# **Regulation of Sodium Absorption by Canine Tracheal Epithelium**

Joseph J. Cullen and Michael J. Welsh

Laboratory of Epithelial Transport and Pulmonary Division, Department of Internal Medicine, University of Iowa College of Medicine, Iowa City, Iowa 52242

### Abstract

To regulate the quantity of respiratory tract fluid, the airway epithelium either secretes chloride, Cl<sup>-</sup>, or reabsorbs sodium, Na<sup>+</sup>. Many secretagogues inhibit Na<sup>+</sup> absorption, but the decrease may result from a fall in the electrochemical gradient for Na<sup>+</sup> absorption. We examined regulation of Na<sup>+</sup> absorption independent of Cl<sup>-</sup> secretion, by bathing canine tracheal epithelium in Cl<sup>-</sup>-free, gluconate Ringers solution. Prostaglandin E<sub>2</sub>, 2chloroadenosine, and isoproterenol increased short-circuit current (Isc) and the rate of Na<sup>+</sup> absorption. In contrast, indomethacin, which inhibits endogenous prostaglandin production, decreased Isc. These agents regulate cellular levels of cAMP; direct addition of 8-Br-cAMP also acutely increased Isc. We examined chronic regulation of Na<sup>+</sup> absorption in cell monolayers grown on permeable supports in serum-free media. Exposure to aldosterone for two days increased baseline Isc by 50% and the amilorideinhibitable current by 55%. These data indicate that Na<sup>+</sup> absorption is both acutely and chronically regulated in the airway epithelium.

### Introduction

Tracheal epithelium has the capacity for both active Cl<sup>-</sup> secretion and active Na<sup>+</sup> absorption (1). The relative magnitude of these two transport processes determines whether the epithelium will secrete or absorb fluid. Although many neurohumoral agents are known to regulate Cl<sup>-</sup> secretion, much less is known about the direct regulation of Na<sup>+</sup> absorption. Previous reports show that many agents that stimulate Cl<sup>-</sup> secretion produce a simultaneous fall in the rate of Na<sup>+</sup> absorption (2–4). However, such a decrease might be secondary to a decrease in the electrochemical gradient favoring Na<sup>+</sup> entry into the cell across the apical membrane: secretagogues depolarize the electrical potential difference across the apical membrane (5) and increase intracellular Na<sup>+</sup> activity (6).

The purpose of this study was to directly examine the regulation of Na<sup>+</sup> absorption. To examine acute regulation we bathed tissues in Cl<sup>-</sup>-free media so that Na<sup>+</sup> absorption would not be secondarily altered by changes in Cl<sup>-</sup> secretion or Cl<sup>-</sup> conductance. We examined several neurohumoral agents known to have receptors on the epithelial cells and known to regulate

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Cl<sup>-</sup> secretion. To examine chronic regulation we grew cultured cells in aldosterone-containing media.

### Methods

The posterior membranous portion of dog tracheal epithelium was prepared as previously described (7). The bathing solution was a modified Cl<sup>-</sup>-free Ringer's solution containing (in mM): 139.3 Na-gluconate, 0.6 KH<sub>2</sub>PO<sub>4</sub>, 2.4 K<sub>2</sub>HPO<sub>4</sub>, 1.2 MgSO<sub>4</sub>, 10 glucose, and 10 Hepes (buffered to pH 7.4). In our first experiments we used 1.2 mM CaSO<sub>4</sub>, but because gluconate buffers  $Ca^{2+}$  (8), in most experiments we used 10 mM CaSO<sub>4</sub>. Tissues bathed in media that contained 10 mM CaSO<sub>4</sub> had a more stable resistance, but the response to addition of hormones was the same. This later finding is consistent with previous observations that the active transport properties of trachea show little dependence on extracellular  $Ca^{2+}$  (9). After removal from the animal, the tissue was placed in Cl<sup>-</sup>free Ringer's solution. When the tissues were mounted in chambers they were bathed with the Cl<sup>-</sup>-free solution, which was replaced twice at halfhour intervals to remove residual Cl<sup>-</sup>. Salt bridges were 140 mM Nagluconate/4% agar. Solutions were bubbled with 100% O2 and maintained at 37°C.

Tracheal epithelial cells were isolated and grown in tissue culture as previously described (10, 11), except that the filters were attached to the bottom of a Lucite tube (exposed area  $0.64 \text{ cm}^2$ ). The media was a 1:1 mixture of Dulbecco's modified Eagle's medium and Ham's F-12 containing 10% fetal calf serum for the first 5–6 d in culture. Then the fetal calf serum was deleted from the media and aldosterone was added to some of the monolayers. The media of both control and aldosterone-treated monolayers was changed after 1 d and the monolayers were studied 1 d later. During measurement of the electrical properties, monolayers were bathed symmetrically with a phosphate-buffered NaCl-Ringer's solution (37°C, equilibrated with room air, pH = 7.4).

For measurement of transepithelial electrical properties and radioisotope fluxes, tissues were mounted in Ussing chambers (1.5 or 0.2 cm<sup>2</sup> surface area) and monolayers were mounted in a specially adapted Ussing chamber. The transepithelial electrical potential difference (referenced to the mucosal solution) was automatically clamped to zero (the shortcircuit condition) by automatic voltage-current clamps. Transepithelial slope conductance ( $G_t$ ) was calculated from the change in current required to clamp transepithelial voltage to  $\pm 10$  mV (duration 1 s; period 50 s). For the measurement of unidirectional transepithelial fluxes of Na<sup>+</sup>, 5  $\mu$ Ci of <sup>22</sup>Na was added to the appropriate side of the tissue (the volume of fluid on each side of the tissue was 8 ml). 1 h was allowed for fluxes of Na<sup>22</sup> to reach steady state and then three to four samples of both bathing solutions were taken at 10- to 20-min intervals during each control and experimental period.

Chloride concentrations were measured with a chloridometer (Laboratory Glass and Instruments Corp., New York). Chemicals used were isoproterenol (Elkins-Sinn, Inc., Cherry Hill, NJ); PGE<sub>2</sub>, 8-Br-cAMP, 2-chloroadenosine, indomethacin (Sigma Chemical Co., St. Louis, MO); propranolol (Ayerst Laboratories Inc., New York), phenylephrine (Winthrop Laboratories, New York), and A-23187 (Calbiochem-Behring Corp., La Jolla, CA).

Results are presented as means $\pm$ SEM. Statistical significance was evaluated using a paired *t* test; a *P* value < 0.05 was considered statistically significant.

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# Results

Effect of hormones and mediators on short circuit current (Isc).<sup>1</sup> Prostaglandin  $E_2$  (PGE<sub>2</sub>) stimulates the rate of Cl<sup>-</sup> secretion and prostaglandin production by the epithelium regulates the baseline rate of Cl<sup>-</sup> secretion (2, 5, 12). To determine if prostaglandins had an effect on Na<sup>+</sup> absorption, we examined the effect of PGE<sub>2</sub> on the electrical properties of tissues bathed in Cl<sup>-</sup>-free media. Fig. 1 shows that PGE<sub>2</sub> added to the submucosal solution increased Isc, suggesting an increase in Na<sup>+</sup> absorption. In contrast to its effect on Cl<sup>-</sup> secretion which is quite rapid, PGE<sub>2</sub> produced a slow increase in Isc, with Isc reaching a maximum at ~1 h.

We also examined the effect of two other agents that regulate ion transport in tracheal epithelium (13, 14). Table I shows that the  $\beta$ -adrenergic agonist, isoproterenol (5 × 10<sup>-6</sup> M, submucosal solution) and the adenosine analogue, 2-chloroadenosine (10<sup>-5</sup> M, mucosal solution) also increased Isc. Although the changes in Isc were small, they were consistent.

To determine if endogenous prostaglandin production by the epithelium may play some role in regulating Na<sup>+</sup> absorption, we examined the effect of adding the prostaglandin synthesis inhibitor indomethacin  $(10^{-6} \text{ M})$  to the mucosal solution. Fig. 2 shows that indomethacin decreased Isc, suggesting a decrease in the rate of Na<sup>+</sup> absorption. Fig. 3 shows that the magnitude of the decrease in Isc produced by indomethacin was dependent on the initial value of Isc, providing further support for the notion that endogenous prostaglandin production by the epithelium regulates the baseline rate of Na<sup>+</sup> absorption. To be sure that indomethacin did not produce a nonspecific decrease in Isc or a toxic effect, we examined the Isc response to isoproterenol in indomethacin-treated tissues. Table II shows that indomethacin decreased Isc compared to control tissues but did not prevent the response to subsequent addition of isoproterenol. Following addition of isoproterenol, Isc was the same for tissues that did and did not receive indomethacin.

Prostaglandin  $E_2$ , isoproterenol, and 2-chloroadenosine increase and indomethacin decreases cellular levels of cAMP in tracheal epithelium (2, 12, 14). It thus seemed likely that cAMP might also be responsible for the increase in Isc observed in Cl<sup>-</sup>free media. To test this possibility, we examined the effect of exogenous addition of cAMP. Table III shows that 8-Br-cAMP ( $5 \times 10^{-5}$  M) added to the mucosal and submucosal solutions also increased Isc. These observations suggest that cAMP may increase Na<sup>+</sup> absorption.

We also examined the effect of the  $\alpha$ -adrenergic agonist phenylephrine. Tissues were pretreated with propranolol ( $10^{-5}$  M mucosal and submucosal solutions) to block any  $\beta$ -adrenergic effects. Subsequent addition of phenylephrine ( $10^{-6}$  M, submucosal solution) did not alter the Isc: Isc was  $34.7\pm6.0$  before and  $34.3\pm8.0$  after the addition of phenylephrine (n = 7).

An increase in cell calcium concentration is thought to inhibit Na<sup>+</sup> absorption in a variety of epithelia (15, 16), primarily by inhibiting Na<sup>+</sup> entry at the apical membrane (17). To determine if an increase in cell calcium might inhibit Na<sup>+</sup> absorption in airway epithelium, we first stimulated Isc with 8-Br-cAMP and then added A-23187. Table IV shows that under these conditions the calcium ionophore decreased Isc, suggesting that an increase in cell calcium concentration inhibits Na<sup>+</sup> absorption.



Time (min)

Figure 1. Effect of PGE<sub>2</sub> on Isc. PGE<sub>2</sub>  $(10^{-6} \text{ M})$  was added to the submucosal solution at time zero. "Control" tissues received no intervention. Values are means±SEM for seven tissues.

Changes in Isc result from changes in  $Na^+$  absorption. The data presented above suggest that changes in Isc resulted from changes in the rate of  $Na^+$  absorption. The direction of the change in Isc is, in most cases, however, similar to that which would be expected for stimulation of  $Cl^-$  secretion. To confirm that the changes in Isc resulted from changes in Na<sup>+</sup> absorption and not  $Cl^-$  secretion, we did four studies.

First, we measured the Cl<sup>-</sup> concentration of the bathing solutions taken from the chamber at the end of an experiment: in 10 samples, the highest Cl<sup>-</sup> concentration was 0.1 mM. Moreover, in tissues that were stimulated with isoproterenol or PGE<sub>2</sub>, addition of 0.5 mM Cl<sup>-</sup> to the mucosal and submucosal solutions did not alter Isc (n = 4). These findings are consistent with the high concentrations of Cl<sup>-</sup> required to produce a half-maximal rate of Cl<sup>-</sup> secretion (unpublished observations).

Second, we examined the effect of the loop diuretic bumetanide. Loop diuretics inhibit Cl<sup>-</sup> secretion in tracheal epithelium by blocking Cl<sup>-</sup> uptake into the cell at the basolateral membrane (7, 18). In three tissues that had been stimulated with 8-Br-cAMP ( $5 \times 10^{-5}$  M, mucosal and submucosal solution), addition of bumetanide ( $10^{-4}$  M) to the submucosal solution did not alter Isc. Isc was  $6.7\pm 2.7$  before and  $6.8\pm 3.0$  after bumetanide, indicating that there was no appreciable Cl<sup>-</sup> secretion.

Third, we examined the effect of amiloride, which inhibits electrogenic Na<sup>+</sup> absorption in tracheal epithelium (4). Amiloride reversed the increase in Isc produced by isoproterenol. Fig. 4 shows that amiloride ( $10^{-4}$  M, added to the mucosal solution) decreased both Isc and  $G_t$ , consistent with the conclusion that the increase in Isc resulted from electrogenic Na absorption. We also found that amiloride ( $10^{-4}$  M, mucosal solution) prevented the increase in Isc produced by 8-Br-cAMP (n = 4).

For the studies shown in Fig. 4 we used an amiloride concentration ( $10^{-4}$  M), which provides maximal inhibition of Na<sup>+</sup> absorption. Fig. 5, however, shows that amiloride decreased the Isc at concentrations similar to those required to inhibit electrogenic Na<sup>+</sup> absorption in other systems (19). Half-maximal inhibition of the Isc was observed at an amiloride concentration of  $1.1 \times 10^{-7}$  M.

Fourth, the most direct evidence that  $Na^+$  absorption increased came from an examination of the effect of isoproterenol on <sup>22</sup>Na fluxes. Table V shows that isoproterenol significantly

<sup>1.</sup> Abbreviations used in this paper: CF, cystic fibrosis; Isc, short circuit current;  $PGE_2$ , prostaglandin  $E_2$ .

	Baseline		Test		Δ	
	Isc	Gı	Isc	Gt	Isc	Gt
Control	37.3±6.9	4.11±0.52	29.0±7.0	4.16±0.57	-8.3±2.2*	0.05±0.18
PGE <sub>2</sub> (10 <sup>-6</sup> M)	34.1±10.2	3.23±0.49	38.8±14.8	3.15±0.42	+4.8±4.8 <sup>‡</sup>	$-0.08 \pm 0.23$
Control	19.0±6.9	2.69±0.47	19.2±7.2	2.65±0.47	+0.2±0.5	-0.04±0.01
Isoproterenol (5 $\times$ 10 <sup>-6</sup> M)	19.8±4.8	3.77±1.16	23.8±4.9	3.76±1.19	+4.0±1.2 <sup>‡*</sup>	$-0.01\pm0.05$
Control	15.9±6.3	2.92±0.15	15.3±6.2	2.86±0.92	-0.6±1.6	-0.04±0.13
2-chloroadenosine (10 <sup>-5</sup> M)	16.8±7.3	3.26±0.92	21.1±8.5	3.24±0.86	+4.3±2.1 <sup>‡</sup>	-0.02±0.16

Table I. Effect of Prostaglandin E2, Isoproterenol, and 2-Chloroadenosine on Transepithelial Electrical Properties

Isc, short-circuit current and  $G_t$  to transepithelial conductance. Tissue pairs from the same animal were studied in two consecutive periods: a baseline period and a test period. Control tissues received no intervention between the first and second period while experimental tissues received the agents indicated. Values represent means±SEM for eight pairs of tissues for PGE<sub>2</sub>, six pairs for isoproterenol and five pairs for 2-chloroadeno-sine. \* Indicates a statistical difference between baseline and test periods, P < 0.05. \* Indicates a statistical difference between control and experimental tissues, P < 0.05.

increased both net Na<sup>+</sup> absorption and the Na<sup>+</sup> flux from the mucosal to submucosal solution.

Effect of aldosterone on  $Na^+$  absorption. The data presented above indicates that  $Na^+$  absorption can be acutely regulated.



Figure 2. Effect of indomethacin on Isc. Indomethacin  $(10^{-6} \text{ M})$  was added to the mucosal solution at the time zero. "Control" tissues received no intervention. Values are means±SEM for 10 tissues.





To determine if Na<sup>+</sup> absorption can also be chronically regulated we examined the effect of aldosterone. We chose to examine the effect of aldosterone because it is known to chronically regulate electrogenic Na<sup>+</sup> absorption in a variety of renal and intestinal epithelia (20). For these studies we used cultured epithelial cells because of the ease of manipulating the hormonal environment, i.e., the cells were grown in serum-free media with or without aldosterone ( $10^{-6}$  M). Table VI shows that monolayers exposed to aldosterone had a 50% higher Isc than those that did not receive aldosterone. Moreover, the inhibition of Isc by amiloride was 55% greater in the aldosterone-treated monolayers. After addition of amiloride, we added forskolin to stimulate Cl<sup>-</sup> secretion. The equivalent increase in Isc in both groups suggests that the Cl transport properties remained intact and were not altered by aldosterone.

# Discussion

This study examined the acute and chronic regulation of Na<sup>+</sup> absorption in canine tracheal epithelium. We found that several

Table II. Effect of Isoproterenol in the Presence of Indomethacin

	Isc	Isc
	μA cm <sup>-2</sup>	μA cm <sup>-2</sup>
Period 1	Baseline	Baseline
	15.6±6.5	19.7±2.46
Period 2	Control	Indomethacin
	15.5±7.1	10.1±1.9*
Period 3	Isoproterenol 19.4±7.1*	Indomethacin and isoproterenol $17.3\pm2.2^*$

Tissue pairs were studied in three consecutive periods: period 1, a baseline period; period 2, in which one of the tissue pairs received indomethacin ( $10^{-6}$  M, mucosal solution); and period 3, in which isoproterenol ( $5 \times 10^{-6}$  M) was added to the submucosal solution (n = 9 pairs of tissues). \* Indicates a statistical difference compared to the preceding period, P < 0.05.

	Baseline		Test	Test		Δ	
	Isc	Gt	Isc	Gt	Isc	Gt	
	μA cm <sup>-2</sup>	mS cm⁻²	μA cm <sup>-2</sup>	mS cm <sup>−2</sup>	μA cm <sup>-2</sup>	mS cm <sup>−2</sup>	
Control	31.7±7.8	3.99±0.59	27.4±8.0	4.10±0.61	-4.3±2.5	+0.11±0.21	
8-Br-cAMP	29.9±9.5	3.00±0.60	38.2±13.7	3.11±0.58	+8.3±5.2*	+0.11±0.16	

Table III. Effect of 8-Br-cAMP on Transepithelial Electrical Properties

8-Br-cAMP ( $5 \times 10^{-5}$  M) was added to both the submucosal and mucosal bathing solutions. (n = 9 pairs of tissues). \* Indicates a statistical difference between the control and experimental tissues, P < 0.05.

Table IV. Effect of A-23187 on Transepithelial Electrical Properties

	Baseline		Test		Δ	
	Isc	Gt	Isc	Gt	Isc	Gt
	μA cm <sup>-2</sup>	mS cm⁻²	μA cm <sup>-2</sup>	mS cm⁻²	μA cm <sup>-2</sup>	mS cm <sup>-2</sup>
Control	41.9±18.8	2.97±1.70	43.3±17.5	2.89±1.63	+1.4±1.6	-0.08±0.09
A-23187 (10 <sup>-6</sup> m)	47.8±17.4	4.82±0.81	41.1±15.3	4.75±0.88	-6.7±3.1*	-0.07±0.17

All tissues were pretreated with 8-Br-cAMP ( $5 \times 10^{-5}$ ). Tissue pairs were studied in two consecutive periods: a baseline and a test period, during which Ca<sup>2+</sup> ionophore A-23187 ( $10^{-6}$  M) was added to the mucosal solution. Values represent means±SEM for five pairs of tissues. \* Indicates a statistical difference between control and experimental tissues, P < 0.05.

neurohumoral mediators, including isoproterenol,  $PGE_2$ , and 2-chloroadenosine, acutely stimulate Na<sup>+</sup> absorption. The previous observation that these agents increase cellular levels of cAMP (12, 14), together with the finding that exogenous addition of cAMP also stimulates Na<sup>+</sup> absorption, suggests that cAMP may be the intracellular mediator responsible for regulating Na<sup>+</sup> absorption. The data also suggest that endogenous prostaglandin production by the epithelium may regulate the baseline rate of Na<sup>+</sup> absorption under these conditions. The prostaglandin-synthesis inhibitor indomethacin decreased the spontaneous rate of Na<sup>+</sup> absorption without blocking the increase in Na<sup>+</sup> absorption produced by isoproterenol. Previous studies have shown that indomethacin decreases intracellular levels of cAMP in canine tracheal epithelium (2, 12).

The parallels between the acute regulation of Na<sup>+</sup> absorption in this study and the acute regulation of Cl<sup>-</sup> secretion are interesting. Isoproterenol, PGE<sub>2</sub>, and 2-chloroadenosine stimulated Na<sup>+</sup> absorption in Cl<sup>-</sup>-free solutions; the same agents stimulate Cl<sup>-</sup> secretion in Cl<sup>-</sup>-containing solutions (2, 13, 14). Exogenous cAMP stimulated Na<sup>+</sup> absorption in this study; it stimulates Cl<sup>-</sup> secretion in the presence of Cl<sup>-</sup> (3). Prostaglandin production by the epithelium probably regulates the baseline rate of Na<sup>+</sup> absorption in the absence of Cl<sup>-</sup>; prostaglandins also control the spontaneous rate of Cl<sup>-</sup> secretion in Cl<sup>-</sup>-containing media (2, 5, 12). In contrast to these parallels, in Cl<sup>-</sup>-containing solutions, agents that stimulate Cl<sup>-</sup> secretion usually decrease or do not alter the rate of Na<sup>+</sup> absorption. The inhibition of Na<sup>+</sup> absorption associated with stimulation of Cl<sup>-</sup> secretion may be indirect, however, resulting from a decrease in the electrochemical gra-



Figure 4. Effect of isoproterenol  $(5 \times 10^{-6} \text{ M}, \text{submucosal solution})$  and amiloride  $(10^{-4} \text{ M}, \text{mucosal solution})$  on Isc and  $G_t$ . Drugs were added at the times indicated (n = 6).



Figure 5. Effect of increasing concentrations of mucosal amiloride on the Isc of tissues treated with 8-Br-cAMP (5  $\times$  10<sup>-5</sup> M, mucosal solution) (n = 3).

dients for Na<sup>+</sup> absorption (5, 6), thereby masking any direct effect of hormones on the Na<sup>+</sup> transport pathway. The advantage of this study is that the use of Cl<sup>-</sup>-free solutions allowed us to directly examine the effect of these agents on Na<sup>+</sup> absorption without secondary effects produced by changes in electrochemical gradients.

The observation that cAMP acutely stimulates Na<sup>+</sup> absorption in airway epithelia is similar to findings in another lung epithelium, cultured monolayers of alveolar type II cells. Exogenous addition of cAMP or  $\beta$ -adrenergic agonists increased the rate of dome formation in type II cells grown on impermeable supports (21) and increased the Isc of cells grown on permeable supports (22). These results suggest stimulation of Na<sup>+</sup> absorption by alveolar epithelium. Cyclic-AMP mediated stimulation of Na<sup>+</sup> absorption has also been observed in amphibian urinary bladder and amphibian skin (23–25). In these epithelia, antidiuretic hormone stimulation of electrogenic Na<sup>+</sup> absorption is mediated by an increase in cellular levels of cAMP.

The data also suggest that an increase in intracellular  $Ca^{2+}$  may inhibit Na<sup>+</sup> absorption. When the rate of Na<sup>+</sup> absorption was increased by isoproterenol, the calcium ionophore A-23187 decreased the Isc. An inhibition of Na<sup>+</sup> absorption by an increase in the intracellular Ca<sup>2+</sup> concentration has also been observed in renal epithelia and is thought to result from inhibition of apical Na<sup>+</sup> channels (15–17).

One other study that suggests acute regulation of Na absorption in the airway epithelium is that of Legris et al. (26). In baboon bronchus mucosal addition of harmaline, which is present in airway APUD (amine-precursor-uptake-and-decarboxylation) cells, inhibited Na<sup>+</sup> absorption. The inhibition by harmaline may result from a direct, amiloride-like block of apical Na<sup>+</sup> channels.

In addition to acute regulation, the data indicate that Na<sup>+</sup> absorption is chronically regulated by aldosterone. This result is consistent with the observations of Hopfer and Legris (personal communication). They observed that volume depletion in patients with cystic fibrosis was associated with an increase in serum aldosterone concentration and an increase in the transepithelial voltage of the upper airway.

Our study has some limitations. First, the conditions for studying acute regulation are artificial, in that the bathing solutions contain no Cl<sup>-</sup>. On the one hand, Cl<sup>-</sup>-free media might alter intracellular metabolism or unknown intracellular regulatory steps. On the other hand, the study would not have been possible without the use of Cl<sup>-</sup>-free solutions. We considered the alternative of using agents that block Cl<sup>-</sup> secretion. Unfortunately, however, neither loop diuretics which block Cl<sup>-</sup> entry into the cell (7, 18) nor carboxylic acid analogues that block the Cl channel (27), completely abolish Cl secretion. Moreover the use of loop diuretics to inhibit Cl secretion would not have prevented the cAMP-induced increase in apical Cl<sup>-</sup> conductance with possible effects on apical membrane voltage. Finally, these drugs might have other unrecognized effects; certainly carboxylic acid analogues have injurious effects with prolonged incubation (27). Second, this study does not allow us to address the mechanism by which Na<sup>+</sup> absorption is increased. Although an increase in the apical Na<sup>+</sup> permeability is a reasonable explanation, stimulation of the Na-K-ATPase or an increase in basolateral K<sup>+</sup> permeability (which would increase the electrical driving force for Na<sup>+</sup> entry) might also account for stimulation of Na<sup>+</sup> absorption.

One unexplained observation in this study is that Isc did not decrease to zero following addition of amiloride. If all of the current were due to electrogenic Na<sup>+</sup> absorption we would have expected amiloride to completely inhibit it. However, it seems unlikely that other transport processes would be accounting for the current:  $HCO_3^-$  secretion is unlikely as the solutions are  $HCO_3^-$  and  $CO_2$  free; H<sup>+</sup> secretion would produce a negative value of Isc; significant K absorption seems unlikely based on previous studies (28, 29) and the low concentration of K in the solutions; and finally, most other electrically neutral transport processes that might indirectly be involved either require Cl<sup>-</sup> or  $HCO_3^-$  or are inhibited by high (1 mM) concentrations of amiloride (i.e., Na<sup>+</sup> – H<sup>+</sup> exchange, see Fig. 5). It is possible that,

Table V. Effect of Isoproterenol on Electrical Properties and Na<sup>+</sup> Fluxes

	Isc	G	J <sup>Na</sup> ms	J <sup>Na</sup>	J <sup>Na</sup> Net
	$\mu eq \cdot cm^{-2} h^{-1}$	mS⋅cm <sup>-2</sup>	$\mu eq \cdot cm^{-2} h^{-1}$	$\mu eq \cdot cm^{-2} h^{-1}$	$\mu eq \cdot cm^{-2} h^{-1}$
Control	0.34±0.12	2.67±0.39	2.45±0.68	2.31±0.46	0.14±0.52
Isoproterenol	0.68±0.20	2.89±0.45	3.22±0.77	2.70±0.50	0.53±0.51
Δ	0.31±0.09*	0.22±0.06*	0.77±0.24*	0.39±0.23*	0.39±0.16*

Indomethacin (10<sup>-6</sup> M) was present in the mucosal solution during both periods.  $J_{Nas}^{Nas}$ ,  $J_{Nas}^{Na}$ , and  $J_{Nas}^{Na}$  refer to Na<sup>+</sup> flux from mucosal to submucosa, submucosa to mucosa, and the calculated net flux, respectively (n = 9 tissue pairs). \* Value different from zero, P < 0.05.

Table VI. Effect of Aldosterone on Isc

	Baseline	Amiloride	ΔAmiloride	Forskolin	ΔForskolin
Control	2.6±0.3	0.7±0.3	-1.8±0.2	9.3±0.8	+8.6±0.8
Aldosterone	3.9±0.5*	1.1±0.4	-2.8±0.3*	9.1±1.0	+8.0±0.8

Values of Isc (in microamperes per square centimeter) were measured in cultured monolayers under baseline conditions, following addition of amiloride ( $10^{-4}$ ) to the mucosal solution and then after forskolin ( $10^{-5}$ ) was added to the mucosal solution.  $\Delta$ Amiloride and  $\Delta$ forskolin refer to the change in Isc following addition of these agents. Values are from 11 control monolayers and 9 monolayers in which the media contained aldosterone (1  $\mu$ M). \* Value different from control, P < 0.02.

under these conditions, amiloride does not inhibit all of the electrogenic Na<sup>+</sup> absorption. In support of this suggestion are the observations that in canine and bovine tracheal epithelium bathed in Cl<sup>-</sup> containing solution amiloride did not completely inhibit electrogenic Na<sup>+</sup> absorption (4, 30).

These observations may be important in understanding abnormalities of airway epithelial ion transport in cystic fibrosis (CF). The airway epithelia from patients with CF are Cl<sup>-</sup> impermeable (31, 32), a defect that has been localized to the apical cell membrane (33). An increase in the rate of Na<sup>+</sup> absorption has also been observed (34). In addition, Boucher et al. (34) found that in CF nasal epithelia isoproterenol stimulated amiloride-sensitive Na<sup>+</sup> absorption, rather than stimulating Cl<sup>-</sup> secretion as it does in normal airways. We speculate that the stimulation of Na<sup>+</sup> absorption in a Cl<sup>-</sup> impermeable CF epithelium may have some similarities to stimulation of Na<sup>+</sup> absorption in a normal tracheal epithelium in which Cl<sup>-</sup> has been removed. cAMP-mediated stimulation of Na<sup>+</sup> absorption thus might be a normal process in airway epithelia that is uncovered by the Cl<sup>-</sup> impermeability in CF.

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# References

1. Olver, R. E., B. Davis, M. G. Marin, and J. A. Nadel. 1975. Active transport of Na<sup>+</sup> and Cl<sup>-</sup> across the canine tracheal epithelium *in vitro*. *Am. Rev. Respir. Dis.* 112:811–815.

2. Al-Bazzaz, F., V. P. Yadava, and C. Westenfelder. 1981. Modification of Na and Cl transport in canine tracheal mucosa by prostaglandins. *Am. J. Physiol.* 240:F101-F105.

3. 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.

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

5. Welsh, M. J., P. L. Smith, and R. A. Frizzell. 1982. Chloride

secretion by canine tracheal epithelium. II. The cellular electrical potential profile. J. Membr. Biol. 70:227–238.

6. Shorofsky, S. R., M. Field, and H. A. Fozzard. 1986. Changes in intracellular sodium with chloride secretion in dog tracheal epithelium. *Am. J. Physiol.* 250:C646-C650.

7. Welsh, M. J. 1983. Inhibition of chloride secretion by furosemide in canine tracheal epithelium. J. Membr. Biol. 71:219-226.

8. Christoffersen, F. G. J., and L. H. Skibstedt. [1975]. Calcium ion activity in physiological salt solutions. Influence of anions substituted for chloride. *Comp. Biochem. Physiol.* 52A:317-322.

9. Marin, M. G., and M. M. Zaremba, 1978. Effect of calcium on ion transport and electrical properties of tracheal epithelium. J. Appl. Physiol. (Respir. Environ. Exercise Physiol.) 44:900-904.

10. Coleman, D, L., I. K. Tuet, and J. H. Widdicombe. 1984. Electrical properties of dog tracheal epithelial cells grown in monolayer culture. *Am. J. Physiol.* 246:C355–C359.

11. Welsh, M. J. 1985. Ion transport by primary cultures of canine tracheal epithelium. Methodology, morphology and electrophysiology. *J. Membr. Biol.* 88:149–163.

12. Smith, P. L., M. J. Welsh, J. S. Stoff, and R. A. Frizzell. 1982. Chloride secretion by canine tracheal epithelium. Role of intracellular cAMP levels. *J. Membr. Biol.* 70:217–226.

13. Al-Bazzaz, F. J., and E. Cheng. 1979. Effect of catecholamines on ion transport in dog tracheal epithelium. J. Appl. Physiol. 47:397-403.

14. Pratt, A. D., G. Clancy, and M. J. Welsh. 1986. Mucosal adenosine stimulates chloride secretion in canine tracheal epithelium. *Am. J. Physiol.* 251:C167-C174.

15. Taylor, A., and E. E. Windhager. 1979. Possible role of cytosolic calcium and Na-Ca exchange in regulation of transpithelial sodium transport. *Am. J. Physiol.* 236:F505-F512.

16. Windhager, E. E., and A. Taylor. 1983. Regulatory role of intracellular calcium ions in epithelial Na transport. *Annu. Rev. Physiol.* 45: 519-532.

17. Chase, H. S. 1984. Does calcium couple the apical and basolateral membrane permeabilities in epithelia? *Am. J. Physiol.* 247:F869–F876.

18. Widdicombe, J. H., I. T. Nathanson, and E. Highland, 1983. Effects of "loop" diuretics on ion transport by dog tracheal epithelium. *Am. J. Physiol.* 245:C388-C396.

19. Cuthbert, A. W., J. M. Edwardson, J. Aceves, and S. A. Wilson. 1979. Amiloride as a membrane probe. *In* Amiloride and Epithelial Sodium Transport. A. W. Cuthbert, G. M. Fanelli, Jr., and A. Scriabine, editors. Urban and Schwarzenbert, Inc., Baltimore-Munich. 113–123.

20. Scott, W. N., and D. B. P. Goodman, editors. 1981. Hormonal regulation of epithelial transport of ions and water. *Ann. NY Acad. Sci.* Vol. 372.

21. Crandall, E. D., S. E. S. Brown, and B. E. Goodman. 1983. Regulation of transport across alveolar epithelial cell monolayers by cAMP. Am. Rev. Respir. Dis. 127:306.

22. Cott, G. R., K. S. Sugahara, and R. J. Mason. 1983. Cyclic adenosine monophosphate is a modulator of the bioelectric properties of alveolar type II cell monolayers. *Physiologist*. 26:A125.

23. Orloff, J., and J. S. Handler. 1962. The similarity of effects of vasopressin and adenosine 3',5'-phosphate (cyclic AMP) and theophylline on the toad bladder. J. Clin. Invest. 41:702-709.

24. Baba, W. I., A. J. Smith, and M. M. Towsend. 1967. The effect of vasopressin, theophylline, and cyclic 3',5'-adenosine monophosphate (cyclic AMP) on sodium transport across the frog skin. Q. J. Exp. Physiol. 52:410-421.

25. Els, W. J., and S. I. Helman. 1981. Vasopressin, theophylline, PGE<sub>2</sub>, and indomethacin on active Na transport in frog skin. Studies with microelectrodes. *Am. J. Physiol.* 241;F279–F288.

26. Legris, G. J., P. C. Will, and V. Hopfer. Inhibition of amiloridesensitive sodium conductance by indoleamines. 1982. *Proc. Natl. Acad. Sci. USA*. 79:2046–2050. 27. Welsh, M. J. 1984. Anthracene-9-carboxylic acid inhibits an apical membrane chloride conductance in canine tracheal epithelium. *J. Membr. Biol.* 78:61–71.

28. Welsh, M. J. 1983. Evidence for a basolateral membrane potassium conductance in canine tracheal epithelium. *Am. J. Physiol.* 244: C377-C384.

29. Smith, P. L., and R. A. Frizzell. 1984. Chloride secretion by canine tracheal epithelium: basolateral membrane K permeability parallels secretion rate. *J. Membr. Biol.* 77:187–199.

30. Langridge-Smith, J. E. 1986. Interaction between sodium and chloride transport in bovine tracheal epithelium. J. Physiol. 376:299-319.

31. Knowles, M. R., M. J. Stutts, A. Spock, N. Fischer, J. T. Gatzy,

and R. C. Boucher. 1983. Abnormal ion permeation through cystic fibrosis respiratory epithelium. *Science (Wash. DC)*. 221:1067-1070.

32. Knowles, M., J. Gatzy, and R. Boucher. 1983. Relative ion permeability of normal and cystic fibrosis nasal epithelium. J. Clin. Invest. 71:1410-1417.

33. Widdicombe, J. H., M. J. Welsh, and W. E. Finkbeiner. 1985. Cystic fibrosis decreases the apical membrane chloride permeability of monolayers cultured from cells of tracheal epithelium. *Proc. Natl. Acad. Sci. USA*. 82:6167–6171.

34. Boucher, R. C., M. J. Stutts, M. R. Knowles, L. Cantlye, and J. T. Gatzy. 1985. Net transport in cystic fibrosis nasal epithelia: Abnormal basal rate and response to adenylate cyclase activation. *Clin. Res.* 33: 467*a*. (Abstr.)