J. Membrane Biol. 70, 217–226 (1982)

The Journal of
Membrane Biology

Chloride Secretion by Canine Tracheal Epithelium: I. Role of Intracellular cAMP Levels

Philip L. Smith*, Michael J. Welsh**, Jeffrey S. Stoff, and Raymond A. Frizzell***

Department of Physiology and Cell Biology, University of Texas Medical School at Houston, Houston, Texas 77025, and Charles A. Dana Research Institute, The Harvard-Thorndike Laboratory and Department of Medicine, Beth Israel Hospital, Boston, Massachusetts 02215

Summary. We measured the short-circuit current (I_{sc}) across canine tracheal epithelium and the intracellular cAMP levels of the surface epithelial cells in the same tissues to assess the role of cAMP as a mediator of electrogenic Cl secretion. Secretogogues fall into three classes: (i) epinephrine, prostaglandin (PG) E_1, and theophylline increase both I_{sc} and cellular cAMP levels; (ii) $PGF_{2\alpha}$ and calcium ionophore, A23187, increase I_{sc} without affecting cell cAMP levels at the doses employed; and (iii) acetylcholine, histamine, and phenylephrine do not alter either I_{sc} or cAMP levels.

These findings indicate that: (i) increases in cAMP or Ca activity stimulate electrogenic Cl secretion by the columnar cells of the surface epithelium; (ii) cAMP mediates the effects of PGE_1 and β-adrenergic agonists; (iii) a strict correlation between cAMP levels and Cl secretion rate is not apparent from spontaneous variations in these parameters or from dose-response relations of I_{sc} and cAMP to epinephrine concentration; and (iv) acetylcholine, histamine, and phenylephrine, agents that stimulate electrically-neutral NaCl secretion by submucosal glands, do not evoke cAMP-mediated responses by the surface epithelium.

Addition of 10^{-6} M indomethacin (or other prostaglandin synthesis inhibitors) to the mucosal solution decreases I_{sc} and cellular cAMP levels and reduces the release of PGE_2 into the bathing media by 80%. Indomethacin does not interfere with the subsequent secretory response to PGE_1. This suggests that endogenous prostaglandin production underlies the spontaneous secretion of Cl across canine tracheal epithelium under basal conditions.

Key Words tracheal epithelium · cAMP · prostaglandins · short-circuit current · chloride secretion · secretogogues

Introduction

The rate of salt and water transport across airway epithelia is thought to be an important determinant of the thickness of the fluid layer that lines the surface of the pulmonary airways. This, in turn, appears to be a critical factor governing the efficacy of pulmonary mucociliary clearance (Nadel, 1980; Widdicombe & Welsh, 1980). Studies aimed at clarifying the mechanisms responsible for fluid and electrolyte transport across the airways have focussed on the posterior membranous portion of the canine trachea since this epithelium can be studied, in vitro, as a flat sheet under short-circuit conditions. In the absence of exogenous agents added to the bathing media (hereafter referred to as "basal conditions") canine tracheal epithelium actively transports Cl from submucosa-to-mucosa at rates of 1–3 $\mu eq/cm^2$ hr and Na from mucosa-to-submucosa at rates of 0.5–1.0 $\mu eq/cm^2$ hr (Olver, Davis, Marin & Nadel, 1975; Al-Bazzaz & Al-Awqati, 1979). Although there are fairly wide variations in the spontaneous rates of net Na and Cl movement across the epithelium under basal conditions, the combined rates of Na absorption and Cl secretion account entirely for the simultaneously-determined short-circuit current (I_{sc}).

A variety of humoral, neurohumoral, and pharmacologic agents have been shown to enhance the rate of Cl secretion across canine trachea, and two distinct patterns of activity have emerged from the influences of these secretogogues: (i) agents that increase both Cl secretion and the I_{sc} (electrogenic Cl secretion) with little effect on Na transport include epinephrine (Al-Bazzaz & Cheng, 1979); the β-adrenergic agonist, terbutaline (Davis, Marin, Yee & Nadel, 1979); protaglandins E_1, E_2 and $F_{2\alpha}$ (Al-Bazzaz, Yadava & Westerfelder, 1981); theophylline (Al-Bazzaz & Al-Awqati, 1979); cyclic AMP (Al-Bazzaz, 1981) and the calcium ionophore, A23187 (Al-Bazzaz & Jayaram, 1981); (ii) agents that enhance Cl secretion and reverse the direction of net Na movement from

* *Present address:* Department of Physiology, University of Kansas Medical Center, College of Health Sciences and Hospital Rainbow Boulevard at 39th, Kansas City, Kansas 66103.

** *Present address:* Laboratory of Epithelial Transport and Kidney Physiology, Department of Internal Medicine, University of Iowa Hospitals, Iowa City, Iowa 52242.

*** *Present address:* Department of Physiology and Biophysics, University of Alabama in Birmingham, University Station, Birmingham, Alabama 35294.

0022-2631/82/0070-0217 $02.00

absorption to secretion while having little, if any, effect on the I_{sc} (neutral Cl secretion)[1] include acetylcholine (Marin, Davis & Nadel, 1976); histamine (Marin, Davis & Nadel, 1977) and the α-adrenergic agonist, phenylephrine (Al-Bazzaz & Cheng, 1979). In the companion paper (Welsh, Smith & Frizzell, 1982), we describe the results of microelectrode studies indicating that epinephrine and prostaglandins of the E series stimulate electrogenic Cl secretion by the surface epithelial cells. In contrast, acetylcholine, parasympathetic nerve stimulation (Borson, Chino, Davis & Nadel, 1980), and phenylephrine (Phipps, Nadel & Davis, 1980; Ueki, German & Nadel, 1980) have been shown to elicit secretion of electrolytes and fluid from submucosal glands that lie beneath the surface epithelium. Thus, these two distinct patterns of secretory activity appear to be localized to different anatomic regions of the tissue.

Since both theophylline and cAMP (Al-Bazzaz & Al-Awqati, 1979; Al-Bazzaz, 1981) increase electrogenic Cl secretion across tracheal epithelium, alterations in intracellular cAMP levels may be an important determinant of Cl secretion rate, as proposed for other Cl-secreting epithelia (Frizzell, Field & Schultz, 1979). This suggestion is supported by the finding of Al-Bazzaz et al. (1981) that prostaglandins E_1 and E_2 increase intracellular cAMP levels in epithelial cells isolated from canine trachea.

To further examine the role of cAMP as an intracellular mediator of Cl secretion, we measured the I_{sc} across canine tracheal epithelium (which largely reflects Cl secretion rate) and the cyclic AMP levels of the surface epithelium in the presence and absence of agents that both enhance and inhibit Cl secretion rate. This approach permitted a direct comparison of intracellular cAMP levels and I_{sc} of individual tissues. Determinations of cAMP levels were carried out using only the surface epithelial cells so that the regional localization of secretogogue activity could be further explored.

Methods and Materials

Tissue Preparation

Trachea obtained from mongrel dogs (20–40 kg) anesthetized with nembutal (25 mg/kg) were immediately placed in ice-cold Ringer's solution gassed with 95% O_2–5% CO_2. The posterior muscle layer was removed, and the posterior membranous portion was mounted in Lucite Ussing chambers having an exposed surface area of 1.25 cm^2. Tissues were bathed on each side with 10 ml of a Ringer's solution having the following composition in mmol/liter: Na, 143.0; K, 5.4; Ca, 1.2; Mg, 1.2; Cl, 123.7; HCO_3, 25; HPO_4, 2.4; H_2PO_4, 0.6; and D-glucose, 10.0. This solution was circulated by a gas-lift system and maintained at 37 °C with water-jacketed reservoirs. The pH was 7.4 when gassed with 95% O_2–5% CO_2.

Electrical Measurements

The transepithelial electrical potential difference (Ψ_t), short-circuit current (I_{sc}), and tissue resistance (R_t) were determined as described previously (Frizzell, Koch & Schultz, 1976).

cAMP Measurements

After mounting, both the I_{sc} and R_t were monitored until a steady-state was obtained. Test agents were then added and after 10 min (*see* below) tissues were quickly removed from the chambers and the exposed surface area scraped with a glass microscope slide to remove the epithelial cells. These were placed in ice-cold 1 N HCl within 1 min of their removal from the chamber. They were sonicated in a bath-type sonicator for 10 sec and centrifuged in a Brinkman microfuge for 25 min. The supernate was then removed and dried under a stream of air at room temperature. The residues were redissolved in 1 ml of 50 mM sodium acetate buffer (pH 6.2) and the cAMP present was determined in duplicate by radioimmunoassay using kits purchased from Becton-Dickinson (Orangeburg, NJ). The HCl precipitates were dissolved in 1 N NaOH and assayed for protein by the method of Lowry, Rosebrough, Farr and Randall (1951). Results are expressed as pmol cAMP per mg protein. The degree of variability encountered in the cellular cAMP levels determined under basal conditions required that the effects of secretogogues be compared against control values obtained using tissues from the same animals. Part of this variability may arise from differences in spontaneous Cl secretion rate; this and other possibilities are detailed in the discussion.

Figure 1*a* shows a photomicrograph of canine tracheal epithelium. The tissue is comprised of a pseudo-stratified columnar epithelium and a submucosal layer of collagen. Figure 1*b* is a micrograph showing unstained epithelial cells, scraped from the tracheal surface, for determinations of their cAMP content. This yielded both individual cells and clumps of cells, showing motile cilia. Figure 1*c* shows a section of the scraped tissue which retains only the submucosal collagen layer and, in this section, a submucosal gland. These figures indicate that the scraping procedure removed only the surface cells for assay of their cAMP content and that the underlying submucosa with its glandular epithelium was not included in these measurements.

In preliminary experiments, we found that both cAMP levels and I_{sc} reached maximal values between 3 and 7 min after stimulation by PGE_1 or epinephrine and remained constant for at least 20 min thereafter. Since some of the agents employed in this study did not measureably affect the I_{sc}, we routinely removed tissues for cAMP assay 10 min after addition of the test agent. At this time, regardless of the agent employed, the I_{sc} was in a steady-state.

Assay of Prostaglandin (PG) Release

Tissues were prepared and incubated as described earlier. Indomethacin was then added to the mucosal medium of half the tissues and the incubation continued an additional 45–60 min.

[1] It is as yet unclear whether neutral Cl secretion results from a mechanistically-neutral transport process involving Na cotransport or is the result of electrogenic Cl transport into a confined glandular structure where the resulting electrical potential difference is entirely dissipated by passive flow of counterion (Na) so that no net Cl current emerges from the gland orifice.

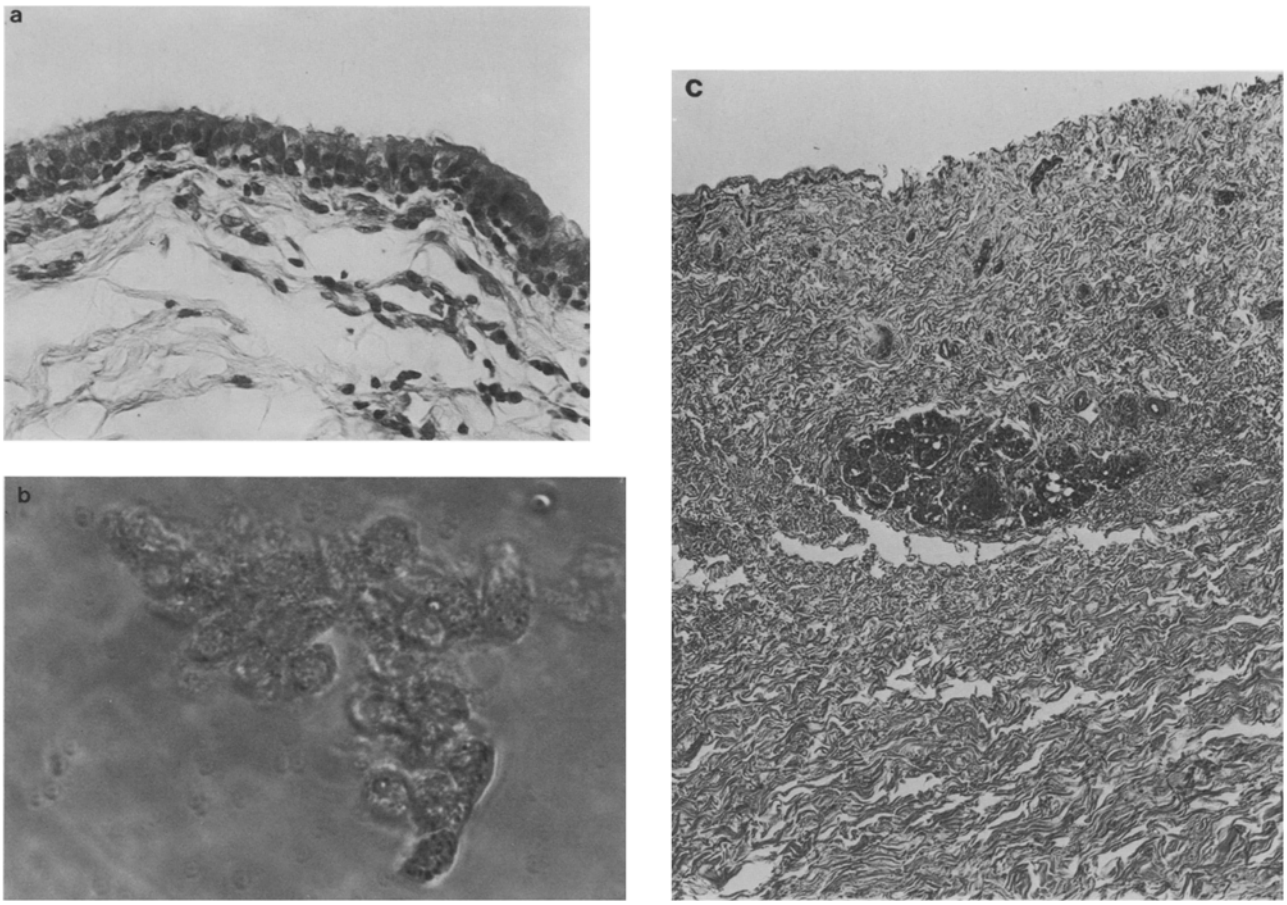

Fig. 1. Morphology of the posterior membranous portion of canine trachea (*a*): Surface epithelium and submucosal collagen layer fixed with formalin and stained with hematoxylin and eosin. The submucosa hydrates during incubation *in vitro*. (*b*): Unstained epithelial cells scraped from the preparation shown in *a* as described in Methods. (*c*): Remaining submucosal collagen layer containing a submucosal gland (center), stained with Periodic-Acid-Schiff stain. The scraping procedure presses fluid out of the submucosa yielding a more compact appearance than that shown in *a*

The serosal and mucosal media were then replaced with fresh media containing indomethacin where appropriate. Thirty min thereafter, the serosal and mucosal media were collected separately, frozen and stored at −20 °C until assay. PGE_2 concentrations in the serosal and mucosal solutions were assayed separately by one of us (J.S.S.) as follows: 1 ml aliquots were extracted in 20-ml glass tubes with Teflon-lined caps. ^3H-PGE_2 (1–2 pg) was added to monitor recovery. All samples were acidified to pH 3.0 with 50% citric acid and extracted twice with three volumes of a mixture of cyclohexane/ethylacetate (1:1). The organic phase was separated by centrifugation and reduced to dryness under a stream of nitrogen at 40 °C. Samples were resuspended in 0.2 ml benzene/ethylacetate/methanol (60:40:10) diluted with 0.8 ml benzene/ethylacetate (60:40) and applied to microcolumns (0.5 × 6 cm) of 0.5 gm nonactivated silicic acid and eluted as previously described (Dray, Charbonnel & Maclouf, 1975). Eluates were dried down under nitrogen, resuspended in assay buffer, and stored 24–48 hr under nitrogen at −20 °C until assayed (Stoff et al., 1980).

PGE_2 was assayed with a specific, high affinity antiserum prepared by immunization of a thyroglobulin-PGE_2 conjugate in rabbits (Dray et al., 1975). The antiserum detects the presence of 2–5 pg PGE_2 with intra-assay and inter-assay coefficients of variation of <10% and <20% respectively. The antiserum shows affinities for other prostaglandins as follows: 5% for PGE_1, 1.5% for 6-keto $F_{1\alpha}$ and less than 0.1% for PGA, PGD_2, $PGF_{2\alpha}$ and PGI_2.

Data is presented as the mean and SEM. Statistical significance was tested using a paired *t*-test where a value of $P<0.05$ was considered significant.

Materials

Epinephrine, acetylcholine, histamine, prostaglandins, phenylephrine, propranolol, indomethacin, and 8-Br-cGMP were obtained from Sigma (St. Louis, MO); theophylline from Matheson, Coleman and Bell (Los Angeles, CA) and A23187 from Calbiochem (La Jolla, CA). Silicic acid was obtained as Silic AR, CC-4 from Mallinckrodt (St. Louis, MO).

Results

Effects of Secretogogues that Increased I_{sc}

The effects of theophylline (5×10^{-4} M), epinephrine (10^{-5} M) and PGE_1 (10^{-6} M) added to the

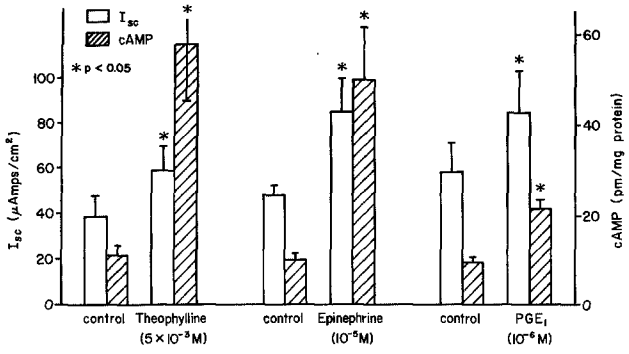

Fig. 2. Effects of theophylline, epinephrine, and PGE_1 on I_{sc} and cellular cAMP levels. Number of paired tissues studied: theophylline, 4; epinephrine and PGE_1, 5. Brackets represent 1 SEM. Significance evaluated with respect to paired control value

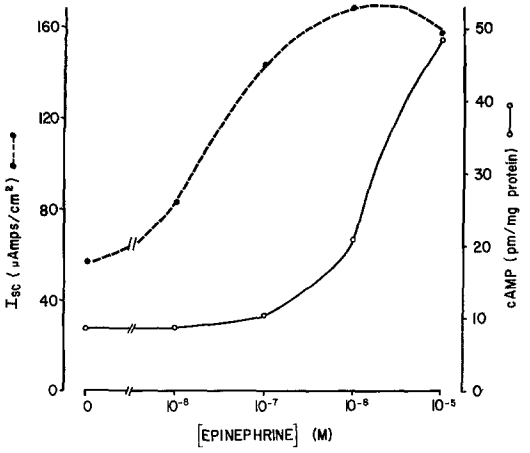

Fig. 3. Cellular cAMP levels and I_{sc} as a function of serosal solution epinephrine concentration. Values from a typical experiment are presented

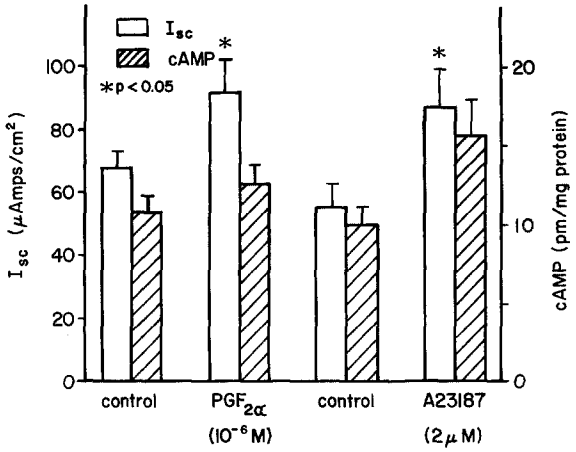

Fig. 4. Effects of $PGF_{2\alpha}$ and A23187 on I_{sc} and cellular cAMP levels. Number of paired tissues studied: $PGF_{2\alpha}$, 6; A23187, 8

serosal solution alone on the I_{sc} across canine tracheal epithelium and intracellular cAMP concentrations of the surface epithelial cells are shown in Fig. 2. These agents increased both I_{sc} (reflecting the rate of electrogenic Cl secretion) and intracellular cAMP levels, but the increases in I_{sc} were not directly proportional to the absolute levels of cAMP achieved. As shown in Fig. 2, the greatest changes in cellular cAMP concentrations were produced by theophylline or epinephrine while a maximal stimulation of I_{sc} was obtained following addition of PGE_1 or epinephrine.

The relation of I_{sc} and cAMP levels to epinephrine concentration was examined in a separate series of experiments. Figure 3 shows that 10^{-6} M epinephrine elicits a maximal increase in I_{sc}. However, the concentration-response relation for the corresponding cellular cAMP levels is displaced to the right of that for the I_{sc}. That is, low epinephrine concentrations produced a measureable increase in I_{sc} but no detectable change in cAMP levels. The results illustrated in Figs. 2 and 3 indicate that these secretogogues increase both cAMP levels and the I_{sc} but that a close correlation between these parameters is not observed; the significance of this will be discussed below.

Figure 4 shows the effects of calcium ionophore, A23187, and $PGF_{2\alpha}$ on I_{sc} and intracellular cAMP concentrations. Although these agents are as effective as epinephrine and PGE_1 in elevating the I_{sc}, they do not significantly alter intracellular cAMP levels at the concentrations employed in these studies. The effect of A23187 on the I_{sc} suggests that in canine tracheal epithelium, as in other Cl secreting tissues (Bolton & Field, 1977; Frizzell, 1977; Prince, Rasmussen & Berridge, 1973), elevation of intracellular Ca activity elicits electrogenic Cl secretion.

The intracellular mediator(s) of the response to $PGF_{2\alpha}$ in canine tracheal epithelium are not known with certainty. This agent stimulates adenylate cyclase of canine gastric mucosa, but the maximal cyclase activity obtained with $PGF_{2\alpha}$ was only 15–20% of that observed with prostaglandins of the E series (Dozois, Kim & Dousa, 1978). Attempts to study the possibility that the increase in I_{sc} elicited by $PGF_{2\alpha}$ requires extracellular Ca were unsuccessful due to the deleterious effect of reduced external Ca concentrations on integrity of tracheal mucosa. Exposure of the tissue to either Ca-free media or EGTA-buffered solutions having

Ca levels of $10^{-6}-10^{-5}$ M decreased tissue resistance to unacceptably low levels.

Effects of Secretogogues that Do Not Alter I_{sc}

As discussed earlier, acetylcholine, histamine and phenylephrine stimulate an electrically-neutral secretion of NaCl across canine tracheal epithelium (Al-Bazzaz & Cheng, 1979; Marin et al., 1976, 1977). Values for I_{sc} and surface cell cAMP levels in the presence or absence of acetylcholine, histamine, or phenylephrine are presented in Fig. 5. Tissues treated with phenylephrine were pre-treated with propranolol (added to the serosal solution) 10 min prior to addition of phenylephrine to prevent changes in ion transport that might result from β-adrenergic effects of phenylephrine (Al-Bazzaz & Cheng, 1979). From Fig. 5, it can be seen that these agents had no significant effect on I_{sc} or cAMP concentrations. The results are consistent with prior observations showing acetylcholine, parasympathetic nerve stimulation, and phenylephrine stimulate glandular secretion (Borson et al., 1980; Phipps et al., 1980; Ueki et al., 1980), and indicate that a cAMP-mediated response of the surface epithelium is not involved in their actions.

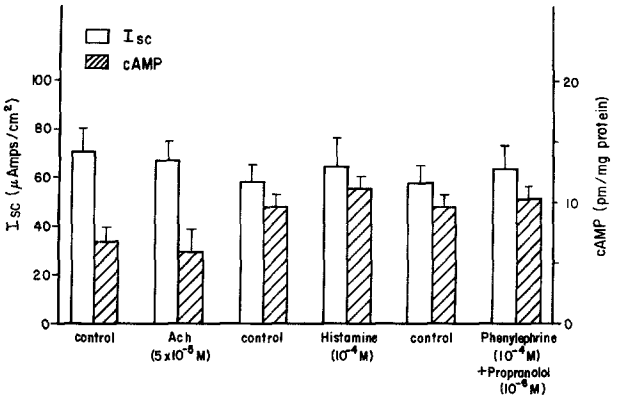

Fig. 5. Effects of acetylcholine, histamine, and phenylephrine plus propranolol on I_{sc} and cellular cAMP levels. Each bar represents the mean of 6 determinations

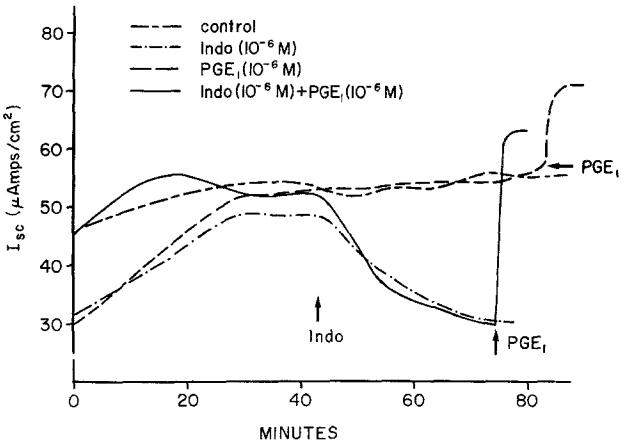

Fig. 6. Effects of indomethacin (mucosal addition) and PGE_1 (serosal addition) on the time-course of I_{sc} across canine tracheal epithelium. Results of a typical experiment are illustrated. Values of I_{sc} at the end of each experimental tracing are recorded for comparison with cAMP levels; at that time, the tissues were removed from the chambers for assay (Figs. 7 and 8)

Effects of Indomethacin on I_{sc}, Intracellular cAMP Levels and PGE_2 Production

Al-Bazzaz et al. (1981) recently reported that addition of indomethacin (10^{-6} M) to the solution bathing the mucosal surface of canine tracheal epithelium decreased Cl secretion and the I_{sc} with no effect on Na absorption. To study the possible role of prostaglandins in regulating Cl secretion across tracheal epithelium under basal conditions, we measured the changes in I_{sc} produced by three inhibitors of prostaglandin synthesis: indomethacin, naproxen, and ibuprofen. These compounds are not closely related on a structural basis and, as such, were chosen in the hope of obtaining one or more agents capable of inhibiting fatty acid cyclooxygenase without directly effecting the Cl transport mechanism (Smith, Blumberg, Stoff & Field, 1980). As seen in Fig. 6, indomethacin (10^{-6} M) added to the mucosal solution decreased I_{sc}; this was accompanied by an increase in R_t (data not shown, *see* Al-Bazzaz et al., 1981; Welsh et al., 1982). The time at which I_{sc} reached a lower steady-state level after indomethacin addition varied from tissue to tissue; in most cases 45–60 min was sufficient. Subsequent addition of PGE_1 (10^{-6} M) to the serosal bathing medium increased I_{sc} and decreased R_t to values identical to those observed in the absence of indomethacin (Fig. 6), and this was also true of prolonged indomethacin treatment (up to 3 hr). Figure 7 summarizes the results obtained from all tissues studied, indicating that indomethacin reduced both I_{sc} and cAMP levels but did not interfere with the subsequent increase in these parameters induced by addition of PGE_1. Similar results were obtained with naproxen and ibuprofen, as shown in Fig. 8; these agents also reduced I_{sc} below basal levels but did not alter the subsequent response of the I_{sc} to PGE_1. In three pairs of indomethacin-treated tissues, we measured the I_{sc} and intracellular cAMP concentrations in the presence and absence of $PGF_{2\alpha}$. The I_{sc} of indomethacin-treated tissues in-

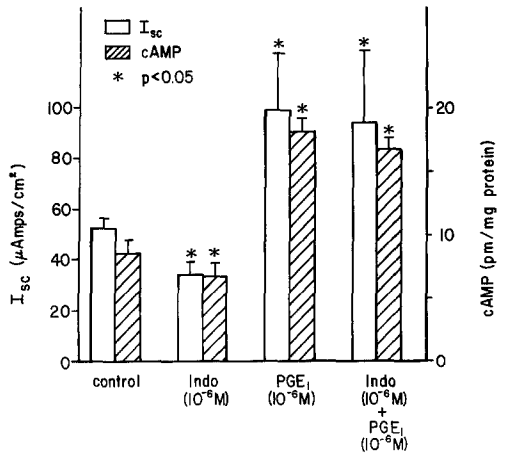

Fig. 7. Effect of PGE_1 on I_{sc} and cellular cAMP levels in control and indomethacin-treated tissues. Significance evaluated with respect to paired control value. Each bar represents the mean of eight determinations

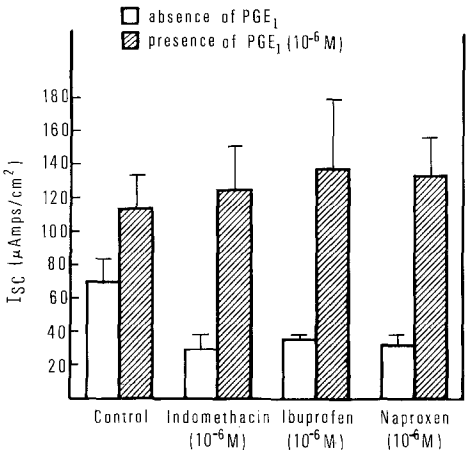

Fig. 8. Effects of PGE_1 on I_{sc} under control conditions and in the presence of inhibitors of prostaglandin synthesis. Experiments conducted as illustrated in Fig. 6. Number of paired tissues studied was 3 in each case

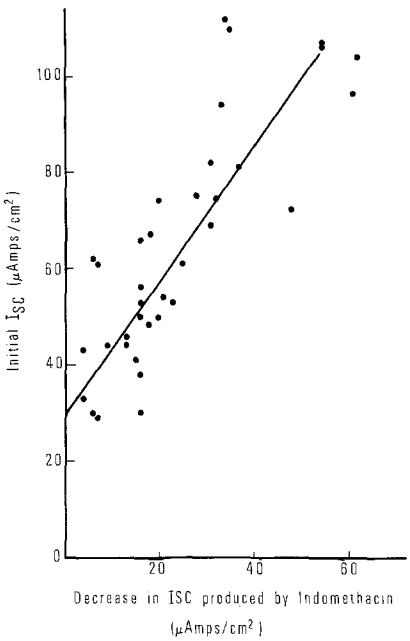

Fig. 9. Relation between the initial basal I_{sc} and the decrease in I_{sc} induced by indomethacin (10^{-6} M) added to the mucosal solution. The line describing these data was obtained from linear regression analysis ($r^2 = 0.63$)

Table 1. PGE_2 production by canine tracheal epithelium

Condition	[PGE_2] (pg/ml)		PGE_2 production (ng/hr)
	Mucosal solution	Serosal solution	
Control	57±22	83±33	2.8±0.8
Indomethacin, 10^{-6} M	8±6[a]	14±8[a]	0.6±0.2[a]

Values are mean±SEM for 5 tissue pairs, PGE_2 concentrations determined as described in Methods. Total PGE_2 production calculated from levels obtained in 10 ml media, each side, during 30 min incubation of 1.25 cm^2 mucosa.
[a] $P < 0.05$.

creased by 30 μA/cm^2 when $PGF_{2\alpha}$ (10^{-6} M) was added to the serosal solution, and, as was observed under basal conditions, intracellular cAMP levels were unaffected (5.67 *vs.* 5.60 pmol cAMP/mg protein).

These findings, and those of Al-Bazzaz et al. (1981) suggest that the basal rate of Cl secretion is determined by endogenous production of prostaglandins. Further support for this notion is provided by the relation between the basal I_{sc} and the steady-state decrease in I_{sc} produced by addition of indomethacin to the mucosal bathing solution. This relation is illustrated in Fig. 9 and shows that the magnitude of the basal I_{sc} determines the inhibitory effect of indomethacin. Moreover, the slope of this relation is not significantly different from unity (1.2±0.3), suggesting that variations in the basal I_{sc} reflect the spontaneous rate of Cl secretion, which can be entirely suppressed by indomethacin. Indeed, transepithelial Cl fluxes determined 3 h following indomethacin addition yielded a net Cl flux of 0.07±0.09 μeq/cm^2 hr ($n = 6$) while the simultaneously determined I_{sc} was 1.3±0.2 μeq/cm^2 hr.

The influence of indomethacin on prostaglandin production by the tissue was examined by determining the concentrations of PGE_2 in the mucosal and serosal solutions during a 30-min incubation period in the presence and absence of the fatty acid cyclooxygenase inhibitor as described

in Methods. The data are given in Table 1. PGE_2 was released to both bathing solutions in a nonpreferential manner. The total rate of PGE_2 production was reduced approximately 80% by indomethacin.

Discussion

The results of this study clearly demonstrate that intracellular cAMP is a physiologic mediator of electrogenic Cl secretion across canine tracheal epithelium. This conclusion applies uniformly to epithelia that carry out electrogenic Cl secretion (Frizzell et al., 1979) including: small intestine (Field, 1971), colon (Frizzell et al., 1976), cornea (Zadunaisky, 1966; Zadunaisky, Lande, Chalfie & Neufeld, 1973), salivary gland (Berridge, Lindley & Prince, 1975; Prince, Berridge & Rasmussen, 1972), gastric mucosa (Sachs, Spenney & Lewin, 1978), dogfish rectal gland (Silva et al., 1977, 1979), and teleost gill (Cuthbert & Pic, 1973) and opercular epithelium (Degnan, Karnaky & Zadunaisky, 1977). In all of these tissues, Cl secretion is elicited or enhanced by addition of cAMP or theophylline to the bathing media. In addition, secretogogue-induced elevations of cellular cAMP concentrations have been reported for small (Field, Sheerin, Henderson & Smith, 1975) and large (Frizzell, 1977) intestine, cornea (Klyce, Neufeld & Zadunaisky, 1973), salivary gland (Prince et al., 1972), gills (Cuthbert & Pic, 1973), rectal gland (Stoff et al., 1980), and stomach (Katsumata & Glick, 1975).

Relation of Intracellular cAMP Levels to Cl Secretion Rate

One goal of our study was to correlate cAMP levels with the rate of Cl secretion. To examine the possibility that a *direct* relation existed between these parameters, we measured both I_{sc} and cAMP concentrations under control conditions and in paired tissues following addition of agents that stimulate or suppress Cl secretion. However, we were unable to detect a direct correlation of I_{sc} to cAMP levels under control conditions (Figs. 2 and 5), following stimulation (Figs. 2 and 3) or after inhibition by indomethacin (Fig. 7); this was true of tissues from the same animal as well as among tissues from different animals. For example, increasing concentrations of epinephrine added to the serosal solution stimulated Cl secretion before an increase in cellular cAMP levels could be detected (Fig. 3). In addition, the increase in cAMP content elicited by PGE_1 was substantially less than that obtained with epinephrine or theophylline (Fig. 2). Yet PGE_1 and epinephrine yielded maximal rates of Cl secretion, as judged both by the peak levels of I_{sc} they evoked, and by the failure of epinephrine to further enhance the I_{sc} of prostaglandin-treated tissues (data not shown). Finally, $PGF_{2\alpha}$ which has been shown to elevate cAMP levels in other systems (Dozois et al., 1978), increased I_{sc} without altering cyclic nucleotide levels.

The absence of a closer correlation between I_{sc} and cell cAMP concentrations could have several possible explanations. First, cAMP is labile and some loss may have occurred during sample processing; however, this would not account for the failure of spontaneous I_{sc} and cAMP levels to correlate. In addition, the increase in cAMP levels stimulated by theophylline was much greater than that obtained with PGE_1 (Fig. 2), yet the I_{sc} response was less. This is also difficult to explain as a fractional loss of cAMP during sample processing. Second, other mediators (e.g., Ca) may exert a parallel control over secretory rate (*see* below). The presence of multiple mediators and differences in the extent to which each participates in the response to extracellular stimuli would obscure a close relation between secretion rate and cAMP levels alone. Third, a small elevation of cAMP content may yield a maximal transport rate, so that under most of the experimental conditions we examined, cAMP may be generated in vast excess. The excess of cyclic nucleotide produced may govern other cellular processes unrelated, or poorly-related, to transport. Finally, the cAMP content of a specific cellular compartment may be critical in determining transport rate so that the transport-related pool of cAMP is a minor component of the total epithelial pool. This possibility has been raised previously for both Na-absorbing (Omachi, Robbie, Handler & Orloff, 1974) and Cl-secreting (Field et al., 1975) epithelia.

Regional Localization of the Responses to Secretogogues

In the present study, we analyzed the cAMP content of the surface epithelium alone. Comparison of the results illustrated in Figs. 2 and 5 indicates that the surface epithelium is involved in cAMP-mediated, electrogenic Cl secretion. Epinephrine and prostaglandins of the E series stimulate both electrogenic Cl secretion across the whole tissue as well as cAMP accumulation within the surface epithelium (Fig. 2). Inasmuch as phenylephrine and propranolol had no effect on either I_{sc} or cellular cAMP levels, the stimulation of elec-

trogenic Cl secretion by epinephrine must be due to β-receptor activation (Al-Bazzaz & Cheng, 1979). In contrast, α-adrenergic agonists (Phipps et al., 1980) and acetylcholine or parasympathetic nerve stimulation (Borson et al., 1980) elicit a neutral NaCl secretion from the submucosal glands and have no effect on cAMP content of the surface cells (Fig. 5). This indicates that humoral control over secretions of the surface epithelium and the glands is governed by different extracellular mediators and, in the case of catecholamine activation, by different receptor types. The results of our study do not permit conclusions regarding a possible role for cAMP in mediating neutral Cl secretion from the submucosal glands, although the known stimuli of gland secretion (cholinergic and α-adrenergic agonists) are not generally thought to evoke increases in cellular cAMP levels. The differential responses of surface and gland epithelia to neurohumoral agents correlates with the distribution of autonomic fibers to these regions (Rhodin, 1966). Using histochemical techniques, parasympathetic fibers have been identified only in the area of the submucosal glands while sympathetic fibers have been identified in both regions.

Within the surface epithelium, there are several cell types (Rhodin, 1966); ciliated columnar cells, goblet cells and, beneath these, basal and intermediate cells. But the most common of these is the columnar epithelial cell comprising 80–85% of the total population (Rhodin, 1966). The results of microelectrode studies, reported in the companion paper (Welsh et al., 1982), indicate that the first cells encountered when electrodes are advanced from the mucosal solution into the surface epithelium are involved in electrogenic Cl secretion, as judged by the responses of membrane potential and resistance to secretogogues. This would seem to rule out involvement of the basal and intermediate cells in Cl transport unless they are electrically-coupled to the columnar cells. Thus, of the cells present in the surface epithelium, it seems most likely that the columnar cells are responsible for cAMP-mediated, electrogenic Cl secretion.

Calcium as an Intracellular Mediator of Cl Secretion

The finding that ionophore A23187 stimulates electrogenic Cl secretion (Al-Bazzaz & Jayaram, 1981) without altering cell cAMP concentrations (Fig. 4) suggests that changes in cell Ca activity influence Cl secretion rate. Ca-dependent Cl secretion has also been suggested for small (Bolton & Field, 1977) and large (Frizzell, 1977) intestine, salivary gland (Prince et al., 1972, 1973; O'Doherty & Stark, 1981), and cornea (Candia, Montoreano & Podos 1977) and may be a general phenomenon in Cl-secreting tissues.

Prostaglandin $F_{2\alpha}$ mimics the action of A23187 in stimulating electrogenic Cl secretion while not altering cellular cAMP levels. However, due to the lack of a strict correlation between cAMP levels and I_{sc}, the intracellular mediator(s) of $PGF_{2\alpha}$-induced secretion cannot be identified. Both PGE_1 and $PGF_{2\alpha}$ at concentrations of 10^{-6} M maximally stimulate the I_{sc} (Figs. 2 and 4 and Al-Bazzaz et al., 1981) and subsequent addition of 10^{-5} M epinephrine to tissues treated with this concentration of PGE_1 or $PGF_{2\alpha}$ elicits no further increase in I_{sc} (data not shown). However, if the effect of 10^{-6} M $PGF_{2\alpha}$ were equivalent to that of 10^{-7} M epinephrine (Fig. 3), a near-maximal stimulation of I_{sc} with no apparent change in cAMP levels would be observed.

It is possible that prostaglandins of the E series are more potent stimuli of adenylate cyclase activity of tracheal mucosa than is $PGF_{2\alpha}$, as Dozois et al. (1978) have shown for canine gastric mucosa. PGE_2 is a much more potent relaxer of tracheal smooth muscle than $PGF_{2\alpha}$ (Anggard & Bergström, 1963; Main, 1964) and this physiologic difference in activity is paralleled by the effects of these agents on cAMP levels (Murad & Kimura, 1974). PGE_1 increased cAMP levels fivefold while $PGF_{2\alpha}$ at the same concentration was without effect. However, in canine tracheal epithelium, these prostaglandins elicited similar maximal values of I_{sc} (Figs. 2 and 4), yet $PGF_{2\alpha}$ had no detectable effect on cAMP levels. Thus, the possibility that factors other than cAMP contribute to stimulation of Cl secretion by $PGF_{2\alpha}$ requires further study.

We found that addition of 8-Br cGMP (10^{-3} M) to the serosal medium had no influence on the I_{sc}, suggesting that cGMP is not a mediator of electrogenic Cl secretion in this tissue and therefore would not be expected to account for the secretory responses elicited by A23187 or $PGF_{2\alpha}$. Studies carried out using rabbit ileum (P.L. Smith and M. Field, *unpublished observations*) indicate that A23187 does not alter cellular cGMP levels.

Prostaglandins as Regulators of Spontaneous Cl Secretion

Our results indicate that prostaglandins are effective secretogogues for tracheal epithelium and suggest that their actions are of physiologic signifi-

cance. This conslusion is supported by several findings: (1) addition of PGE_1 or $PGF_{2\alpha}$ increase Cl secretion rate (Figs. 2 and 4 and Al-Bazzaz et al., 1981). (2) fatty acid cyclooxygenase inhibitors, e.g., indomethacin, inhibit PGE_2 production by tracheal epithelium (Table 1), decrease cAMP levels and basal Cl secretion rate (Figs. 7 and 8). This demonstrates that the enzymes necessary for prostaglandin synthesis are present, and active. Finally, (3) in several pilot experiments, indomethacin prevented increases in I_{sc} and cAMP accumulation elicited by addition of arachadonic acid (10^{-5} M) to the serosal solution.

These observations indicate that endogenous prostaglandin production is responsible for the spontaneous secretion of Cl across canine tracheal epithelium observed under basal conditions. Whether endogenous synthesis of prostaglandins occurs within the secretory cells or whether it is a paracrine function of another cell type cannot be discerned from the present results. In addition, it is possible that a prostaglandin other than PGE_2 is responsible for basal Cl secretion and that its production is reduced by indomethacin in parallel with that of PGE_2.

The decrease in I_{sc} elicited by indomethacin was directly related, on a one-for-one basis, to the magnitude of the spontaneous I_{sc} observed under basal conditions (Fig. 9). Extrapolation of this relation to the ordinate yields the fraction of the basal I_{sc} that is insensitive to indomethacin. The magnitude of this current, 32 $\mu A/cm^2$ or 1.2 $\mu eq/cm^2$ hr, is in good agreement with the spontaneous rate of electrogenic Na absorption reported in other studies (Olver et al., 1975; Al-Bazzaz & Al-Awqati, 1979) and with the I_{sc} after prolonged indomethacin treatment (1.3 $\mu eq/cm^2$ hr) when Cl secretion has been abolished (*see* Results, p. 222). Experimentally, indomethacin affords the advantage of suppressing basal Cl secretion so that the effects of exogenous secretogogues are magnified (Welsh et al., 1982).

This research was supported by grants from the NIH: NIAMDD (AM 26702 and AM 27524) and Merck and Co.

Dr. Smith was supported by a National Research Service Award (AM 05973), Dr. Welsh by a National Pulmonary Faculty Training Award (HL-07159), and Dr. Frizzell by a research Career Development Award (AM 00173).

References

Al-Bazzaz, F.J. 1981. Role of cyclic AMP in regulation of chloride secretion by canine tracheal mucosa. *Am. Rev. Respir. Dis.* **123**:295–298

Al-Bazzaz, F.J., Al-Awqati, Q. 1979. Interaction between sodium and chloride transport in canine tracheal mucosa. *J. Appl. Physiol.: Respirat. Environ. Exercise Physiol.* **46**:111–119

Al-Bazzaz, F.J., Cheng, E. 1979. Effect of catecholamines on ion transport in dog tracheal epithelium. *J. Appl. Physiol.: Respirat. Environ. Exercise Physiol.* **47**:397–403

Al-Bazzaz, F.J., Jayaram, T. 1981. Ion transport by canine tracheal mucosa: Effect of elevation of cellular calcium *Exp. Lung Res.* **2**:121–130

Al-Bazzaz, F.J., Yadava, V.P., Westerfelder, C. 1981. Modification of Na and Cl transport in canine tracheal mucosa by prostaglandins. *Am. J. Physiol.* **240**:*F*101–*F*105

Anggard, E., Bergström, S. 1963. Biological effects of an unsaturated trihydroxy acid ($PGF_{2\alpha}$) from normal swine lung. *Acta Physiol. Scand.* **58**:1–12

Berridge, M.J., Lindley, B.D., Prince, W.T. 1975. Membrane permeability changes during stimulation of isolated salivary glands of *Calliphora* by 5-hydroxytryptamine. *J. Physiol. (London)* **244**:549–567

Bolton, J.E., Field, M. 1977. Ca ionophore-stimulated ion secretion in rabbit ileal mucosa: Relation to actions of cyclic 3′,5′-AMP and carbamylcholine. *J. Membrane Biol.* **35**:159–173

Borson, D.B., Chino, R.A., Davis, B., Nadel, J.A. 1980. Adrenergic and cholinergic nerves mediate fluid secretion from tracheal glands of ferrets. *J. Appl. Physiol.: Respirat. Environ. Exercise Physiol.* **49**:1027–1031

Candia, O.A., Montoreano, R., Podos, S.M. 1977. Effect of the ionophore A23187 on chloride transport across the isolated frog cornea. *Am. J. Physiol.* **233**:*F*94–*F*101

Cuthbert, A.W., Pic, P. 1973. Adenoreceptors and adenylcyclase in gills. *Brit. J. Pharmacol.* **49**:134–137

Davis, B., Marin, M.G., Yee, J.W., Nadel, J.A. 1979. Effect of terbutaline on movement of Cl and Na across the trachea of the dog *in vitro*. *Am. Rev. Resprirat. Dis.* **120**:547–552

Degnan, K.J., Karnaky, K.J., Zadunaisky, J.A. 1977. Active chloride transport in the *in vitro* opercular skin of a teleost (*Fundulus heteroclitus*), a gill-like epithelium rich in chloride cells. *J. Physiol. (London)* **271**:155–191

Dozois, R.R., Kim, J.K., Dousa, T.P. 1978. Interaction of prostaglandins with canine gastric mucosal adenylate cyclase – cyclic AMP system. *Am. J. Physiol.* **4(5)**:*E*546–*E*551

Dray, F., Charbonnel, B., Maclouf, G. 1975. Radioimmunoassay of prostaglandin F, E_1, E_2 in human plasma. *Eur. J. Clin. Invest.* **5**:311–318

Field, M. 1971. Ion transport in rabbit ileal mucosa. II. Effects of cyclic 3′,5′-AMP. *Am. J. Physiol.* **221**:992–997

Field, M., Sheerin, H.E., Henderson, A., Smith, P.L. 1975. Catecholamine effects on cyclic AMP levels and ion secretion in rabbit ileal mucosa. *Am. J. Physiol.* **229**:86–92

Frizzell, R.A. 1977. Active chloride secretion by rabbit colon: Calcium-dependent stimulation by ionophore A23187. *J. Membrane Biol.* **35**:175–187

Frizzell, R.A., Field, M., Schultz, S.G. 1979. Sodium-coupled chloride transport by epithelial tissues. *Am. J. Physiol.* **236**:*F*1–*F*8

Frizzell, R.A., Koch, M.J., Schultz, S.G. 1976. Ion transport by rabbit colon. I. Active and passive components. *J. Membrane Biol.* **27**:297–316

Katsumata, Y., Glick, D. 1975. Effects of drugs influencing gastric secretion on the quantitative histological distribution of cyclic adenosin 3′:5′-monophosphate in the rat stomach. *Gastroenterology* **69**:409–415

Klyce, S.D., Neufeld, A.H., Zadunaisky, J.A. 1973. The activation of chloride transport by epinephrine and DB cyclic-AMP in the cornea of the rabbit. *Invest. Opthamol.* **12**:127–133

Lowry, O.H., Rosebrough, N.J., Farr, A.L., Randall, R.J. 1951. Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* **193**:265–275

Main, I.H.M. 1964. The inhibitory actions of prostaglandins on respiratory smooth muscle. *Brit. J. Pharmacol. Chemother.* **22**:511–519

Marin, M.G., Davis, B., Nadel, J.A. 1976. Effect of acetylcholine on Cl^- and Na^+ fluxes across dog tracheal epithelium *in vitro*. *Am. J. Physiol.* **231**:1546–1549

Marin, M.G., Davis, B., Nadel, J.A. 1977. Effect of histamine on electrical and ion transport properties of tracheal epithelium. *J. Appl. Physiol.: Respirat. Environ. Exercise Physiol.* **42**:735–738

Murad, F., Kimura, H. 1974. Cyclic nucleotide levels in incubations of guinea pig trachea. *Biochim. Biophys. Acta* **343**:275–286

Nadel, J.A. 1980. Mucus secretion and ion transport in airways. *Fed. Proc.* **39**:3061

O'Doherty, J., Stark, R.J. 1981. Transmembrane and transepithelial movement of calcium during stimulus-secretion coupling. *Am. J. Physiol.* **241**:*G*150–*G*158

Olver, R.E., Davis, B., Marin, M.G., Nadel, J.A. 1975. Active transport of Na^+ and Cl^- across the canine tracheal epithelium. *Am. Rev. Resp. Dis.* **112**:811–815

Omachi, R.S., Robbie, D.E., Handler, J.S., Orloff, J. 1974. Effects of ADH and other agents on cyclic AMP accumulation in toad bladder epithelium. *Am. J. Physiol.* **266**:1152–1157

Phipps, R.J., Nadel, J.A., Davis, B. 1980. Effect of alpha-adrenergic stimulation on mucus secretion and on ion transport in cat trachea *in vitro*. *Am. Rev. Respir. Dis.* **121**:359–365

Prince, W.T., Berridge, M., Rasmussen, H. 1972. Role of calcium and adenosine-3′:5′-cyclic monophosphate in controlling fly salivary gland secretion. *Proc. Natl. Acad. Sci. USA* **69**:553–557

Prince, W.T., Rasmussen, H., Berridge, M.J. 1973. The role of calcium in fly salivary gland secretion analyzed with the ionophore A23187. *Biochim. Biophys. Acta* **329**:98–107

Rhodin, J.A. 1966. Ultrastructure and function of the human tracheal mucosa. *Am. Rev. Respirat. Dis.* **93**:1–15

Sachs, G., Spenney, J.G., Lewin, M. 1978. H^+ transport: Regulation and mechanism in gastric mucosa and membrane vesicles. *Physiol. Rev.* **58**:106–173

Silva, P., Stoff, J., Field, M., Fine, L., Forrest, J.N., Epstein, F.H. 1977. Mechanism of active chloride secretion by shark rectal gland: Role of Na–K–ATPase in chloride transport. *Am. J. Physiol.* **233**:*F*298–*F*306

Smith, P.L., Blumberg, J.B., Stoff, J.S., Field, M. 1980. Antisecretory effects of indomethacin on rabbit ileal mucosa *in vitro*. *Gastroenterology* **80**:356–365

Stoff, J.S., Stemerman, M., Steer, M., Salzman, S., Brown, R.S. 1980. A defect in platelet aggregation in Barter's Syndrome. *Am. J. Med.* **68**:171–180

Ueki, I., German, V.F., Nadel, J.A. 1980. Micropipette measurement of airway submucosal gland secretion: Autonomic effects. *Am. Rev. Respirat. Dis.* **121**:351–357

Welsh, M.J., Smith, P.L., Frizzell, R.A. 1982. Chloride secretion by canine tracheal epithelium: II. The cellular electrical potential profile. *J. Membrane Biol.* **70**:227–238

Widdicombe, J.H., Welsh, M.J. 1980. Ion transport by dog tracheal epithelium. *Fed. Proc.* **39**:3062–3066

Zadunaisky, J.A. 1966. Active transport of chloride in frog cornea. *Am. J. Physiol.* **211**:506–512

Zadunaisky, J.A., Lande, M.A., Chalfie, M., Neufeld, A.H. 1973. Ion pumps in the cornea and their stimulation by epinephrine and cyclic AMP. *Exp. Eye Res.* **15**:577–584

Received 6 January 1982; revised 28 May 1982