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The cellular mechanism of active chloride secretion in vertebrate epithelia: studies in intestine and trachea

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The cellular mechanism of active chloride secretion, as it is manifested in the intestine and trachea, appears to possess the following elements: (1) NaCl co-transport across the basolateral membrane; (2) Cl^- accumulation in the cell above electrochemical equilibrium due to the Na^+ gradient; (3) a basolateral Na^+–K^+ pump that maintains the Na^+ gradient; (4) a hormone-regulated Cl^- permeability in the apical membrane; (5) passive Na^+ secretion through a paracellular route, driven by the transepithelial potential difference; and (6) an increase in basolateral membrane K^+ permeability occurring in conjunction with an increase in Na^+–K^+ pump rate. Electrophysiological studies in canine trachea support this model. Adrenalin, a potent secretory stimulus in that tissue, increases apical membrane conductance through a selective increase in Cl^- permeability. Adrenalin also appears to increase basolateral membrane K^+ permeability. Whether or not adrenalin also increases paracellular Na^+ permeability is unclear. Some of the testable implications of the above secretion model are discussed.

INTRODUCTION

Our purpose here is to discuss cellular mechanisms of Cl^- secretion by vertebrate epithelia as these are manifested in intestine and trachea. Although neither is primarily thought of as a secretory epithelium, secretion plays a crucial role in their normal functioning and each has a large secretory capacity.

PHYSIOLOGICAL AND PATHOLOGICAL ASPECTS OF ACTIVE SECRETION OF Cl^- IN INTESTINE AND TRACHEA

In the small intestine, this secretory capacity is dramatically evident in the disease cholera (Field 1978). An adult with cholera may excrete per rectum more than 1 l of fluid per hour. The diarrhoeal fluid contains very little protein and no blood or inflammatory cells. Provided that the patient's fluid losses are adequately replaced, intestinal biopsy reveals no evidence of epithelial damage, even at an ultrastructural level, and this morphological judgement is reinforced by demonstrations that nutrients (sugars, amino acids, oligopeptides, lipids, vitamins, etc.) are absorbed normally. The addition of easily absorbed nutrients such as glucose to oral replacement solutions has made it possible, in the vast majority of cases, to replace diarrhoeal fluid losses without recourse to intravenous solutions.

We know a great deal about the mechanisms by which *Vibrio cholerae* elicit water and electrolyte secretion in the small intestine (Fishman 1979; Moss & Vaughan 1979); we present here the briefest possible synopsis of these events. Cholera is truly an active transport disease. The bacteria release a protein which, through one of its subunits, binds tightly to a single, ubiquitous glycolipid receptor in the intestinal epithelial cell membrane, the so-called M1 ganglioside.

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Consequent on surface binding, another subunit of cholera toxin, an ADP ribosylase, enters the lipid phase of the cell membrane and gains access to the cell interior. There it covalently bonds the ADPR moiety of NAD to a particular protein acceptor, the GTP-dependent regulatory subunit of the plasma membrane's adenylate cyclase complex. The covalent bond blocks the GTPase activity in the cyclase complex, thereby 'freezing' the cyclase in its GTP-bound, active state. The resulting cellular accumulation of cyclic AMP (cAMP) then triggers the secretory process. This entire sequence of events can be reproduced with intact mucosa mounted *in vitro*, beginning with addition of the toxin and, in an hour or less, culminating in active secretion of Cl^- (Field 1978). Active secretion in the intestine can also be elicited by hormones, such as vasoactive intestinal peptide and the E prostaglandins and other bacterial enterotoxins, including one (*Escherichia coli* heat-stable enterotoxin) that stimulates guanylate cyclase (Field 1981).

The normal physiological role of intestinal secretion is less obvious than its role in diarrhoeal diseases because the secreted fluid is reabsorbed and nothing remains to be collected and measured. None the less, active secretion appears to be important for maintaining fluidity of the contents of the small bowel and for propelling outwards immunoglobulins that are initially secreted into intestinal crypts (O'Daly *et al.* 1971). Electrolytes and water appear to be secreted in the crypt region of the intestines, whereas they are absorbed in the villus or more superficial region (Field 1981).

Because active secretion in the intestines appears to originate in cells recessed in crypts that are not readily accessible to impalement with microelectrodes, we turned to another epithelium, the trachea, in which the electrolyte-secreting cells are exposed on the luminal surface, to learn more about the cellular mechanism of active secretion of Cl^-. The tracheal and bronchial surfaces are lined with a ciliated, pseudostratified columnar epithelium, the purpose of which is to clear the airways of inhaled particles, microorganisms and secreted substances (Nadel *et al.* 1979). By actively secreting electrolytes, the tracheal epithelial cells maintain an aqueous layer on their surface within which cilia can beat in a coordinated fashion, thereby propelling outwards an overlying mucoid layer containing trapped particles. If the aqueous layer shrinks, the cilia come into contact with the viscous and adhesive mucoid layer and their rhythmic movements cease. One can also reasonably speculate that, if the aqueous layer becomes too large because it is produced at an excessive rate, then ciliary movements become ineffective and the more superficial mucoid layer is no longer cleared from the airways. Thus in both intestine and trachea, secretion must be finely regulated to be physiologically useful. In both organs, this regulation involves a balance between electrolyte absorption and secretion. Table 1 compares the changes in Na^+ and Cl^- fluxes produced by secretory stimuli in distal rabbit colon, distal rabbit ileum and canine trachea. In all three tissues, stimulation of secretion increases short-circuit current (I_{sc}) and results in a net serosa-to-mucosa flux of Cl^-. Effects on Na^+ fluxes are variable. In the distal colon and in the trachea, both of which are relatively 'tight' epithelia, electrolyte absorption is a consequence of an amiloride-inhibitable Na^+ transport mechanism (Schultz 1981; Widdicombe & Welsh 1980). This absorptive process is not greatly affected by secretory stimuli. On the other hand, in the ileum, electrolyte absorption is by a cAMP-inhibitable NaCl co-transport mechanism and therefore secretagogues inhibit active salt absorption as well as stimulating active secretion (Field 1981). In both colon and small intestine absorptive and secretory regions appear to be anatomically separate entities. In the trachea (or, at least, in canine trachea), however, absorption and secretion both appear to be properties of a single epithelial cell. In what follows we shall focus our attention on the cellular mechanism of secretion.

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A general model for NaCl secretion in vertebrate epithelia

The secretory processes of the intestines and the trachea exhibit striking similarities. In both, the ion actively secreted is Cl^- and the accompanying Na^+ movement appears to be largely passive. The same relation between Cl^- and Na^+ movements also holds true for most other secretory epithelia in vertebrates (Frizzell *et al.* 1979) although the choroid plexus is an exception (Quinton *et al.* 1973). At first thought, the fact of 'active' anion secretion may seem paradoxical since only *cation*-translocating ATPases have been demonstrated in vertebrate epithelial cell plasma membranes. On further reflection this paradox disappears, however, because coupling between anion and cation fluxes across biological membranes has been repeatedly demonstrated and the associated concepts are now commonplace. 'Active' transport of Cl^- can be classified as 'secondarily active' if its transport is coupled to the transport of Na^+. That this is so for Cl^--secreting epithelia, such as intestine and trachea, is strongly suggested from the dependence of Cl^- secretion on the presence of Na^+ in the bathing medium (and particularly the serosal bathing medium) and from the inhibition of Cl^- secretion by serosally added ouabain, which specifically inhibits the Na^+ and K^+-translocating ATPase present on the basolateral membrane of secretory cells (Frizzell *et al.* 1979). Our preliminary measurements of intracellular Cl^- activities in canine trachea indicate that Cl^- accumulates there above electrochemical equilibrium (unpublished results). This appears to be also true for other salt-secreting vertebrate epithelia such as dogfish rectal gland and salivary glands (Frizzell *et al.* 1979; Young 1973). Thus the *uphill* step in Cl^- transport appears to be at the basolateral or serosal membrane and the *downhill* step at the luminal or apical membrane. These observations have led to the general model for NaCl secretion illustrated in figure 1. Its features are the following. (1) Cl^- entry across the basolateral membrane is coupled to Na^+ entry. This is the postulated site of inhibition by furosemide and its more potent analogues; these drugs have been shown to inhibit NaCl co-transport in the brush border of certain absorptive epithelia (Frizzell *et al.* 1979). (2) As a consequence of the Na^+ gradient, Cl^- accumulates in the cell above electrochemical

Table 1. Effects of secretory stimuli on ion transport across rabbit distal colon, rabbit ileum and canine trachea

(All values are in microequivalents per hour per square centimetre. In tracheal experiments 10^{-6} M indomethacin was present in both control and PGE_1-treated tissues.)

	Na^+ fluxes			Cl^- fluxes			
	ms	sm	net	ms	sm	net	I_{sc}
(*a*) distal colon (from Frizzell 1977)							
control	3.4	1.5	1.9	6.3	4.9	1.4	2.2
A23187	3.4	1.6	1.8	6.1	7.5*	−1.4*	4.6*
(*b*) distal ileum (from Bolton & Field 1977)							
control	11.1	10.8	0.3	8.8	8.0	0.8	1.8
theophylline	8.8**	10.8	−2.0**	6.6**	11.4**	−4.8**	4.8**
(*c*) trachea (from Al-Bazzar *et al.* 1981)							
control	0.43	0.08	0.35	0.29	0.85	−0.57	0.95
10^{-6} M PGE_1	0.42	0.24*	0.18*	2.08*	5.96*	−3.88*	4.70*

* $p < 0.05$; ** $p < 0.01$.

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equilibrium. (3) Secretagogues (i.e. in trachea, adrenalin) stimulate Cl^- secretion by increasing the apical membrane permeability to Cl^-, thereby allowing diffusion into the lumen of the Cl^- that accumulates intracellularly. (4) The Na^+ which enters the cell with Cl^- is recycled to the serosal side by the Na^+–K^+ exchange pump. The recycled Na^+ then diffuses to the lumen by a paracellular route, the driving force being the transepithelial electric potential difference.

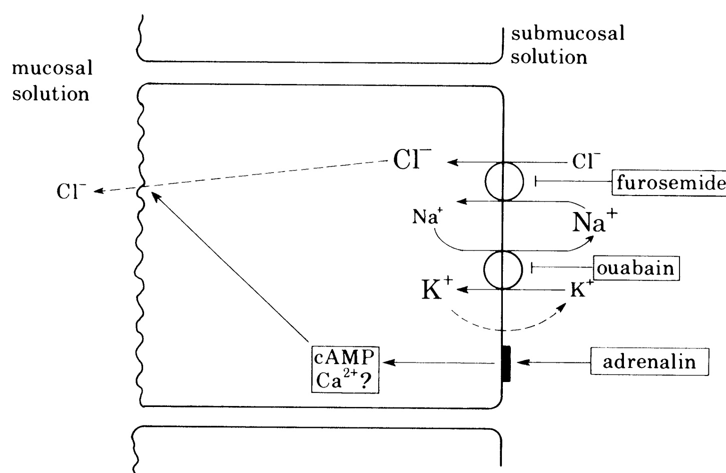

Figure 1. Cellular model for Cl^- secretion (canine trachea). See text for details.

Table 2. Steady-state effects of adrenalin on epithelial parameters in canine trachea

(Means ± 1 s.e. for 17 experiments. Potentials were measured at their first subscript with reference to their second subscript. Subscripts m, c, and s refer to mucosa, cell and serosa. $R_a/(R_a+R_b)$ is equal to β (i.e. $\Delta V_{cm}/\Delta V_{sm}$ in the presence of 60 $\mu A\ cm^{-2}$ current or less). Tissues were bathed in the HCO_3^- and CO_2-free amine-buffered Ringer solution described by Shorofsky *et al.* (1982). Glucose (5 mM) and indomethacin (1 μM) were also present.)

V_{sm}/mV	V_{cm}/mV	V_{cs}/mV	$R_{ms}/(\Omega\ cm^2)$	$I_{sc}/(\mu A\ cm^{-2})$	$R_a/(R_a+R_o)$
			control		
20 ±1.5	−43.9 ±1.8	−63.9 ±1.3	777 ±81	28.7 ±3.1	0.71 ±0.03
			adrenalin		
38.9* ±1.9	−29.3* ±1.5	−68.2* ±1.7	379* ±55	126.5* ±15.7	0.35* ±0.03

* $p < 0.01$.

(5) Finally, the K^+ that enters in exchange for Na^+ diffuses back across the basolateral membrane, the K^+ permeability of which increases as the Na^+–K^+ pump rate increases.

Electrophysiology of tracheal secretion: cell potentials and membrane resistances

This model or paradigm for salt secretion explains a number of the readily observable features of secretion by intestine, trachea and other vertebrate epithelia. To assess its validity, however, we cannot rely only on its explanatory power; intracellular ion activities and cell

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membrane potentials, resistances and specific ionic permeabilities, both coupled and uncoupled, must be measured in secretory cells both at rest and during secretion. We have made a beginning towards this goal by using microelectrode techniques on canine trachea. Our findings, which are summarized here, are described in greater detail elsewhere (Shorofsky *et al.* 1982). Mucosa obtained from the posterior, cartilage-free portion of canine trachea and stripped of underlying muscle and much of the connective tissue was mounted in a modified Ussing-type chamber

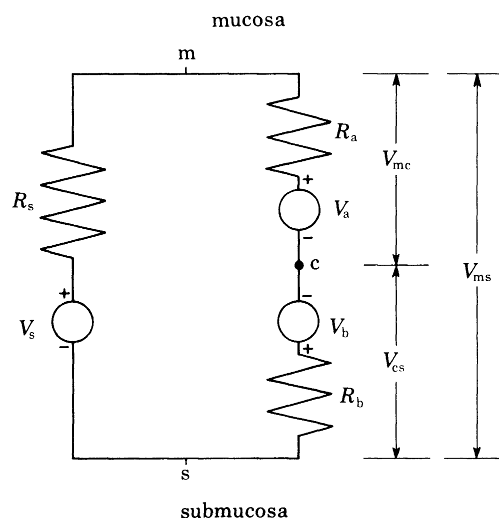

FIGURE 2. Lumped-parameter equivalent circuit model of the epithelium: m, c and s represent the mucosal solution, cell and serosal solution respectively; R_a, R_b and R_s represent the resistances and V_a, V_b and V_s the electromotive forces (e.m.fs) of the apical and basolateral membranes and shunt respectively. Polarities of batteries are as indicated. V_{mc}, V_{cs} and V_{ms} are the measured potentials (second subscript is the reference potential). The model is characterized by the following equations:

$$R_{ms} = (R_a + R_b)\,R_s/(R_a + R_b + R_s); \qquad (a)$$

$$\beta = \Delta V_{mc}/\Delta V_{ms} \text{ at } 60\ \mu\text{A cm}^{-2} \text{ current or less } = R_a/(R_a + R_b); \qquad (b)$$

$$V_a = V_{mc} + (V_{ms} - V_s)\quad R_a/R_s; \qquad (c)$$

$$V_b = V_{sc} - (V_{ms} - V_s)\quad R_b/R_s. \qquad (d)$$

with the apical surface facing up. Tissues were treated with indomethacin (1 μM), which suppresses spontaneous secretion (Al-Bazzaz *et al.* 1981), and allowed to stabilize for 1–2 h before microelectrode impalements. Table 2 shows the effect of adrenalin on the electric potential profile (V_{sm}, V_{cm} and V_{cs}), transepithelial electric resistance (R_{ms}), short-circuit current (I_{sc}) and the voltage-divider ratio (β), which, in terms of the equivalent circuit model shown in figure 2, is formally equal to $R_a/(R_a+R_b)$. Adrenalin caused almost a fivefold increase in I_{sc}, which was associated with both an increase in V_{sm} and a decrease in R_{ms}. The increase in I_{sc} is due to adrenalin's stimulation of Cl^- secretion (Al-Bazzaz & Cheng 1979). With respect to the intracellular potentials, V_{cm} depolarized by about 15 mV whereas V_{cs} hyperpolarized by about 4 mV. β decreased by about 50 %, signifying a large decrease in R_a relative to R_b.

A better way to study the action of adrenalin on this epithelium is to record from a single cell during the complete time course of the drug response. This proved difficult because impalements could seldom be maintained for a long enough time. We were able to obtain a few such recordings, however, and the results for one, expressed in terms of I_{sc}, R_{ms} and the ratio R_a/R_b, are shown in figure 3. It can be seen that the changes in R_a/R_b are biphasic whereas the changes

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in I_{sc} are monotonic. The delayed increase in R_a/R_b 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).

Since the stimulation of Cl^- secretion by adrenalin was associated with decreases in both R_{ms} and β, it is tempting to conclude that adrenalin stimulates Cl^- secretion by increasing the Cl^- conductance of the apical membrane. Furthermore a secondary decrease in R_b could represent

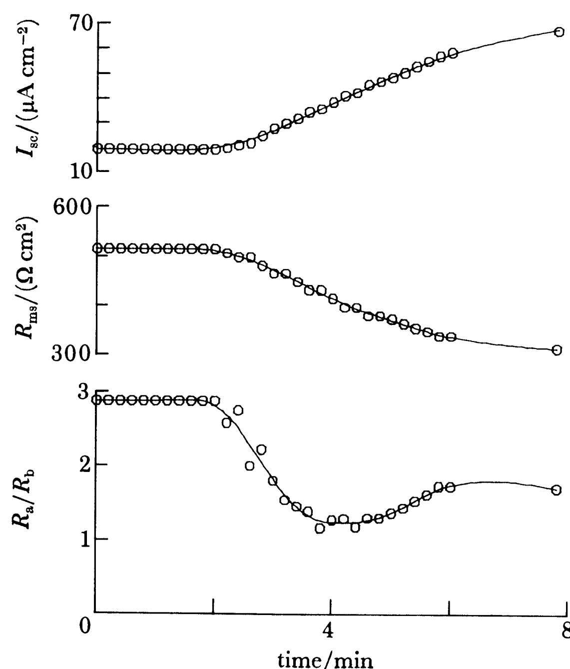

FIGURE 3. Responses of tissue parameters to adrenalin (2×10^{-5} M). Results are from a single representative experiment.

an increase in the K^+ conductance of the basolateral membrane without which the cell would accumulate K^+ as the Na^+–K^- pump rate increases. The existence of such a mechanism for cell K^+ homoeostasis seems almost inevitable and it has been proposed to occur in other ion-transporting epithelia (Schultz 1982). How pump rate and K^+ permeability are coordinated is not known.

Although the data in table 2 and figure 3 are consistent with the above formulation, they by no means prove it. As can readily be seen from the equivalent circuit model shown in figure 2, a direct measure of R_a can only be obtained in very tight epithelia (i.e. when $R_s \gg R_a$, R_b). The data also do not provide any specific information about the Cl^- permeability of the apical membrane. None the less, application of the equivalent circuit model shown in figure 2 (see legend for the relevant equations) to the results of the individual experiments for which averages are shown in table 2, does permit some qualitative statements to be made about the number of resistances that must change and about the directions of changes. For a more detailed description of the necessary calculations, see Shorofsky *et al.* (1982). The assumption that adrenalin treatment does *not* decrease R_a requires that either V_b must be greater than 150 mV or R_s must decrease from more than 30 kΩ cm² to 500 Ω cm². Since neither of these occurrences is at all likely, we conclude that adrenalin must decrease R_a. Upon further analysis it also becomes clear

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that adrenalin could not have affected R_a *alone*. In half of the experiments negative values were obtained for at least one of the derived resistances if it is assumed that only R_a changes. The conclusion that adrenalin affects more than one membrane resistance is supported by the delayed increase in R_a/R_b shown in figure 3. This change could be due either to a secondary increase in R_a accompanied by a continuing decrease in R_s or to the delayed decrease in R_b discussed above. In the latter case no alteration in R_s need be stipulated. Additional support

Table 3. Effect of adrenalin on the ion-selectivity of the apical membrane

(Means ± 1 s.e. for (n) determinations of ΔV_{cm} or midpoints of ranges ± ranges for T_i (calculated from the experiments for which ΔV_{cm}'s are shown). T_i is the ion-dependent partial potential ratio defined by equation (2) in the text. K^+ and Na^+ were replaced by tris(hydroxymethyl)aminomethane and Cl^- by methane sulphonate. The Na^+ concentration was 100 mM in the $[K^+]$ change experiments but was 145 mM in the $[Cl^-]$ change experiments.)

change in luminal-side ion concentration /mM	ΔV_{cm}/mV		T_i	
	control	adrenalin	control	adrenalin
$[K^+]$ (5 → 50)	3.9 ± 0.5 (9)	1.3 ± 0.6 (5)	0.17 ± 0.03	0.05 ± 0.01
$[Na^+]$ (145 → 20)	−4.7 ± 0.6 (8)	−2.1 ± 0.4 (6)	0.28 ± 0.08	0.05 ± 0.03
$[Cl^-]$ (146 → 49)	−9.1 ± 0.3 (7)	−17.7 ± 0.8 (7)	0.57 ± 0.12	0.74 ± 0.09

for a delayed decrease in R_b can be gleaned from the fact that adrenalin also hyperpolarizes V_{sc} (table 2). If this change is due to an increase in V_b, then it may signify a delayed increase in the P_{K^+} of the basolateral membrane: if the postulated decrease in R_b were selective for K^+, V_b should increase to more closely approximate E_{K^+}.

Electrophysiology of tracheal secretion: ion selectivity of the apical membrane

In order to examine the nature of the adrenalin-induced change in apical membrane conductance, we determined the effects of changes in the ionic composition of the mucosal bathing solution on intracellular potential. Measurements were made both before and after adding adrenalin. Results are shown in table 3. It should be noted that the magnitudes of changes in ion concentrations differed for each of the three ions tested; thus for K^+ the change was tenfold, for Na^+ sevenfold and for Cl^- only threefold. It can be seen that the changes in V_{cm} caused by changes in mucosal solution K^+ and Na^+ concentrations were relatively small under control conditions and were further reduced by adrenalin. The change in Cl^- concentration, on the other hand, has a relatively large effect on V_{cm} under control conditions, and this effect was further increased by adrenalin. It therefore appears that adrenalin causes a selective increase in apical membrane Cl^- permeability. However, the ion permeability properties of the apical membrane are reflected by changes in V_a (the e.m.f. across the apical membrane), not V_{cm}. Since current can flow intraepithelially, V_{cm} may differ from V_a according to the following equation:

$$-V_{cm} = \frac{V_a(R_b + R_s) + (V_b + V_s)\,R_a}{R_a + R_b + R_s}. \tag{1}$$

Since changes in V_{cm} can result not only from changes in V_a but also from changes in V_b, V_s or any of the three resistances, V_a itself must be estimated to determine the ionic permeabilities of the apical membrane. On the basis of the data shown we can only accomplish this in part: we

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can estimate changes in V_a (ΔV_a) and from these we can estimate relative ionic permeabilities (i.e. P_{Cl^-}/P_{K^+}, P_{Na^+}/P_{K^+}, P_{Cl^-}/P_{Na^+}). To do this we first applied our data to the equivalent circuit model in figure 2, making the following two assumptions: (1) $V_s = 0$ when identical solutions bathe both sides of the tissue, and (2) R_b and V_b do not change in the time required (less than 1 min) to make measurements after a change in the mucosal bathing solution. Using these assumptions, the validity of which is discussed below, along with the equations in the legend to figure 2, we were able to determine a range of possible values for ΔV_a (the change in V_a due to the solution change). For details see Shorofsky *et al.* (1982). Using these ranges of values for ΔV_a, we calculated for each ion the ion-dependent partial potential ratio (T_i) as defined by Strickholm & Wallin (1967), i.e.

$$T_i = \frac{\Delta V_a}{(RT/F)\ln(C_1/C_2)}, \tag{2}$$

where C_1 and C_2 are the concentrations of ion (i) in the two mucosal bathing solutions and R, T and F have their usual meanings. In this way experiments involving different magnitudes of changes in ion concentrations 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 that ion. Means and range for T_i's are shown in table 3. The ratio T_{Cl^-}/T_{K^+} is 3.3 in the control state and 15.4 in the adrenalin-stimulated state, suggesting that adrenalin causes an almost fivefold increase in the relative Cl^- permeability of the apical membrane.

The above calculations were based on the two assumptions mentioned above. The first assumption, that $V_s = 0$, 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 were present, however, it would have almost no effect on the calculated T_i's.

The assumption that V_b is constant over the time required to make measurements after changing the mucosal solutions is difficult to test directly. V_b should remain constant if the intracellular ion concentrations do not change significantly during the time required to make the relevant measurements (usually less than 1 min). In the presence of significant alterations in these ion concentrations, a progressive change in V_{cm} would be expected. In almost all experiments, however, ΔV_{cm} quickly reached a stable value that lasted until the solution change was reversed. The assumption that R_b remains constant is difficult to prove, but even if R_b were to decrease by as much as 50% after a mucosal solution change, there would be almost no change in the calculated T_i's. Although the ionic permeabilities cannot be directly calculated from the T_i's, permeability ratios can be estimated by using the Goldman–Hodgkin–Katz equation (see Shorofsky *et al.* 1982). The resulting value for P_{Na^+}/P_{K^+} in the control state is 0.36 and in the adrenalin-stimulated state 0.39, or essentially unchanged from the control state.

To calculate P_{Cl^-}/P_{K^+} the intracellular Cl^- activity must be known. In preliminary experiments with Cl^--sensitive ion-selective microelectrodes, we have found $a_{Cl_i^-}$ in the control state to be 46 mM (unpublished observations). From this value, P_{Cl^-}/P_{K^+} in the control state is 0.83. We have not yet estimated $a_{Cl_i^-}$ for the adrenalin-stimulated state, but if the model of secretion outlined in figure 1 is correct, $a_{Cl_i^-}$ should either decrease or remain the same. Therefore if 46 mM is taken as the maximum possible value for $a_{Cl_i^-}$ in the adrenalin-stimulated state, the minimum estimate for P_{Cl^-}/P_{K^+} in that state would be 5.45, which represents an increase of greater than sixfold over the control state.

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In summary, then, it appears nearly certain that there is a large increase in the apical membrane's permeability to Cl^- relative to its permeability to either Na^+ or K^+ when secretion is stimulated by adrenalin. Since adrenalin also increases total conductance of the apical membrane, there must be an absolute as well as a relative increase in Cl^- permeability.

Conclusions and questions

The observations summarized above show that adrenalin stimulates active Cl^- secretion in canine tracheal epithelium by increasing the Cl^- permeability of the apical membrane. A similar conclusion has been arrived at by Welsh *et al.* (1982) and has also been suggested by electrophysiological studies of the corneal epithelium (Klyce & Wong 1977; Nagel & Reinach 1980). We have shown that this increase in Cl^- permeability is selective in that there does not appear to be a comparable increase in Na^+ and K^+ permeabilities. Since intracellular Cl^- activity, measured under basal conditions, is above electrochemical equilibrium, the adrenalin-stimulated increase in Cl^- permeability produces net Cl^- secretion. Adrenalin also alters other cell parameters, one of which may be a decrease in basolateral membrane resistance, reflecting the expected increase in K^+ permeability believed to occur in conjunction with an increase in turnover rate of the Na^+/K^+ pump. These observations are consistent with the model for Cl^- secretion depicted in figure 1. Further analysis of this model will require determinations of intracellular ion activities and the application of one or more additional techniques for resolving unique values for each circuit element in the model's electrical analogue. It will also be necessary to define the electrically silent or coupled membrane permeabilities, especially those in the basolateral membrane.

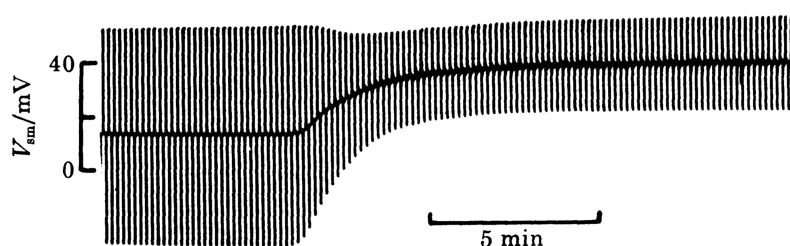

Figure 4. Sample recording during the time course of an adrenalin response. The transepithelial recording is shown in the upper trace, the intracellular in the lower trace. Owing to the delay in the flow apparatus, the exact time that adrenalin reached the tissue cannot be independently determined. Note the slower return of the current-induced voltage to baseline after the onset of the adrenalin effect.

An interesting area for future investigation is the nature of the stimulus-induced Cl^- permeability in the apical membrane. What are the electrical, kinetic and steric properties of these Cl^- channels? Do existing membrane channels open in response to adrenalin or are new channels recruited from intracellular vesicles, or both? Recruitment of new channels implies that adrenalin promotes vesicle–membrane fusion with a resulting increase in membrane surface area. An increase in membrane area should cause an increase in tissue capacitance. Figure 4 shows that such an increase in capacitance takes place, although we cannot at present quantitate the change or localize it to the apical membrane.

Another interesting area for future investigation is the nature of the NaCl co-transport process assumed to be present in the basolateral membrane. Is this coupled permeability enhanced by secretory stimuli? Are Na^+ and Cl^- the only ions translocated? Intracellular Na^+

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and Cl^- concentrations are almost invariably lower than their extracellular concentrations. If Na^+ and Cl^- are the only participating ions, a net inward flux of NaCl through an electrically neutral co-transport pathway would occur, even in the absence of net secretion. The Na^+/K^+ pump would then be required to turn over to keep up with the inward flux of NaCl even when the cell is not secreting. One possible resolution of this paradox would be a regulated co-transporter that is switched on when secretion is stimulated and switched off when secretion stops. Another possible resolution is the participation of a third ion, probably K^+, in the co-transport so that one of the three ion gradients is directed from cell to medium. The co-transport might then be able to reach equilibrium when the cell is not secreting. With the onset of secretion, cell [Cl^-] would drop, upsetting the equilibrium in favour of an inward flux of ions. The presence of Na^+, K^+, Cl^- co-transport has recently been reported for several cell types, including epithelial cells (Palfrey & Greengard 1981; Dunham *et al.* 1980; McRoberts *et al.* 1982; Greger & Schlatter 1981; Musch *et al.* 1982; Sackin *et al.* 1982).

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