithelial current pulses of less than 60 μA/cm^2 (I). The short-circuit current (I_{sc}) was calculated by dividing the open circuit, spontaneous transepithelial potential (V_{sm}) by the calculated transepithelial resistance (R_t). This was possible because it has been found that for current densities of less than 100 μ amp/cm^2, the I vs. V_{sm} plot for the tissue is linear.

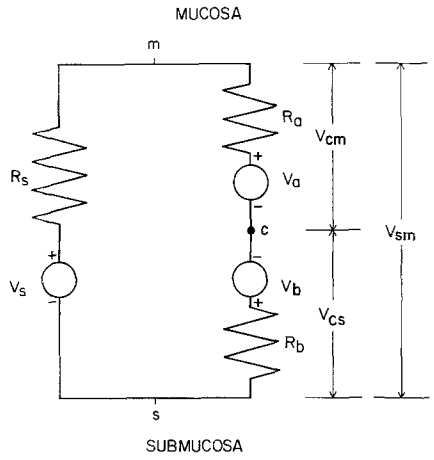

Fig. 3. Lumped-parameter equivalent circuit model of the epithelium. *m*, *c*, and *s* represent the mucosal solution, cell, and submucosal solution, respectively. R_a, R_b and R_s represent the resistances and V_a, V_b, and V_s the electromotive forces (emf's) of the apical membrane, basolateral membrane and shunt, respectively. Polarities of batteries are as indicated. V_{cm}, V_{cs} and V_{sm} are the measured potentials. *See* text for further explanation

The potential measurements reported in this paper are defined as follows: V_{sm} is the potential of the submucosal solution referenced to the mucosal solution. V_{cm} is the potential of the cell referenced to the mucosal solution and V_{cs} is the potential of the cell referenced to the submucosal solution.

Impalements were performed from the mucosal side with mechanical micromanipulators under visualization with a dissecting microscope (Wild, Switzerland). The criteria used to determine a successful impalement were as follows:

1) Upon impaling the cell, the potential changes must be abrupt.

2) The intracellular potential recorded by the microelectrode must reach a stable value (± 2 mV) for at least 30 sec and this value must be no more than the initial deflection.

3) The voltage divider ratio ($\Delta V_{cm}/\Delta V_{sm}$) (*see below*) must be stable and measurably different from its value when the microelectrode is in either bathing solution.

4) Upon withdrawal of the electrode, the potential change must be abrupt and return to within 2 mV of its value prior to the impalement.

Model Analysis

The data were analyzed according to the lumped parameter, equivalent circuit model shown in Fig. 3. The equations used to characterize this model are as follows:

$$R_t = \frac{(R_a + R_b) R_s}{R_a + R_b + R_s} \tag{1}$$

$$\beta = \frac{R_a}{R_a + R_b} \tag{2}$$

$$V_a = -V_{cm} - (-V_{sm} + V_s)\frac{R_a}{R_s} \tag{3}$$

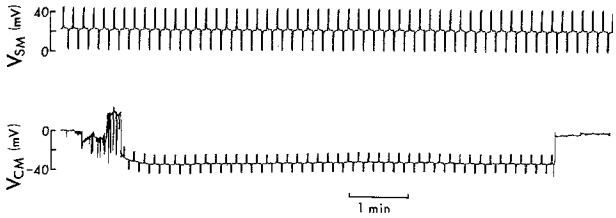

Fig. 4. A representative intracellular recording from an epinephrine-treated tissue is shown in the lower trace. The upper trace is the corresponding transepithelial recording. The periodic potential deflections in both traces were due to the passage of current ($\pm 50\,\mu$amp) across the tissue. Nonstable potentials occurred as the electrode was advanced into the tissue. Cell impalement occurred with a sharp negative potential deflection which stabilized at -32 mV. The impalement was lost spontaneously. When the electrode was completely withdrawn from the tissue (25 sec later), the potential returned to within 1 mV of its value prior to the impalement attempt

$$V_b = -V_{cs} + (V_{sm} + V_s)\frac{R_b}{R_s}. \tag{4}$$

V_{cm}, V_{cs} and V_{sm} are the measured potentials described above and β is the voltage divider ratio which is determined from $\Delta V_{cm}/\Delta V_{sm}$ when current is passed across the tissue. V_a, V_b and V_s represent the electromotive forces (emf's) in each of the equivalent circuit elements, with the polarities indicated in Fig. 3. R_a, R_b and R_s represent the equivalent resistances of these elements.

All values reported are means $\pm$ standard errors (paired analysis used where appropriate).

Results

Baseline Recordings

A representative intracellular recording is shown in Fig. 4. The tissue had been previously treated with epinephrine. The cell maintained a potential of -32 mV relative to the mucosal bath until the impalement was spontaneously lost. The voltage divider ratio (β) was 0.45, suggesting that R_a was only slightly less than R_b. The slow potential change seen in the beginning of the impalement (-26 to -32 mV) was often seen and may have been due to sealing of the cell membrane around the microelectrode tip.

In all cases, when an electrode was advanced through the tissue a two-step potential profile was observed. Under baseline conditions (87 punctures in 24 tissues), the transepithelial potential (V_{sm}) averaged 21.3 ± 1.0 mV (mucosal reference), the transapical potential (V_{cm}) averaged -41.2 ± 1 mV (mucosal reference), and the transbasal potential (V_{cs}) averaged -62.8 ± 0.8 mV (serosal reference). Since V_{cs} varied

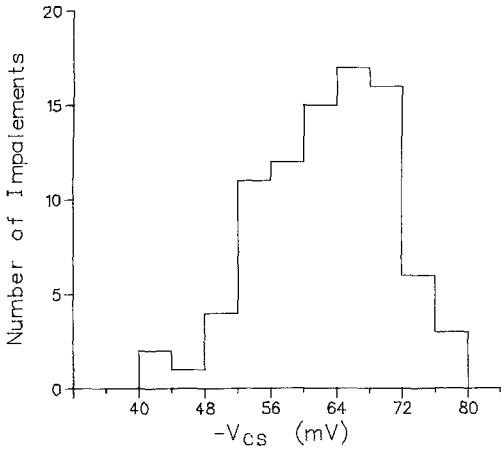

Fig. 5. Distribution of $-V_{cs}$ from 87 impalements in 24 control tissues

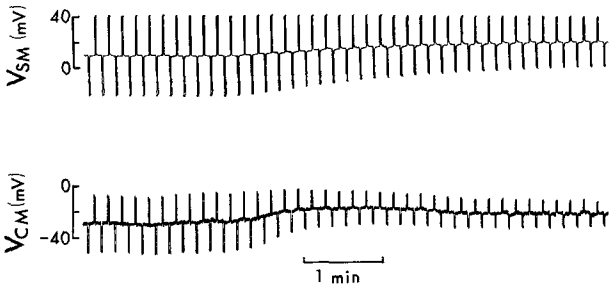

Fig. 6. Sample recordings during the time course of an epinephrine response. The transepithelial recording is shown in the upper trace, the intracellular one in the lower trace. Epinephrine (2×10^{-5} M) was administered to the submucosal reservoir prior to the recordings shown. Due to the delay in the flow apparatus, the exact time that the drug reached the tissue cannot be determined

Table 1. Steady-state effects of epinephrine on tissue parameters[a]

	V_{sm} (mV)	V_{cm} (mV)	V_{cs} (mV)	R_t ($\Omega\,cm^2$)	I_{sc}[b] (μamp/cm^2)	$\frac{R_a}{R_a+R_b}$
control	20.0 ±1.5	−43.9 ±1.8	−63.9 ±1.3	777 ±81	28.7 ± 3.1	0.71 ±0.03
epinephrine	38.9[c] ±1.9	−29.3[c] ±1.5	−68.2[c] ±1.7	379[c] ±55	126.5[c] ±15.7	0.35[c] ±0.03

[a] ±SEM; $n=17$. [b] I_{sc} calculated as described in Materials and Methods. [c] $P<0.01$ for paired data.

less among tissues with differing I_{sc}'s than did either V_{sm} or V_{cm}, its distribution is shown in Fig. 5. This distribution appears to be unimodal.

Response to Epinephrine

The increase in short-circuit current caused by epinephrine was found to be relatively stable, declining only 10% over 1 hr and 40% over 3 hr. Therefore it was possible to examine the effect of epinephrine on each tissue by averaging the results of multiple impalements. These experiments are summarized in Table 1. The almost fivefold increase in I_{sc} was associated with both an increase in V_{sm} and a decrease in R_t. V_{cm} depolarized by 14.6 mV whereas V_{cs} hyperpolarized by 4.3 mV. The voltage divider ratio decreased to about 50% of its initial value, indicating a large decrease in R_a relative to R_b.

A more ideal way to study the action of epinephrine on this epithelium is to record from a single cell during the complete time course of the drug response. This proved difficult, however, because impalements could seldom be maintained for a long enough time. Three such recordings were obtained and the results for one are shown in Fig. 6. Upon addition of epinephrine, the I_{sc} increased from 19.4 to 59.1 μamp/cm^2. V_{cm} depolarized from −38 to −27 mV and then slowly repolarized to −31 mV. In both traces, the deflections in potential produced by the injection of current decreased monotonically with time. In Fig. 7, values for the ratio of membrane resistances ($\alpha = R_a/R_b$), R_t and I_{sc}, all calculated from Fig. 6, are shown as functions of time. The change in α is biphasic, first decreasing from 2.88 to 1.25 and then increasing again to 1.75. During the same time period R_t decreases monotonically from 514 to 315 $\Omega\,cm^2$. When taken in the context of a continuous decrease in R_t, the delayed increase in α suggests that there is a slow or delayed decrease in R_b as well as a more rapid decrease in R_a. Similar results have been obtained by Welsh et al. (1982).

Ion Selectivity of Apical Membrane

In order to examine the nature of the epinephrine-induced change in apical membrane permeability, experiments were performed in which the intracellular potential was monitored during changes in the ionic composition of the mucosal bathing solution. Recordings and solution changes were made before and after add-

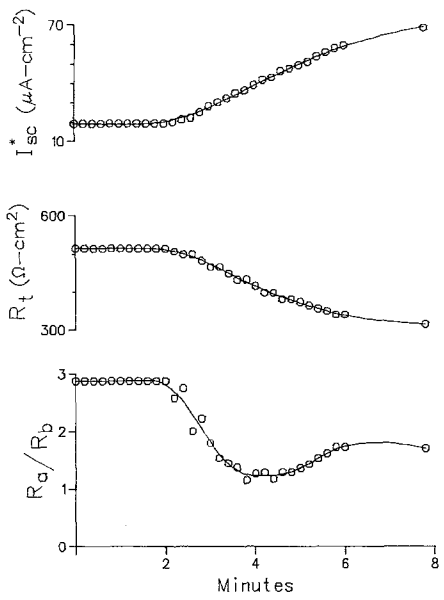

Fig. 7. Responses of tissue parameters to epinephrine. Values shown are derived from the experiment illustrated in Fig. 6. $*I_{sc}$ calculated as described in Materials and Methods

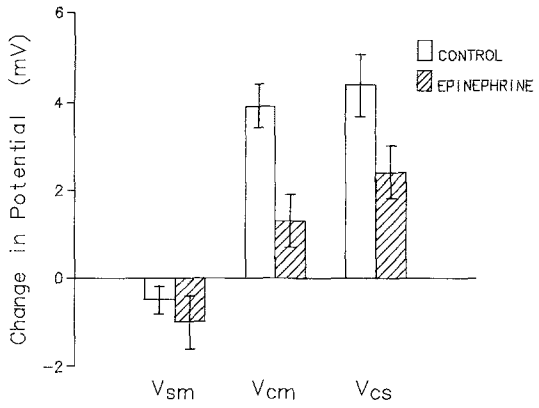

Fig. 8. K^+ substitution. Changes in cell potentials due to increasing [K] from 5 to 50 mM ([TRIS] decreasing reciprocally) both in presence and absence of epinephrine. Results are for nine control and five epinephrine-treated tissues. Error bars indicate $\pm$SE (paired analysis)

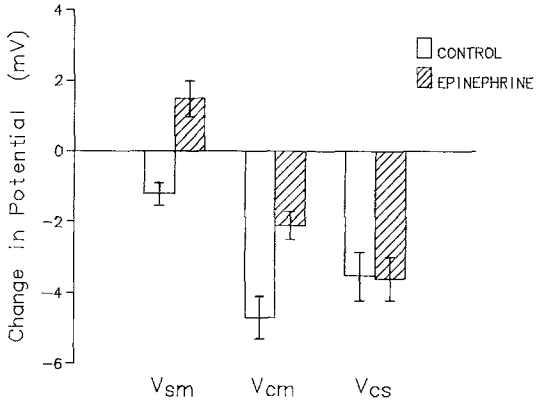

Fig. 9. Na^+ substitution. Changes in cell potential due to decreasing mucosal [Na] from 145 to 20 mM ([TRIS] increased reciprocally). Error bars indicate $\pm$SE (paired analysis). Results are for eight control and six epinephrine-treated tissues

ing epinephrine. Results are shown in Figs. 8 through 10. Corrections were made for the junctional potentials that develop in the reference electrodes in response to the solution change[1].

Figure 8 shows the changes in the cell potentials that occurred in both epinephrine-altered and control states when [K^+] in the mucosal bathing solution (modified to contain 45 mM TRIS and 100 mM Na) was increased from 5 to 50 mM ([TRIS] decreased reciprocally, [Na] kept constant). In the control state, the the cell depolarized about 4.0 mV relative to either bathing solution. Although not shown, R_t and β both decreased slightly ($16 \pm 4\,\Omega\,cm^2$ and 0.02 ± 0.006, respectively). In the presence of epinephrine, the K^+-dependent change in V_{cm} was only 1.3 ± 0.6 mV and there were no detectable changes in R_t and β. The change in V_{cs} also appears to have been less than in the control state although this difference is not statistically different from zero (paired analysis). In these experiments, the lower [Na] may have been responsible for the lower value of V_{sm} in the control state (12.9 ± 2 mV) and for the diminished response to epinephrine (I_{sc} increased to $47.3 \pm 4.3\,\mu$amp/cm^2, V_{sm} to 24.5 ± 2 mV and V_{cs} to -59.5 ± 2 mV; β decreased only to 0.50 ± 0.04; the other parameters were unaffected).

The potential changes that were observed when mucosal [Na] was decreased from 145 to 20 mM ([TRIS] increased reciprocally) are shown in Fig. 9. Epinephrine decreased the change in V_{cm} (-4.7 to -2.1 mV), reversed the change in V_{sm} (-1.6 to 1.5 mV) but had no effect on the change in V_{cs}. Reducing [Na] from 145 to 20 mM had little effect on R_t, increasing it from 642 to $690\,\Omega\,cm^2$ in the absence of epinephrine. In the presence of epinephrine, altering mucosal Na had no effect on R_t (287 to $292\,\Omega\,cm^2$).

Finally, Fig. 10 shows the effect of decreasing [Cl] in the mucosal bath from 146 to 49 mM (replacement with methane sulfonate). In the control state, this solution change hyper-

[1] The junction potentials that developed in the reference bridges were measured using a flowing-junction calomel reference electrode. The measured potentials were 0, -1 and 3.5 mV for the K, Na and Cl substitutions, respectively.

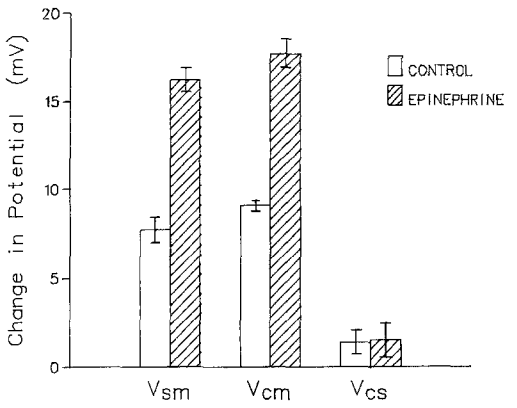

Fig. 10. Cl^- substitution. Change in cell potential due to decreasing mucosal [Cl] from 146 to 49 mM ([methane sulfonate] increased reciprocally). Error bars indicate $\pm$SE (paired analysis). $n=7$ for both control and epinephrine-treated states

polarized V_{sm} (21.5 to 29.2 mV) and depolarized V_{cm} (-44.5 to -35.4 mV). Although not shown, β increased by 0.029 ± 0.004 and R_t increased $68 \pm 9\,\Omega\,cm^2$. Epinephrine magnified each of these changes: V_{sm} hyperpolarized by 16.2 ± 0.7 mV, V_{cm} depolarized by 17.7 ± 0.8 mV, and β increased by 0.053 ± 0.008 mV. No change in V_{cs} was detected in either the absence or presence of epinephrine.

Discussion

Validation of Techniques

Microelectrode measurements in tracheal epithelium are subject to the usual uncertainties due to possible impalement artifacts and to not knowing the cell type impaled. Possible impalement artifacts include alterations in tip potentials upon penetration, damage to the cell membrane penetrated (Lindemann, 1975) and changes in the intracellular ion composition due to KCl leak from the electrode (Nelson, Ehrenfeld & Lindemann, 1978). To minimize these errors, strict criteria, described in Materials and Methods, were used to define a successful impalement. If the tip potential was altered by more than 2 mV the recording was discarded. The recording was also required to be stable at a value no less than the original instantaneous deflection. Even though only 30 sec of stability were required, occasional cells in which impalements were maintained for minutes to over 1 hr yielded constant values. Among "successful" impalements, therefore, cell membrane damage is unlikely.

It has also been reported that significant leak of KCl occurs from electrodes filled with 3 M KCl and that this can give rise to artifacts due to altered intracellular ion composition (Nelson et al., 1978; Fromm & Schultz, 1981). In both reports these effects were minimized when the electrode was filled with 0.5 M KCl. Although we used 3 M KCl, the stability of our cell recordings suggests that K leakage was not a significant problem. This is not to say that KCl leak out of our electrodes was insignificant but that, if it did occur, low resistance connections between cells were able to shunt the leaked KCl, maintaining the cell's ion composition constant.

The canine tracheal epithelium contains at least three basic cell types (ciliated columnar, goblet, and basal cells; Widdicomb, Basraum & Highland, 1981). Although we cannot be absolutely certain as to which cell type we impaled, the unimodal distribution of $-V_{cs}$ (Fig. 5) and the uniformity of the responses to epinephrine suggest that we impaled the cell type of interest with respect to Cl secretion. Since the columnar epithelial cells comprise a majority of the mucosal volume, we assume that these are the cells impaled.

Localization of the Effect of Epinephrine on Cell Membrane Resistances

If the model for Cl^- secretion previously proposed is correct, then stimulation of secretion with epinephrine should be accompanied by a decrease in resistance of the apical membrane. The results shown in Table 1 and Fig. 7 support this idea. Epinephrine caused a large decrease in both R_t and β. If one analyzes these results with the assumption that R_a does not decrease, then one must postulate that either V_b is greater than 150 mV in the epinephrine-stimulated state or that R_s decreases from more than 30,000 to $500\,\Omega\,cm^2$ upon epinephrine treatment[2]. Since both of these possibilities seem unlikely, we conclude that R_a must decrease after the addition of epinephrine.

[2] To determine the limits stated, the analysis was performed assuming that R_a remained constant (assuming R_a increases makes it even worse). A near minimum value for R_a ($565\,\Omega\,cm^2$) leads to a calculation for V_b (epinephrine) equal to 150 mV and for a change in R_s upon epinephrine treatment from about $30{,}000\,\Omega\,cm^2$ to about $500\,\Omega\,cm^2$. Higher values for R_a generate smaller changes in R_s but larger values for V_b (epinephrine). Lower values for R_a decrease V_b (epinephrine) but cause even larger changes in the shunt resistance. Eqs. (1)–(4) were used to arrive at these limits.

Table 2. Steady-state responses to epinephrine and corresponding resistances calculated on the assumption that epinephrine alters only R_a

Experiment No.	Measured				Calculated			
	R_{T_1}	R_{T_2}	β_1	β_2	R_b	R_s	R_{a_1}	R_{a_2}
1	584	330	0.46	0.17	220	−1348	187	45*
2	799	495	0.62	0.35	350	5930	571	189
3	1482	1191	0.62	0.55	424	−4541	693	519*
4	365	149	0.705	0.43	69	− 664	166	52*
5	857	246	0.89	0.40	168	1945	1364	112
6	664	332	0.91	0.40	339	806	3424	225
7	437	253	0.55	0.25	181	−4790	220	60*
8	1018	415	0.82	0.54	197	15383	894	231
9	797	327	0.88	0.27	337	1111	2478	125
10	739	307	0.86	0.30	295	1138	1812	127
11	485	230	0.68	0.16	227	1531	482	44
12	1011	482	0.74	0.58	148	−1294	420	204*
13	813	382	0.67	0.39	201	−2447	409	128*
14	448	291	0.80	0.29	423	569	1692	173
15	1038	359	0.71	0.15	308	49459	753	54
16	583	303	0.49	0.26	145	− 554	139	51*
17	1082	359	0.49	0.46	123	− 627	273	105*
average	777	379	0.71	0.35	266	5090	651	143

Subscripts 1 and 2 indicate before and after epinephrine treatment. Resistances in $\Omega\,cm^2$.
* Negative resistances calculated for R_s.

If we assume that epinephrine affects only R_a, and not R_b or R_s, we can use Eqs. (1) and (2) to calculate R_a, R_b and R_s for both control and epinephrine-stimulated states. The results of applying this analysis to the data are shown in Table 2. In eight out of the 17 experiments, the model generated a negative value for one of the derived resistances, suggesting that the assumption that epinephrine affects only R_a is incorrect. In fact, no model in which epinephrine affects only one cell resistance can explain the results.

The conclusion that epinephrine affects more than one cell parameter is supported by Fig. 7. The increase in R_a/R_b that develops toward the end of the response to epinephrine, while R_t is still decreasing, indicates that at least one resistance other than R_a must be altered. The increase in R_a/R_b could be due either to a secondary increase in R_a accompanied by a decrease in R_s or to a decrease in R_b. In the latter case, no alteration in R_s need be stipulated. Although we have presented no evidence to support or refute either possibility, a decrease in R_b is more in keeping with the model for Cl secretion proposed earlier. If the main pathway for K^+ to exit the cells is by diffusion across the basolateral membrane, then, when secretion is increased and thus the Na/K pump rate is increased, a parallel decrease would have to occur in R_b to prevent K^+ accumulation by the cell. This mechanism for cell K homeostasis has been proposed to occur in other ion transporting epithelia (*see* Schultz, 1981). Although the mechanism of this change in K^+ permeability of the basolateral membrane is not clear, one possibility is dependent on an increase in intracellular Na^+. This increase might alter Na/Ca exchange to raise intracellular Ca^{2+}, which would be expected to increase K^+ permeability (Meech, 1976).

Ionic Properties of the Alteration in R_a

The experiments testing the effects of changes in mucosal-solution ion concentrations appear, superficially at least, to support the hypothesis that epinephrine causes a selective increase in apical membrane Cl permeability. Epinephrine decreased the effect on V_{cm} of changes in [Na] and [K] but increased the effect on V_{cm} of a change in [Cl]. However, the ion permeability properties at the apical membrane are better reflected by changes in V_a, not V_{cm}. Since current is permitted to flow intra-epithelially, V_{cm} may differ from V_a according to the following equation:

$$-V_{cm}=\frac{V_a(R_b+R_s)+(V_b+V_s)R_a}{R_a+R_b+R_s}. \tag{5}$$

Because changes in V_{cm} can result from changes in V_b, V_s or any of the various resistances as well as from changes in V_a, to establish actual apical membrane permeability changes we must estimate the effect of ion substitutions on V_a itself.

To accomplish this, we applied our data to the equivalent circuit shown in Fig. 3, making the following assumptions: (1) $V_s=0$ when identical solutions bathe both sides of the tissue, and (2) R_b and V_b do not change within the time required (usually less than 1 min)[3] to make the measurements following a change in the mucosal bathing solution. Using these assumptions, the validity of which we will discuss later, along with Eqs. (1)–(4), it can be shown that:

$$R_{s_1}=\frac{R_{t_1}R_{t_2}[-V_{sm_1}(1-\beta_1)+(V_{sm_2}+V_{s_2})(1-\beta_2)-V_{cs_1}+V_{cs_2}]}{(V_{cs_2}-V_{cs_1})R_{t_2}-(V_{sm_2}+V_{s_2})[R_{t_1}(1-\beta_1)-R_{t_2}(1-\beta_2)]} \tag{6}$$

where subscripts 1 and 2 indicate the value of the parameter before and after the solution change, respectively. Since all parameters except R_{s_1} and V_{s_2} in this equation have been experimentally determined, we calculated R_{s_1} and then all of the model's other parameters as functions of V_{s_2}[4]. In this way we determined a range of possible values for ΔV_a (the change in V_a due to the change in solution). The range was limited at one extreme by the fact that V_{s_2} should be no larger than the theoretical limit for the solution change as expressed by the Nernst potential, and at the other extreme by a point where negative values for R_a, R_b or R_s were generated.

Since in these experiments, the ionic composition of the mucosal bathing solution was altered by one ion at a time, an ion-dependent partial potential ratio (T_i) can be calculated as defined by Strickholm and Wallin (1967), i.e.:

$$T_i=\frac{\Delta V_a}{\frac{RT}{F}\ln\frac{C_1}{C_2}} \tag{7}$$

where C_1 and C_2 are the concentration of ion (i) in the two mucosal bathing solutions, respectively. R, T and F have their usual meaning. In this way, experiments involving different magnitudes of changes in ion concentration can be compared since the T_i's represent the changes in V_a's normalized by the amount of change that would be expected if the membrane were perfectly permselective for the ion. The range of T_i's calculated from the ranges of ΔV_a's are shown in Fig. 11. The error bars indicate the range of possible T_i's and the columns are drawn to the midpoint of each range. At the midpoint of the ranges, the ratios T_{Cl}/T_K and T_{Na}/T_K are 3.3 and 1.6 in the control state and 15.4 and 1.0 in the epinephrine-stimulated state. Although the relation between T_i and the permeability coefficient (P_i) is complex (i.e., *see* Eq. 8) the results nonetheless suggest that epinephrine increases the relative Cl permeability of the apical membrane.

The above calculations were based on the two assumptions mentioned previously. The first assumption, that $V_{s_1}=0$, rests on the premise that the shunt is a passive element that does not generate a potential when bathed on both sides by identical solutions. Even if a spontaneous shunt potential as large as ± 10 mV was present, however, it would have very little effect on the calculated T_i's (if $V_{s_1}=+10$, 0 or -10, T_{Cl} would equal 0.75, 0.74 or 0.73, respectively). Reuss and Finn (1975) came to the same conclusion for *Necturus* gallbladder.

The assumption that V_b and R_b remain con-

[3] The less than 1 min value was the time between turning the valve to switch solutions and the peak of the potential changes that occurred. We could not be sure when the new solution reached the tissue, because of dead space in the flow system. However, if one considers the onset of the potential changes as a reflection of the moment the new solution reached the tissue, then the potential change was completed within 10 to 20 sec.

[4] A computer program was generated that picked a value for V_s and then using Eq. (6) calculated a corresponding value for R_{s_1}. Using these values and the following equations:

$$R_{s_2}=\frac{R_{s_1}R_{t_1}R_{t_2}(1-\beta_1)}{R_{s_1}R_{t_1}(1-\beta_1)+R_{t_1}R_{t_2}(1-\beta_2)-R_{s_1}R_{t_2}(1-\beta_2)}$$

and

$$R_b=\frac{R_{s_2}R_{t_2}(1-\beta_2)}{R_{s_2}-R_{t_2}}=\frac{R_{s_1}R_{t_1}(1-\beta_1)}{R_{s_1}-R_{t_1}}$$

values for R_{s_2} and R_b were calculated. R_{a_1}, R_{a_2}, V_b, V_{a_1} and V_{a_2} were then calculated from Eqs. (1) through (4) using the appropriate values for R_{s_1} and R_b. The computer then systematically varied the value for V_{s_2} and recalculated all of the above parameters. The change in V_a due to the solution change ($\Delta V_a=V_2-V_1$) was then tabulated for each value of V_{s_2}.

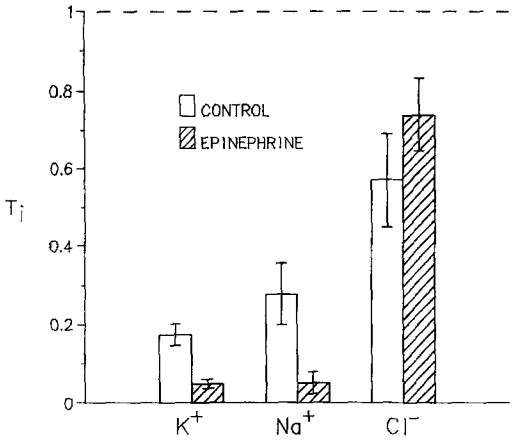

Fig. 11. Ion-dependent partial potential ratios (T_i's) calculated for each ion in the absence and presence of epinephrine. Brackets indicate the ranges of calculated values; columns are drawn to the midpoints of these ranges. *See* text for further explanation

stant is difficult to prove but is of little consequence with respect to the results generated by the model. If V_b was allowed to vary by as much as 10%, the value of T_{Cl} in the normal state would change from 0.57 to 0.52 while the value of T_{Cl} in the epinephrine-stimulated state would change from 0.74 to 0.87. Similarly, if R_b were allowed to decrease by as much as 50%, T_{Cl} would change to 0.65 and 0.82 in the normal and epinephrine-stimulated states, respectively. The effects of variation of V_b and R_b on the ion-dependent partial potential ratios for K and Na (T_K, T_{Na}) are even less than those exhibited for Cl. Therefore alterations in V_b or R_b, unless very large, would have little effect on the conclusions of this analysis.

An implicit assumption used when applying Eq. (7) is that the intracellular ionic composition remains constant during the time required to measure the potential response. Although our measurements were obtained in a short period of time[3] and the potential responses were stable in almost all experiments until the bathing solution was reversed, there is no direct evidence to support this assumption. Because the potential response occurred quickly, the magnitude of the error introduced by this assumption is not likely to be large and the ion-dependent partial potential ratios and the effect of epinephrine on them should at least be qualitatively correct.

Estimation of Permeability ratios

Although a permeability coefficient P_i can be related to T_i by the equation:

$$P_i = \frac{RTT_iG_a}{Z^2F^2\bar{c}} \tag{8}$$

where $\bar{c}$ is the mean ion concentration and G_a = the total "chord" conductance of the apical membrane [Frömter, Müller & Wick, 1971; *see* Helman (1981) or Thompson, Suzuki & Schultz (1982) for a discussion of "chord" conductance]. This equation cannot be used to calculate the permeability ratios for P_{Na}/P_K and P_{Cl}/P_K for two reasons: First, G_a, which represents the total "chord" conductance of the apical membrane is simply not known. Second, Eqs. (1) through (4) which were used to calculate the ΔV_a's (*see* p. 108) assume that the apical membrane is a linear resistor. However, in the derivation of T_i, it is implicitly assumed that the membrane can be modeled by the Goldman-Hodgkin-Katz (GHK) constant field equation whose I–V relationship is, except in unusual circumstances, nonlinear. Therefore the ΔV_a's may not correspond to the true emf's of the apical membranes. (Although from the analysis described below, they are found not to be much in error.)

It is possible, however, to estimate the values of the ratios P_{Na}/P_K and P_{Cl}/P_K by fitting the ΔV_a's calculated by the model (*see* p. 108) to the changes in potentials calculated from the slope-intercepts of I–V plots generated by the GHK equation under conditions identical to those of the experiments. In other words, GHK equation-derived I–V plots were generated for each solution change experiment, the slopes of the plots were estimated at the measured membrane potentials and the intercepts of these lines with the X-axis ($I=0$) were calculated. The changes in these potentials (intercepts) were then compared to the ΔV_a's generated by the computer model for similar experimental conditions. The values of the ratios used in the GHK equation were then altered until the best fit with the calculated ΔV_a's was found.

To perform this type of analysis, one needs to estimate the activity of the ions inside the cell. Smith and Frizzell (1982) have reported intracellular K activities (a_{K_i}) of 66 and 52 mM in control and epinephrine-stimulated tissues, respectively. Initial results in our laboratory indicate that in the control state intracellular chloride activity (a_{Cl_i}) is approximately 46 mM (*unpublished experiments*).[5] Using these values,

[5] This value is comparable to the value measured by ^{36}Cl technique (50 mM) in isolated tracheal cells (Widdicombe et al., 1981); in those experiments, $a_{Cl_i}=37.5$ if an activity coefficient of 0.75 is assumed.

the ΔV_a's calculated from the potential changes in unstimulated tissues observed following mucosal bathing solution alterations were best fit by permeability ratios of $P_{Na}/P_K = 0.48$ and $P_{Cl}/P_K = 1.37$ and an intracellular Na activity (a_{Na_i}) of 10 mM.[6]

In the epinephrine-stimulated state a problem occurs in that there is as yet no good estimate for a_{Cl_i}. If the model for secretion outlined in Fig. 1 is correct, then the gradient for Cl to leave the cell should either stay the same or decrease upon stimulation of secretion with epinephrine. Therefore, if 79.8 mM is taken as a maximal estimate of a_{Cl_i} then the experimental data is best fit when $P_{Na}/P_K = 0.33$ and $P_{Cl}/P_K = 3.4$[6] (the model is relatively insensitive to changes at a_{Na_i} from 10 to 30 mM), indicating at least qualitatively a large increase in relative Cl permeability of the apical membrane upon stimulation of secretion with epinephrine. In fact, one would have to postulate that a_{Cl_i} increases to greater than 200 mM if P_{Cl}/P_K does not increase.

Though the model used to calculate ΔV_a yielded a range of possible values rather than a unique solution and therefore the above permeability ratios should only be regarded as qualitatively correct, we feel this data and analysis strongly support the conclusion that P_{Cl}/P_K for the apical membrane is increased by epinephrine.

With respect to the effects of epinephrine on the potential profile of the tissue, our results are similar to those of Welsh et al. (1982), differing in only two ways: (1) our values for R_t were consistently larger than theirs, due probably to either greater stretch of our tissues when mounting or to less edge damage resulting from our use of Sylgard rings in mounting the tissue and also our use of a chamber with a considerably larger inner diameter; and (2) we found that epinephrine, along with its other effects, caused a small but statistically significant hyperpolarization of V_{cs}. The latter might have been more readily discerned by us than by Welsh et al. (1982), because of the apparently higher values of R_s in our study. As can be seen from Eq. (4), as R_s increases, V_{cs} more closely approximates V_b. There are two reasons why V_b might be expected to hyperpolarize when secretion is stimulated. First, at increased rates of Cl secretion, the rheogenic Na/K pump would be working at a faster rate. Second, if the basolateral membrane is permeable to one or more ions other than K under basal conditions, an increase in P_K would result in hyperpolarization. An increase in P_K in conjunction with the stimulation of Cl secretion is consistent with the general model for secretion outlined in the Introduction. Both of these possibilities are speculative and need to be evaluated by other experimental ways of determining V_b.

In conclusion, we have found that epinephrine stimulates active Cl secretion in tracheal epithelium primarily by increasing the Cl conductance of the apical membrane as reflected by the decrease in R_a and the increase in the P_{Cl}/P_K ratio. Epinephrine also causes alterations in other cell parameters, one of which probably is a decrease in R_b. Finally, the data presented here are consistent with the model for Cl secretion proposed earlier. Further analysis of this model will require determinations of intracellular ion activities and application of a technique for resolving unique values for each circuit element in the model's electrical analog.

[6] If one defines an adequate fit as a deviation of no more than 2 mV for only one of the three ΔV_a's from the solution change experiments, then control tissues can be adequately fit with a P_{Cl}/P_K between 1.3 and 1.4, a P_{Na}/P_K between 0.4 and 0.5, and with an a_{Na_i} of less than 15 mM. Epinephrine-stimulated tissues could be adequately fit with a P_{Cl}/P_K between 3.0 and 4.0 and a P_{Na}/P_K between 0.3 and 0.35.

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