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Inhibition of Chloride Secretion by Furosemide in Canine Tracheal Epithelium

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Summary. Furosemide inhibits Cl transport in a variety of epithelial and nonepithelial cells. To examine the mechanism of Cl secretion in canine tracheal epithelium, the effect of furosemide on transepithelial ion fluxes, membrane resistances, and electromotive forces was determined using intracellular microelectrodes and an equivalent electrical circuit analysis. There were six main observations: First, furosemide was only effective when added to the submucosal solution. Second, inhibition by furosemide (10^{-3} M) was specific for Cl secretion with no effect on Na absorption. Third, furosemide produced a half-maximal inhibition of Cl secretion at a concentration of 7×10^{-6} M. A Hill plot yielded a slope not different from unity, suggesting a one-for-one interaction of furosemide with the Cl transport process. Fourth, despite complete inhibition of Cl secretion, furosemide produced only small changes in transepithelial and apical membrane resistance, indicating that the primary effect was not an inhibition of Cl exit from the cell across the apical membrane. Fifth, basolateral membrane resistance and electromotive force were not altered by furosemide. This finding suggested that the effect of furosemide at the basolateral membrane was on an electrically neutral Cl entry process. Sixth, calculation of the intracellular Cl concentration from the electromotive force across the apical membrane indicated that furosemide decreased intracellular Cl concentration by 50%, consistent with an inhibition of Cl entry into the cell. These results indicate that Cl enters the epithelial cell via an electrically neutral process at the basolateral membrane and that furosemide selectively inhibits that process, resulting in a decreased intracellular Cl concentration and a decrease in the driving force for Cl exit across the apical membrane.

Key words tracheal epithelium · furosemide · Cl secretion · electrophysiology · loop diuretic · equivalent electrical circuit

Introduction

The canine tracheal epithelium secretes Cl via an electrogenic transport process (Olver et al., 1975) in response to a variety of secretagogues (Smith et al., 1982). The mechanism of Cl secretion appears to conform to a cellular model of ion transport that has been recently proposed for tracheal epithelium (Widdicombe & Welsh, 1980; Welsh, Smith & Frizzell, 1982) as well as a diverse group of secretory epithelia (*see* Frizzell, Field & Schultz, 1979, for a review). A central feature of the proposed cellular mechanism of Cl secretion is the entry of Cl into the cell across the basolateral membrane via an electrically neutral NaCl cotransport process which results in the intracellular accumulation of Cl at an activity greater than predicted for electrochemical equilibrium. It has been suggested that furosemide may inhibit the NaCl entry process in Cl-secreting epithelia (Frizzell et al., 1979).

The purpose of this study was to investigate the mechanism of Cl secretion in canine tracheal epithelium by localizing the site and mechanism of furosemide's action. The rationale is based on the observation that addition of furosemide to the submucosal bathing solution decreased the rate of Cl secretion by the canine tracheal epithelium (Davis et al., 1977). Furosemide has also been observed to inhibit Cl secretion in epithelia that appear to share a similar mechanism of Cl transport, including shark rectal gland (Silva et al., 1977), frog corneal epithelium (Candia, 1973), rabbit descending colon (Frizzell et al., 1979), teleost operculum (Degnan, Karnaky & Zadunaisky, 1977) and the ciliary body epithelium of the eye (Saito et al., 1980).

There are three possible mechanisms by which furosemide might inhibit Cl secretion. First, furosemide might inhibit Cl entry into the cells across the basolateral membrane. Since the inhibition of Cl secretion is observed when furosemide is added to the submucosal bathing solution, Frizzell et al. (1979) suggested that furosemide inhibits a Na-coupled Cl entry process. Support for this hypothesis was obtained by Eveloff and co-workers (1978), who observed that Na uptake into plasma membrane vesicles of dogfish rectal gland was inhibited in the absence of Cl or following the addition of furosemide. However, they were unable to show

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either Na dependence of Cl uptake or an ihhibition of Cl uptake by furosemide. Second, furosemide might inhibit Cl exit from the cell at the apical membrane. Candia et al. (1981) have suggested that piretanide and Mk-196 (loop diuretics structurally and/or functionally related to furosemide) inhibit Cl exit from the cell at the apical membrane in frog corneal epithelium. A third possibility is that loop diuretics might interfere with the Na pump (which indirectly provides the nonconjugate energy source for Cl secretion).

In this study, the measurement of transepithelial isotope fluxes indicates that the inhibitory effect of furosemide is specific for Cl secretion. The results of intracellular microelectrode studies and an equivalent electrical circuit analysis localize the inhibition of Cl secretion to an effect of furosemide on a neutral Cl entry step at the basolateral membrane.

List of Symbols

R_t	– transepithelial resistance
I_{sc}	– short-circuit current
ψ_t	– transepithelial electrical potential difference; submucosal solution with respect to mucosal solution
ψ_a	– electrical potential difference across the apical membrane; cell interior with respect to mucosal solution
R_a, R_b, R_p	– electrical resistance of the apical cell membrane, the basolateral cell membrane, and the paracellular pathway, respectively
E_a, E_b	– electromotive force across the apical and the basolateral cell membranes, respectively
J_{ms}, J_{sm}, J_{Net}	– ion flux from the mucosal to submucosal solution, submucosa to mucosa, and net flux. Superscripts Cl and Na refer to the flux of those ions

Materials and Methods

The methods for obtaining and preparing the posterior membranous portion of the canine tracheal epithelium were similar to those previously described (Welsh & Widdicombe, 1980). The bathing solution contained (in mM): 118.9 NaCl, 20.4 $NaHCO_3$, 2.4 K_2HPO_4, 0.6 KH_2PO_4, 1.2 $CaCl_2$, 1.2 $MgCl_2$, and 10 glucose. The solution was maintained at 37 °C and bubbled with 95% O_2 and 5% CO_2 (pH 7.4).

For measurement of transepithelial electrical properties and radioisotope fluxes, tissues were mounted in Ussing chambers with 1.5 cm^2 surface area. The transepithelial electrical potential difference ψ_t (referenced to the mucosal solution) was automatically clamped to zero (the short-circuit condition) by automatic voltage-current clamps (University of Iowa, Bioengineering). Transepithelial resistance R_t was calculated from the change in current required to clamp ψ_t to ± 10 mV (pulses delivered by a pulse generator built into the voltage-current clamp; duration 0.5 or 1 sec; period 60 sec). Na and Cl transport rates were determined from the unidirectional and calculated net transepithelial fluxes of ^{22}Na and ^{36}Cl measured in paired tissues from the same dog. Five μCi of ^{22}Na and 7 μCi of ^{36}Cl were added to the appropriate side of the tissue. Thirty minutes were allowed for isotope fluxes to reach a steady state, and then three samples of both bathing solutions were taken at 20-min intervals during each control and experimental period. Isotope flux measurements were made during the steady state at least 15 to 20 min after an experimental intervention.

Indomethacin (10^{-6} M) (Sigma) was added to the mucosal bathing solution of all tissues to minimize the rate of Cl secretion during control periods and thus to maximize the effects produced by secretagogues. Mucosal addition of indomethacin decreases the endogenous rate of prostaglandin production, decreases intracellular cAMP, and thus decreases the rate of Cl secretion (Al-Bazzaz, Yadova & Westenfelder, 1981; Smith et al., 1982). Addition of indomethacin does not interfere with the subsequent response to secretagogues (Smith et al., 1982). Other drugs used were epinephrine (Elkin-Sinn) and furosemide (Hoechst).

The techniques and methods of preparing the epithelium for intracellular studies, measurement of electrical properties, construction of microelectrodes and performance of cellular impalements were the same as those previously described (Welsh et al., 1982). All measurements were made under short-circuit conditions. Two to six cellular impalements were made during each experimental condition in each tissue in order to measure the electrical potential difference across the apical cell membrane ψ_a and the membrane resistance ratio R_a/R_b where R_a and R_b refer to the resistance of the apical and basolateral membrane, respectively.

The results were analyzed using an equivalent electrical circuit model of the epithelium (Welsh, Smith & Frizzell, 1983, companion manuscript) that is similar to that used in Na-absorbing epithelia (Reuss & Finn, 1975; Frömter & Gebler, 1977; Schultz, Frizzell & Nellans, 1977; Wills, Lewis & Eaton, 1979). The flow of ions is represented as the short-circuit current I_{sc}, the electrochemical driving force for ion flow as an electromotive force or battery E, and permeabilities as electrical resistances R. The apical and basolateral cell membranes are each represented as a resistance and electromotive force in series. The paracellular pathway is represented as a resistance in parallel with the cellular pathway. A value of paracellular resistance was obtained in each tissue by examining the acute response to addition of either epinephrine or amiloride as previously described (Welsh et al., 1983) and then the other circuit parameters were calculated from the measured values of I_{sc}, R_t, ψ_a and R_a/R_b. The determination of the circuit parameters following the addition of furosemide requires the assumption that furosemide does not alter paracellular pathway resistance R_p. This assumption is consistent with the observation (Welsh & Widdicombe, 1980) that furosemide did not alter transepithelial fluxes of radiolabeled mannitol, a marker of paracellular pathway permeability.

All values are presented as means ±SEM. Statistical significance was evaluated using a paired or unpaired t-test, as indicated. $P<0.05$ was considered statistically significant.

Results

The sidedness of the effects of furosemide and its reversibility are indicated by the response of three representative tissues from the same animal, shown in Fig. 1. Addition of epinephrine (10^{-6} M) to the submucosal bathing solution increased the I_{sc} in all three tissues, reflecting an increase in the rate

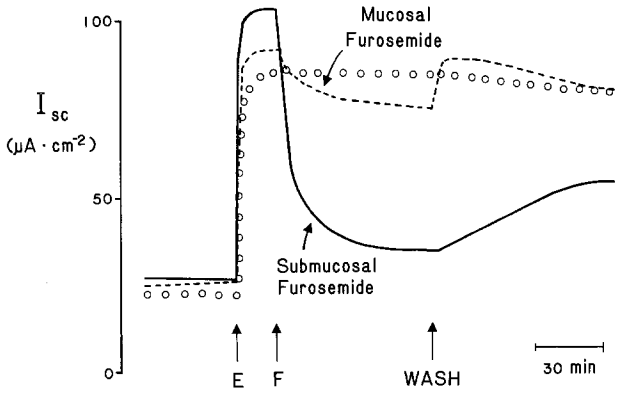

Fig. 1. Time course and sidedness of the effect of furosemide on the short-circuit current I_{sc}. The arrow labeled *E* indicates the time at which epinephrine (10^{-6} M) was added to the submucosal bathing solution. At the arrow labeled *F*, furosemide 10^{-4} M was added to either the submucosal (solid line), mucosal (dashed line), or neither (dotted line) bathing solution. At the arrow marked wash, the solutions containing furosemide were replaced with fresh Ringer's solution

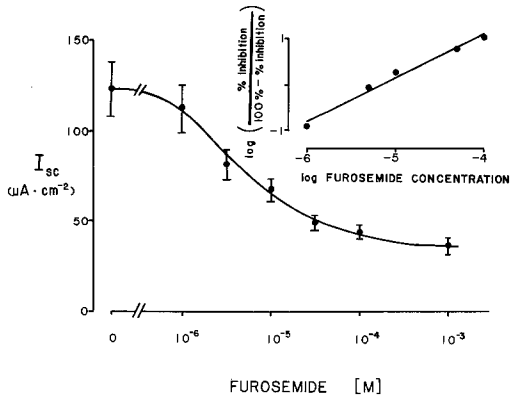

Fig. 2. Effect of the furosemide concentration in the submucosal solution on the short-circuit current I_{sc}. Each point represents the mean ± SEM of five determinations. The inset is a Hill plot of the data. Maximum inhibition was taken as the response at 10^{-3} M (i.e., complete inhibition of Cl secretion). The percent inhibition refers to the percent of maximum inhibition produced by a dose of furosemide. Straight line is least-squares linear regression fit to data. Slope − 0.94. *X*-axis intercept at 6.8×10^{-6} M furosemide. $r = 0.99$

of Cl secretion. The subsequent addition of furosemide (10^{-4} M) to the submucosal bathing solution produced a rapid decrease in I_{sc}. In contrast, addition of furosemide to the mucosal bathing solution produced only a small decrease in I_{sc}. The marked decrease in I_{sc} in response to submucosal addition of furosemide was quite consistent from tissue to tissue; the response to mucosal addition of furosemide was always substantially smaller and varied in magnitude from tissue to tissue. Occasionally there was no effect of mucosal addition of furosemide on I_{sc}. As shown in Fig. 1, the response to submucosal furosemide was partially reversible but usually had a prolonged time course. Since furosemide added to the mucosal bathing solution had little effect on I_{sc}, the drug was added only to the submucosal solution for the remainder of the study.

Figure 2 shows the dependency of the I_{sc} on the concentration of furosemide present in the submucosal bathing solution of tissues in which secretion had been stimulated with epinephrine. I_{sc} progressively decreased with increasing concentrations of furosemide but clearly did not approach zero, even with the highest concentration (10^{-3} M). The I_{sc} that remains following addition of 10^{-3} M furosemide is consistent with the rate of net electrogenic Na absorption (as will be shown subsequently). A Hill plot was made as shown in the inset in Fig. 2 to analyze the kinetics of the effect of furosemide on Cl secretion. The Hill plot was performed using the furosemide-inhibitable current (i.e., the net Cl secretion rate) with 100% inhibition assumed at 10^{-3} M (*see* Table 1). The slope is not different from the slope of 1.0 predicted from a one-for-one interaction between the furosemide molecule and the chloride transport system. The furosemide concentration required to produce a half-maximal inhibition was 6.8×10^{-6} M. For all subsequent studies a dose of 10^{-3} M furosemide was used in order to obtain a maximum inhibition of Cl transport.

The effect of epinephrine and furosemide on I_{sc}, R_t and unidirectional and net Na and Cl fluxes are shown in Table 1. Epinephrine increased I_{sc} and decreased R_t, reflecting an increased rate of Cl secretion and a slight decrease in the rate of Na absorption. The subsequent addition of furosemide reduced I_{sc} to a value nearly identical to the initial, control value and decreased the rate of net Cl secretion (J^{Cl}_{Net}) to a value not significantly different from zero. The net rate of Na absorption was unaltered. Despite the decrease in I_{sc} and J^{Cl}_{Net} to control values, R_t remained substantially lower than the value obtained during the nonsecreting, control condition. This observation suggests that the primary effect of furosemide is not to inhibit an electrically conductive transport process.

Table 2 shows the I_{sc}, R_t, and net and unidirectional fluxes in tissues that did not receive furosemide during the third period, thus providing a time-control for the effect of furosemide. The small increase in R_t observed during the second epinephrine period suggests that a portion of the increase in R_t observed with furosemide might be a time-dependent effect. Small decreases were also ob-

Table 1. Effect of epinephrine and furosemide on transepithelial electrical properties and ion fluxes[a]

	I_{sc}	R_t	J^{Na}_{ms}	J^{Na}_{sm}	J^{Na}_{Net}	J^{Cl}_{ms}	J^{Cl}_{sm}	J^{Cl}_{Net}
Control	1.34 ±26	1294 ±195	1.24 ±0.15	0.31 ±0.05	0.94 ±0.11	0.68 ±0.17	0.97 ±0.21	0.29 ±0.11
Epinephrine	2.80* ±0.30	533* ±40	1.13 ±0.16	0.49* ±0.06	0.65 ±0.13	1.90* ±0.27	4.13* ±0.49	2.23* ±0.33
Furosemide and epinephrine	1.31* ±0.26	776* ±44	1.11 ±0.15	0.32* ±0.04	0.79* ±0.12	1.06* ±0.19	1.21* ±0.13	0.15* ±0.17

[a] I_{sc} refers to the short-circuit current, R_t to the transepithelial resistance, J^{Na} and J^{Cl} to the fluxes of Na and Cl, respectively, and the subscripts *ms*, *sm*, and Net refer to the flux from mucosa to submucosa, submucosa to mucosa, and calculated net fluxes, respectively. The net flux of Na was considered positive in the *ms* direction and the net flux of Cl was taken as negative in the *sm* direction, in accord with the direction of current flow. The I_{sc} and all fluxes are in units of $\mu eq\ cm^{-2}\ hr^{-1}$ and R_t is in units of $\Omega\ cm^2$. Tissues were studied during three consecutive periods: A "Control" period; an "Epinephrine" period (epinephrine 10^{-6} M present in the submucosal solution to stimulate Cl secretion); and an "Epinephrine and Furosemide" period during which furosemide (10^{-3} M) and epinephrine (10^{-6} M) were present in the submucosal solution. Indomethacin (10^{-6} M) was present during the "Control" period to minimize the spontaneous rate of Cl secretion and remained in the mucosal solution during both other periods. Values represent means ±SEM for seven pairs of tissues.
* Indicates a statistical difference from the preceding period ($P<0.05$).

Table 2. Effect of epinephrine on transepithelial electrical properties and ion fluxes[a]

	I_{sc}	R_t	J^{Na}_{ms}	J^{Na}_{sm}	J^{Na}_{Net}	J^{Cl}_{ms}	J^{Cl}_{sm}	J^{Cl}_{Net}
Control	0.90 ±0.12	943 ±124	1.49 ±0.23	0.51 ±0.18	0.97 ±0.28	1.10 ±0.28	1.15 ±0.24	0.04 ±0.18
Epinephrine	1.90* ±0.21	592* ±73	1.52 ±0.29	0.64 ±0.17	0.88 ±0.25	1.85* ±0.32	3.28* ±0.53	1.43* ±0.30
Epinephrine	1.68* ±0.21	666* ±91	1.43 ±0.26	0.67 ±0.17	0.77 ±0.25	1.77 ±0.35	3.10* ±0.51	1.33 ±0.31

[a] Tissues were studied during three consecutive periods: A "Control" period; and two consecutive "Epinephrine" periods during which epinephrine (10^{-6} M) was present in the submucosal bathing solution. Indomethacin (10^{-6} M) added to the mucosal solution was present during the "Control" period to minimize the spontaneous rate of Cl secretion and remained in the mucosal solution during the other two periods. Values represent means ±SEM for seven pairs of tissues.
* Indicates a statistical difference from the preceding period ($P<0.05$). For abbreviations and units, *see* legend of Table 1.

served in the I_{sc} and the unidirectional flux of Cl from submucosa to mucosa; however, the important point is that these changes were substantially less than those observed with furosemide.

To examine the effect of furosemide at the level of the individual cell membrane, intracellular microelectrode techniques and an equivalent electrical circuit analysis were used during the control nonsecreting condition, when Cl secretion was stimulated by epinephrine, and during the subsequent inhibition of secretion with furosemide. Figure 3 shows the effect of epinephrine and furosemide on the transepithelial electrical properties. These results are similar to those shown in Table 1; furosemide completely inhibited the increase in I_{sc} produced by epinephrine (i.e., furosemide inhibited Cl secretion) but produced only a partial return of R_t to the control value.

The effects of epinephrine and furosemide on the electrical potential difference across the apical membrane ψ_a (ψ_a is equal and opposite to the electrical potential difference across the basolateral membrane ψ_b under short-circuit conditions, so that the sum of the two electrical potential differences, arranged in series, is zero) and the membrane resistance ratio R_a/R_b is shown in Fig. 4. The depolarization of ψ_a produced by addition of secretagogue was completely reversed by furosemide with ψ_a returning to the value observed under control conditions. R_a/R_b also decreased during stimulation of secretion, but following the addition of furosemide returned only partially toward the control value.

Figure 5 illustrates the effect of epinephrine and furosemide on the individual membrane resistances. Addition of epinephrine decreased R_a to

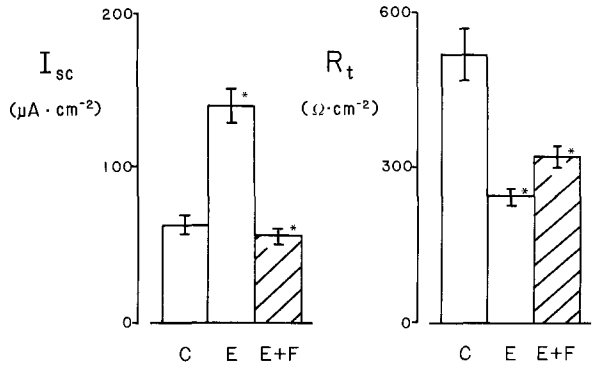

Fig. 3. Effect of epinephrine and furosemide on the short-circuit current I_{sc} and transepithelial resistance R_t. C refers to the control period, E to the steady-state epinephrine-treated condition (10^{-6} M to the submucosal solution), and E and F to the third period during which furosemide (10^{-3} M) and epinephrine (10^{-6} M) were both present in the submucosal bathing solution. Indomethacin (10^{-6} M added to the mucosal solution) was present during the control period to minimize the spontaneous rate of Cl secretion and remained in the mucosal solution during both other periods. Values represent the mean $\pm$SEM from determinations made in eight tissues. * indicates a statistical difference from the preceding period ($P<0.05$)

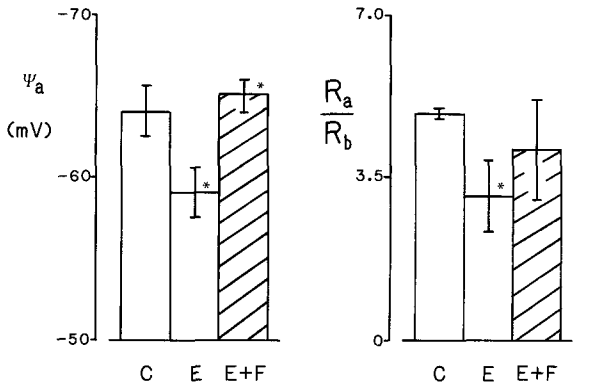

Fig. 4. Effect of epinephrine and furosemide on the electrical potential difference across the apical membrane ψ_a and the membrane resistance ratio R_a/R_b. (*See* legend of Fig. 3.)

15% of the control value. Although subsequent addition of furosemide increased R_a, it remained only 26% of the value observed during control, nonsecreting conditions. This finding indicates that the primary effect of furosemide in inhibiting Cl secretion is not an increase in the resistance of Cl movement across the apical membrane. Figure 5 also shows the response of R_b to stimulation of secretion followed by inhibition with furosemide. As previously observed (Welsh et al., 1983), R_b decreased to approximately one-fourth the control value with stimulation of secretion. This decrease in R_b is most likely secondary to an increase in the potassium permeability of the basolateral membrane (Welsh et al., 1983). Following the addition of furosemide, there was no significant change in R_b. This finding suggests that furosemide does not inhibit an electrically conductive Cl entry step at the basolateral membrane, nor does it appear to alter other basolateral membrane ionic conductances. This finding is best explained by furosemide's inhibition of an electrically neutral transport process at the basolateral membrane.

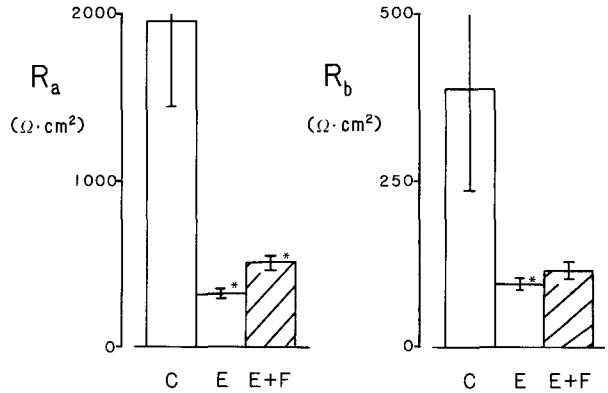

Fig. 5. Effect of epinephrine and furosemide on the resistance of the apical R_a and basolateral R_b membrane. Paracellular pathway resistance R_p was $752 \pm 122\ \Omega \cdot cm^2$. (*See* legend of Fig. 3.)

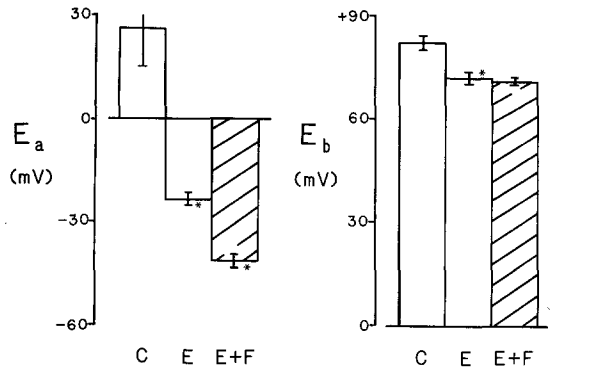

Fig. 6. Effect of epinephrine and furosemide on the electromotive force at the apical E_a and basolateral E_b membrane. (*See* legend of Fig. 3.)

The calculated electromotive forces at the apical E_a and basolateral membrane E_b are shown in Fig. 6. With the addition of epinephrine, E_a decreased to -22 mV. Since, under stimulated conditions, Cl secretion predominates and the apical membrane is primarily Cl selective (Welsh et al., 1982), the value of E_a obtained in the presence of epinephrine is expected to reflect the chemical potential difference for Cl across the apical membrane (Welsh et al., 1983). Thus, cell Cl concentration can be estimated from the Nernst equation:

$$E_a = \frac{RT}{zF} \ln \frac{[Cl]_m}{[Cl]_c} \quad (1)$$

where $[Cl]_m$ and $[Cl]_c$ refer to the concentration of Cl in the mucosal solution and cell interior, respectively, and R, T, z, and F have their usual meanings. This calculation gives a $[Cl]_c$ of approximately 54 mM during steady-state secretion. This estimate of $[Cl]_c$ is in good agreement with the value of 50 mM obtained in isolated cells of the tracheal epithelium (Widdicombe, Basbaum & Highland, 1981). Following addition of furosemide, E_a decreased to -41 mV, yielding a $[Cl]_c$ of 26 mM, a substantial decrease. This estimate is based on the assumption that, following addition of furosemide, Cl is still the major permeant ion at the apical membrane. This is a reasonable assumption based on two observations: first, Na absorption is not significantly altered by furosemide as would be expected if there were an increase in Na permeability; and second, there is only a small increase in R_a after furosemide. If there were a substantial decrease in the apical membrane Cl permeability or an increase in Na permeability, E_a would be expected to become more positive, as is observed under control conditions.

The decrease in the estimated $[Cl]_c$ indicates that furosemide inhibited the entry of Cl into the cell at the basolateral membrane. The lack of change in E_b following the addition of furosemide, shown in Fig. 6, together with the failure of furosemide to alter R_b, indicates that furosemide inhibits an electrically neutral Cl entry step located at the basolateral membrane. These findings also suggest that furosemide has no significant effect on the basolateral membrane electrogenic transport processes, i.e., the Na pump-K backleak process.

Discussion

The results of this study indicate that furosemide is a specific inhibitor of Cl secretion by the canine tracheal epithelium. The absence of an effect on the rate of Na absorption suggests that the inhibition by furosemide was not due to a nonspecific depression of epithelial cell function or an effect on the Na-K-ATPase. The effects were only seen during addition of furosemide to the submucosal side of the tissue, with smaller and less consistent effects observed following addition to the mucosal solution.

Furosemide produced a half-maximal inhibition of Cl secretion at a concentration of approximately 7×10^{-6} M. This concentration is in the range of those reported to produce a half-maximal inhibition of Cl absorption in flounder intestine (10^{-6} to 10^{-5} M) (Frizzell et al., 1979), and thick ascending limb on Henle's loop (10^{-6} to 10^{-5} M) (Burg et al., 1973), and a half-maximal inhibition of Cl secretion in frog cornea (10^{-5} M) (Candia, 1973). A Hill plot of the dose-response relation for furosemide yielded a slope not substantially different from one, suggesting that one furosemide molecule interacts with one Cl transport process. The kinetics of the effect of furosemide on Cl secretion in tracheal epithelium are very similar to the kinetics of the furosemide inhibition of Cl secretion in the ciliary body epithelium of the toad (Saito et al., 1980) which requires a furosemide concentration of 3×10^{-6} M to produce half-maximal inhibition of Cl transport and in which a Hill plot of the dose-response curve yielded a slope of unity.

The results localize the effect of furosemide to the basolateral cell membrane and indicate that furosemide inhibits an electrically neutral Cl entry step. Four lines of evidence support this conclusion: first, although furosemide abolished the epinephrine-induced net Cl secretion, it did not increase transepithelial resistance back up to prestimulation values. This observation suggests that the primary effect of furosemide cannot be due to the inhibition of an electrically conductive transport process since such an effect would be expected to reverse the epinephrine-induced decrease in R_t. More direct evidence in this regard is the observation that, following addition of furosemide, R_a would be expected to increase back to the value observed under control, nonsecreting conditions. Thus, an increase in resistance to Cl movement across the apical membrane cannot account for the abolition of Cl secretion. Second, furosemide did not alter either basolateral membrane resistance or electromotive force, indicating that an electrically neutral process was inhibited. Third, the electromotive force across the apical membrane E_a, which primarily represents a Cl diffusion potential, decreased with addition of furosemide, suggesting a 50% fall in the intracellular Cl concentration. A decrease in intracellular Cl concentration indicates an inhibition of the entry of Cl into the cell. Fourth, the observation that furosemide was only effective when added to the submucosal solution is indirect evidence that the effect is localized to the basolateral membrane.

These conclusions are consistent with previous observations on the mechanism of ion transport in tracheal epithelium and extend the understanding of the mechanisms of Cl secretion. They agree with the previous conclusion (Welsh et al., 1982), based on ion substitution studies, that Cl movement across the basolateral membrane is a neutral process. Two pieces of evidence suggest that neutral Cl movement is the result of cotransport

of Na and Cl: first, removal of Na from the submucosal bathing solution alone inhibits Cl secretion (Al-Bazzaz & Al-Awqati, 1979; Widdicombe et al., 1979), second, Cl secretion is not dependent upon the presence of CO_2 or HCO_3 in the bathing solutions (Al-Bazzaz & Al-Awqati, 1979) and is not inhibited by addition of the carbonic anhydrase inhibitor, acetazolamide, or the substituted stilbene derivative, SITS (*unpublished observation*). These observations suggest that in tracheal epithelium, a Cl-HCO_3 exchange process is not involved. The suggestion that a neutral NaCl cotransport process mediates Cl entry at the basolateral cell membrane is consistent with the finding that in perfused shark rectal gland (which appears to share a similar mechanism of Cl secretion), removal of Na from the perfusate inhibits the intracellular accumulation of Cl (Welsh et al., 1981).

Although the primary effect of furosemide was to inhibit Cl entry into the cell, an increase in apical membrane resistance was observed (Fig. 5). There are two possible explanations for this increase: first, a decrease in $[Cl]_c$ would be expected to increase R_a since the resistance of a membrane is inversely related to the concentration of the ion in the membrane; second, the permeability of the membrane might decrease. An evaluation of the contribution of these two components to the increase in R_a requires a knowledge of the mechanism of the apical Cl conductance (i.e., diffusion, charged-carrier transport, single-file diffusion, etc.), which is currently unknown, and the intracellular Cl concentration, which has been derived from the value of E_a, using a slope resistance. In view of the assumptions that must be made, it is currently not possible to quantitatively separate the contribution of a decrease in ion concentration and a decrease in permeability to the increase in R_a observed with furosemide.

The estimation of $[Cl]_c$ from E_a for these analyses rests on the assumption that the apical membrane is primarily Cl-permeable under stimulated conditions. In support of this assumption is the dependency of ψ_a on the Cl concentration in the mucosal solution (Welsh et al., 1982) and the absence of a change in ψ_a or ψ_t when mucosal Na is decreased or K increased (*unpublished observation*). The estimate of $[Cl]_c$ calculated from E_a is also in excellent agreement with that determined chemically in isolated tracheal epithelial cells (Widdicombe et al., 1981). Finally, it should be pointed out that the estimate of $[Cl]_c$ will be slightly high if a Na diffusion potential makes some contribution to E_a (Welsh et al., 1983), but as discussed earlier, the direction and magnitude of any change in E_a will provide an accurate reflection of a qualitative change in $[Cl]_c$ under these conditions.

In these experiments, stimulation of secretion resulted in a decrease in R_b [which, as discussed in the companion paper (Welsh et al., 1983), is most likely due to an increase in the K permeability of the basolateral membrane]. The failure of furosemide to alter R_b has two implications: first, it suggests that furosemide had either no direct effect on the Na pump (the Na-K-ATPase) and basolateral membrane conductive processes or that it had equal and opposite effects on them. The former suggestion is the most likely in view of the failure of furosemide to inhibit the rate of Na absorption (Table 1) and the observation that in homogenates of tracheal epithelium, Westenfelder, Earnest and Al-Bazzaz (1980) found that addition of 10^{-3} M furosemide had no effect on Na-K-ATPase activity. The second implication of the constancy of R_b following addition of furosemide is that the activity of the Na pump and R_b are not tightly coupled. During stimulation of secretion, the activity of the Na pump probably increases and R_b decreases (Welsh et al., 1983); during treatment with furosemide, the activity of the Na pump probably decreases (due to a decrease in the rate of transport) but there is no change in R_b.

In conclusion, in canine tracheal epithelium, as well as a variety of other secretory epithelia, absorptive epithelia, and nonepithelial cells, furosemide inhibits Cl transport. It seems that a common feature of the effect of furosemide may be the inhibition of neutral transport processes. Furthermore, it would appear that several different types of neutral Cl transport might be inhibited by furosemide. A NaCl cotransport process may be involved in tracheal epithelium and shark rectal gland (Eveloff et al., 1978). In absorptive epithelia, the most direct evidence for the inhibition of a NaCl cotransport process has been obtained in flounder intestine (Frizzell et al., 1979); furosemide, added to the mucosal bathing solution, inhibited the influx of Na and Cl from the mucosal solution into the cell by approximately equal amounts, suggesting the inhibition of a NaCl cotransport process. Evidence for furosemide inhibition of a neutral NaCl cotransport process has also been obtained in a nonepithelial cell, squid giant axon, by Russell (1979). In nonepithelial cells, there is direct evidence that furosemide inhibits two other forms of neutral Cl transport: in human erythrocytes, Brazy and Gunn (1976) found that furosemide inhibited a Cl-HCO_3 exchange mechanism; in Ehrlich ascites cells (Geck et al., 1980) furosemide inhibits a neutral cotransport of 2 Cl, 1 K

and 1 Na. At this time it is not known if this diverse group of Cl transport processes may actually share some common factor. Finally, one exception to this list of cells and tissues in which furosemide appears to inhibit neutral Cl transport processes may be the Cl secretory process in frog corneal epithelium. Candia and coworkers (1981) suggested that furosemide, piretanide, and MK 196 (loop diuretics pharmacologically and functionally related to furosemide) inhibit electrically conductive Cl exit from the cell at the apical membrane. Since corneal epithelium appears to share a common mechanism of Cl secretion with the tracheal epithelium and shark rectal gland, the explanation for this difference is unknown.

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