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Adrenergic Regulation of Ion Transport by Primary Cultures of Canine Tracheal Epithelium: Cellular Electrophysiology

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Summary. We examined the effect of adrenergic agents on the cellular electrical properties of primary cultures of canine tracheal epithelium. Both isoproterenol and epinephrine stimulated Cl secretion, as evidenced by an increase in transepithelial voltage and a fall in transepithelial resistance. Moreover, both agents appear to increase the conductance of apical and basolateral membranes. However, the pattern of response was different. Isoproterenol initially depolarized apical voltage Ψ_a and decreased the fractional resistance of the apical membrane f_R. These changes are consistent with an initial increase in apical Cl conductance. In contrast, epinephrine acutely hyperpolarized Ψ_a and increased f_R, changes consistent with an initial increase in basolateral K conductance. Following the acute effect of epinephrine, Ψ_a depolarized and f_R decreased to values not significantly different from those observed with isoproterenol. The acute increase in basolateral K conductance produced by epinephrine appeared to result from stimulation of α adrenergic receptors because it was reproduced by addition of the α agonist phenylephrine, and blocked by the α antagonist phentolamine. The ability of prazosin but not yohimbine to block the acute epinephrine-induced increase in K permeability indicates the presence of α_1 adrenergic receptors. The acute α adrenergic-induced increase in basolateral K conductance may be mediated by an increase in cell Ca because the response was mimicked by addition of the Ca ionophore A23187. In contrast, the response to isoproterenol was similar to that observed with addition of 8-bromo-cAMP and theophylline. These results indicate that both β and α adrenergic agents mediate the ion transport processes in canine tracheal epithelium. β adrenergic agents have their primary effect on the apical Cl conductance, probably via an increase in cAMP. α adrenergic agents exert their primary effect on the basolateral K conductance, possibly via an increase in cell Ca.

Key Words tracheal epithelium · adrenergic agents · chloride secretion · chloride conductance · potassium conductance · electrophysiology

Introduction

Canine tracheal epithelium secretes Cl from the submucosal to the mucosal surface in response to a variety of neurohumoral agents. Previous studies by Al Bazzaz and Cheng (1979) and Davis et al. (1979) have shown that β-adrenergic agents stimulate transepithelial Cl secretion. Stimulation of secretion by the combined β- and α- adrenergic agonist, epinephrine, was subsequently found to increase cellular levels of cAMP (Smith et al., 1982). Intracellular electrophysiologic studies showed that epinephrine stimulated transepithelial Cl secretion by increasing both the apical membrane Cl conductance (G_a^{Cl}) and the basolateral membrane K conductance (G_b^K) (Welsh et al., 1982, 1983; Shorofsky et al., 1983; Smith & Frizzell, 1984).

The purpose of this study was to further examine the response of tracheal epithelium to adrenergic agents. There were several reasons to do this. First, it was unclear whether there was any effect of α adrenergic agents in tracheal epithelium. In previous studies, the α adrenergic agonist, phenylephrine, had no effect on the rate of transepithelial Cl secretion, as measured by the short-circuit current (I_{sc}) (Al-Bazzaz & Cheng, 1979; Smith et al., 1982), nor did it alter cellular levels of cAMP (Smith et al., 1982). However, histologic studies show α_1-adrenergic receptors on the surface epithelium (Barnes & Basbaum, 1983), suggesting that α agonists may regulate epithelial function. Second, in previous studies we found a different pattern of cellular electrical response to epinephrine and prostaglandin E_1 (Welsh et al., 1982) although both agents increase cellular cAMP levels (Smith et al., 1982). This observation, plus the knowledge that the effects of both β-adrenergic agonists and prostaglandins of the E series are mediated by cAMP in a variety of tissues, raised the possibility that the difference between the two secretagogues might be explained by the fact that epinephrine is also an α-adrenergic agonist. Third, tracheal epithelial monolayers cultured from humans have the same mecha-

nism of Cl transport and hormonal responsiveness as those cultured from dogs (Widdicombe et al., 1985). However, in a monolayer of tracheal epithelial cells cultured from a patient with cystic fibrosis, isoproterenol failed to stimulate Cl secretion (Widdicombe & Welsh, 1985). Thus, it seemed important to further examine the response of the cultured epithelium to adrenergic agonists.

We used monolayers of tracheal epithelium cultured on permeable supports. Previous studies have shown that the cultured cells have the same mechanism of Cl secretion as the intact (or native) epithelium (Coleman et al., 1984; Welsh, 1985). In addition, use of the cultured epithelium has the advantage that other cell types, such as submucosal cells and glands and axon terminals, are not present. To examine the effect of adrenergic agents we used intracellular microelectrode techniques.

Materials and Methods

The methods of isolation and culture of canine tracheal epithelial cells have been previously described (Coleman et al., 1984; Welsh, 1985). Cells were grown on collagen-coated Nucleopore filters. The methods of cell impalement are similar to those we have previously used in the native tracheal epithelium (Welsh et al., 1982) and in cultured epithelial monolayers (Welsh, 1985). The transepithelial voltage (Ψ_t) and the electrical potential difference across the apical membrane (Ψ_a) were referenced to the mucosal solution. Transepithelial resistance (R_t) was measured from the voltage response to transepithelial constant current pulses (± 40 to 160 $\mu A \cdot cm^{-2}$). The fractional resistance of the apical cell membrane (f_R) was calculated as $f_R = \Delta\Psi_a/\Delta\Psi_t = R_a/(R_a + R_b)$, where $\Delta\Psi_a$ and $\Delta\Psi_t$ refer to the changes in Ψ_a and Ψ_t induced by the current pulses, and R_a and R_b refer to apical and basolateral membrane resistance, respectively. In figures which show representative tracings of single experiments, changes in f_R can be appreciated by inspection of the changes in Ψ_a produced by the current pulses (for an example *see* Fig. 1).

The bathing solution contained (in millimolars): 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 bubbled with 95% O_2 and 5% CO_2 (pH 7.4 at 37°C). For some experiments Cl was partially replaced by gluconate or K was increased in exchange for Na. Drugs used were epinephrine (Elkin-Sinn, Inc., Cherry Hill, N.J.), isoproterenol (Elkin-Sinn), phenylephrine (Winthrop Laboratories, New York, N.Y.), prazosin HCl (Pfizer Inc., Brooklyn, N.Y.), phentolamine (Ciba Pharmaceutical Co., Summit, N.J.), A23187 (Sigma Chemical Co., St. Louis, Mo.), 8-bromo-cAMP (Sigma), and theophylline (Sigma).

Results

Comparison of Isoproterenol and Epinephrine

We first examined the responses to isoproterenol and epinephrine; representative examples are shown in Fig. 1. Both agents increased Ψ_t and reduced R_t consistent with stimulation of Cl secretion. Isoproterenol initially depolarized Ψ_a and reduced f_R. In a previous study (Welsh, 1985), we showed that these changes result primarily from an increase in apical Cl conductance (G_a^{Cl}); however, the increase in G_a^{Cl} is followed by an increase in basolateral K conductance (G_b^K). (This is also consistent with the tendency for biphasic changes in Ψa and f_R, Figs. 1 and 2.) In contrast, epinephrine produced an initial hyperpolarization of Ψ_a and increase in f_R, then Ψ_a depolarized and f_R decreased. The initial response to epinephrine is best explained by an increase in G_b^K. An increase in G_b^K would hyperpolarize the cell (as the voltage approached the K equilibrium potential), minimally decrease R_t, and increase f_R (due to a decrease in R_b). There is no other reasonable explanation for this sequence of events. The subsequent depolarization of Ψ_a and fall in f_R can be attributed to an increase in G_a^{Cl} so that the final values of both transmonolayer and cellular electrical properties are the same with the two agents.

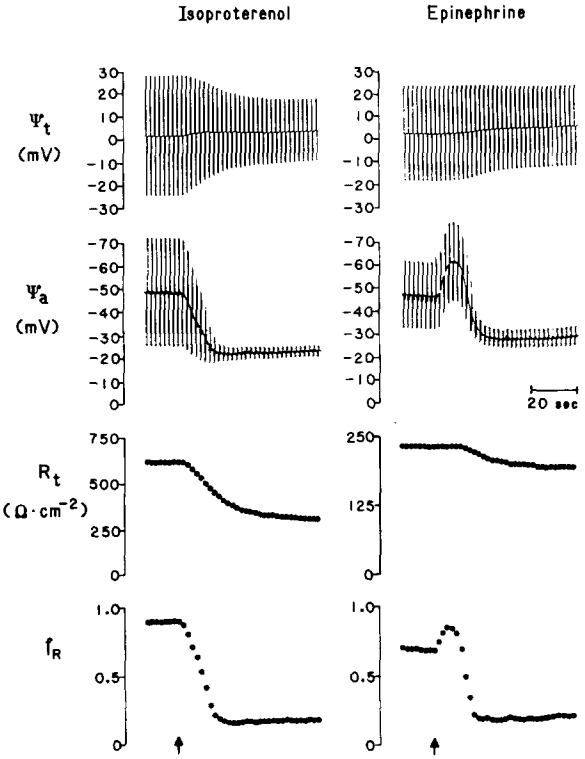

Fig. 1. Effect of submucosal addition of isoproterenol (5×10^{-6} M) and epinephrine (10^{-6} M) on the electrical properties of two representative epithelial monolayers. Figure shows record of transmonolayer electrical potential difference (Ψ_t) and apical membrane voltage (Ψ_a). Transepithelial resistance (R_t) and the fractional resistance of the apical membrane (f_R) were calculated from the voltage response to constant current pulses. Arrows indicate the onset of the response to secretagogue

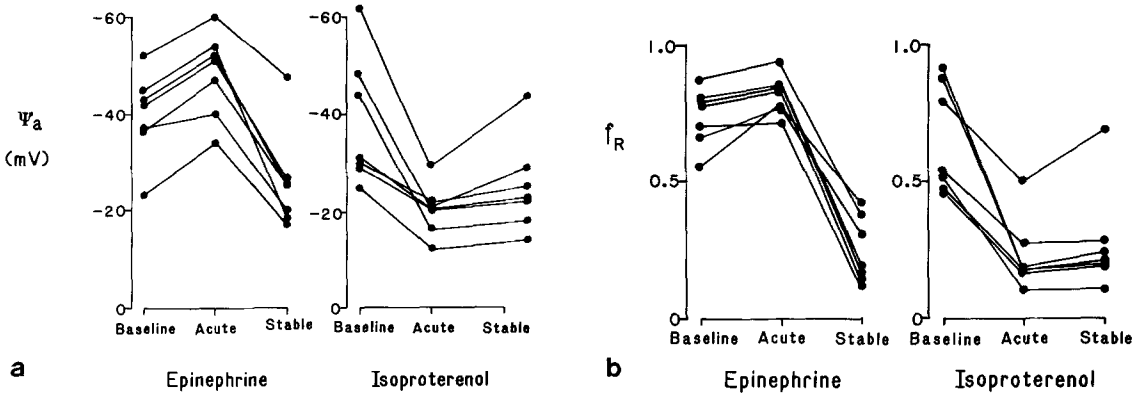

Fig. 2. Effect of epinephrine (10^{-6} M) and isoproterenol (5×10^{-6} M) on apical voltage Ψ_a, Fig. 2*a*, and fractional resistance of the apical membrane (f_R), Fig. 2*b*. Results show "Baseline" values, the maximal "Acute" change following submucosal addition of either agent, and the value obtained after electrical properties were "Stable"). Seven monolayers were studied in each group

Table 1. Effect of mucosal Cl and submucosal K substitutions on the electrical properties of monolayers stimulated with isoproterenol (5×10^{-6} M) or epinephrine (10^{-6} M)[a]

Condition	(*n*)	Isoproterenol				(*n*)	Epinephrine			
		Ψ_t (mV)	Ψ_a (mV)	R_t (Ω cm^2)	f_R		Ψ_t (mV)	Ψ_a (mV)	R_t (Ω cm^2)	f_R
Baseline	(5)	0.8	−28	42	0.26	(4)	0.7	−31	42	0.16
		±0.6	± 4	± 13	±0.05		±1.4	± 4	± 3	±0.04
Mucosal 4.8 mM Cl		7.2	−12	69	0.58		7.9	− 7	63	0.45
		±1.2	± 3	± 20	±0.07		±1.3	± 4	± 3	±0.02
Δ		+6.4*	+16*	+ 28*	+0.32*		+7.2*	+24*	+ 21*	+0.29*
		±0.7	± 2	± 7	±0.03		±0.4	± 3	± 3	±0.03
Baseline	(8)	0.3	−21	122	0.38	(14)	1.0	−28	150	0.38
		±0.7	± 4	± 53	±0.08		±0.5	± 2	± 48	±0.05
Submucosal 50 mM K		−0.7	−14	125	0.48		−0.6	−22	151	0.43
		±0.8	± 2	± 58	±0.09		±0.5	± 1	± 49	±0.06
Δ		−1.0	+ 7*	+ 3	+0.10*		−1.7*	+ 6*	+ 1	+0.05*
		±0.4	± 2	± 6	±0.04		±0.2	± 2	± 6	±0.02

[a] Mucosal Cl was decreased to 4.8 mM by substituting gluconate. Submucosal K was increased to 50 mM in exchange for Na. (*n*) represents the number of monolayers in each group. Δ refers to the change produced by the ion substitution.
* $P < 0.05$ by paired analysis.

The sequence of changes in Ψ_a and f_R following addition of isoproterenol and epinephrine were consistent findings, as shown in Fig. 2, for seven monolayers treated with each agent. Epinephrine consistently produced an initial simultaneous hyperpolarization of Ψ_a and increase in f_R. In contrast, isoproterenol initially depolarized Ψ_a and decreased f_R. Following these initial changes with isoproterenol, there was a small and variable hyperpolarization of Ψ_a.

In both the cultured monolayers (Fig. 2) and the intact epithelium (Al-Bazzaz & Cheng, 1979) isoproterenol and epinephrine produce the same maximal degree of stimulation of Cl secretion: with both agents the steady-state changes in transepithelial and cellular electrical properties, as well as the rate of Cl secretion, are identical. To further substantiate that this is the case with the cultured cells, we examined the effect of mucosal Cl substitution or an increase in submucosal K concentration on the cellular electrical properties and compared the response in monolayers treated with either isoproterenol or epinephrine. Table 1 shows that the changes in cellular electrical properties induced by ion substitutions are the same, irrespective of the specific secretagogue used.

One further way of evaluating the differences between isoproterenol and epinephrine is to examine the effect of sequential addition of maximal concentrations of the two agents. Addition of isopro-

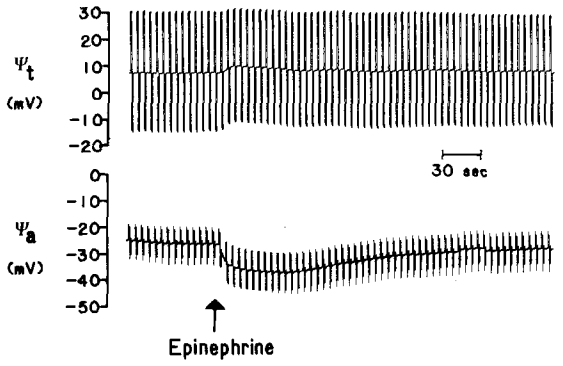

Fig. 3. Representative tracing showing the effect of adding submucosal epinephrine (10^{-6} M) to an isoproterenol-stimulated (5×10^{-6} M) monolayer

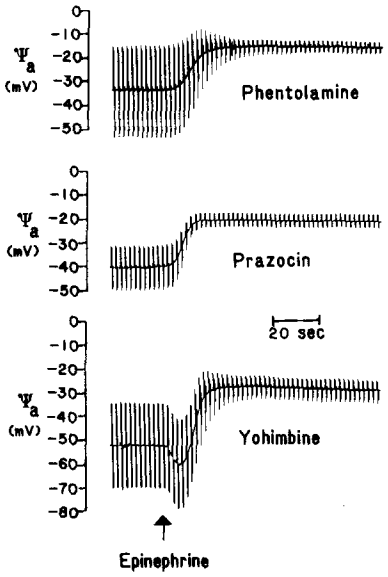

Fig. 4. Effect of α adrenergic antagonists on the response to epinephrine. Three representative monolayers are shown; either phentolamine (10^{-5} M), prazosin (10^{-5} M), or yohimbine (10^{-6} M) was added to the submucosal solution (as indicated) 10 min before the addition of epinephrine (10^{-6} M, submucosal solution)

terenol (5×10^{-6} M) had no effect on monolayers that had been stimulated with epinephrine (10^{-6} M). However, when we added epinephrine to monolayers that had been stimulated with isoproterenol, we saw a hyperpolarization of Ψ_a and increase in f_R, as shown in Fig. 3. The changes in Ψ_a and f_R were transient; both returned to the steady-state values observed before addition of epinephrine. Figure 3 shows that epinephrine also increased Ψ_t and decreased R_t; however, such changes were small, transient, and not always present. These results suggest that both epinephrine and isoproterenol increase G_a^{Cl} and increase G_b^K, but that epinephrine has a more pronounced acute effect on G_b^K than

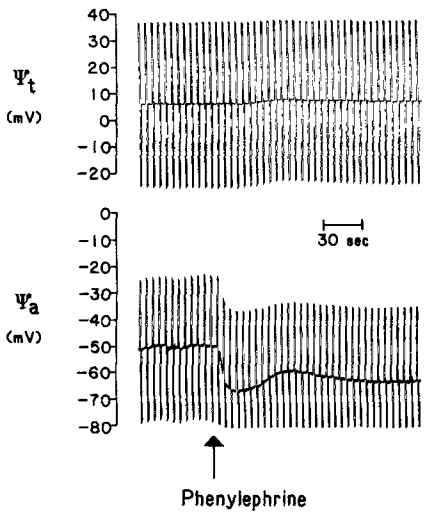

Fig. 5. Effect of phenylephrine (10^{-6} M) on cellular electrical properties. Propranolol (10^{-5} M) was present in both bathing solutions throughout to minimize possible β adrenergic effects of phenylephrine. Following addition of phenylephrine, the voltage deflections of Ψ_a are asymmetric because they were limited by the width of the chart paper

isoproterenol. The most likely explanation for this difference is the fact that epinephrine also stimulates α adrenergic receptors.

Effect of α Adrenergic Stimulation

To determine if the epithelial cells have α adrenergic receptors that regulate ion transport, we examined the effect of specific α adrenergic antagonists and agonists. Figure 4 shows that the α adrenergic antagonist, phentolamine, prevented the acute hyperpolarization of cell voltage and increase in f_R induced by epinephrine (compare with Fig. 1) (n = 5). However, it did not alter the depolarization or decrease in f_R. Thus, in the presence of an α adrenergic blocker, the response to epinephrine resembled the response to the pure β agonist, isoproterenol. To determine whether the α-adrenergic response results from stimulation of α_1 or α_2 receptors, prazosin and yohimbine, respectively, were added to monolayers before stimulating with epinephrine (Fig. 4). The ability of prazosin (n = 5) but not yohimbine (n = 3) to prevent the acute hyperpolarization produced by epinephrine suggest that α_1 adrenergic receptors mediate the acute effect on G_n^K.

We also examined the effect of adding an α-adrenergic agonist, to determine if it would reproduce the hyperpolarization of cell voltage and increase f_R. Figure 5 shows that phenylephrine (10^{-6} M) hyperpolarized Ψ_a and increased f_R (as judged from the increase in voltage deflections in Ψ_a). In

Table 2. Effect of phenylephrine (10^{-6} M) added to the submucosal solution[a]

	Ψ_t (mV)	Ψ_a (mV)	R_t (Ω cm^2)	f_R
Baseline	2.8	−35	160	0.55
	±0.9	± 4	± 37	±0.08
Phenylephrine	3.1*	−41*	159	0.64*
	±0.8	± 5	± 37	±0.08

[a] Values represent the mean ± SEM from nine monolayers taken during the baseline period and during the peak hyperpolarization of Ψ_a following addition of phenylephrine.
* $P < 0.05$ compared to baseline by paired analysis.

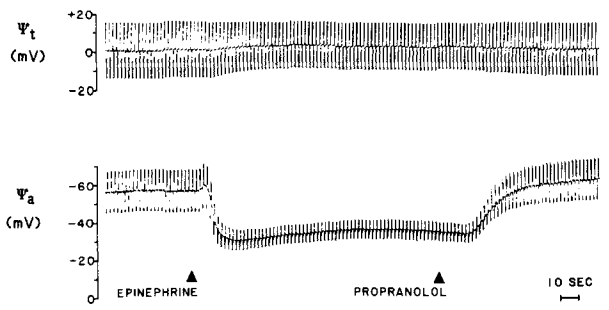

Fig. 6. Effect of sequential addition of epinephrine (10^{-6} M) and propranolol (10^{-5} M) to the submucosal bathing solution

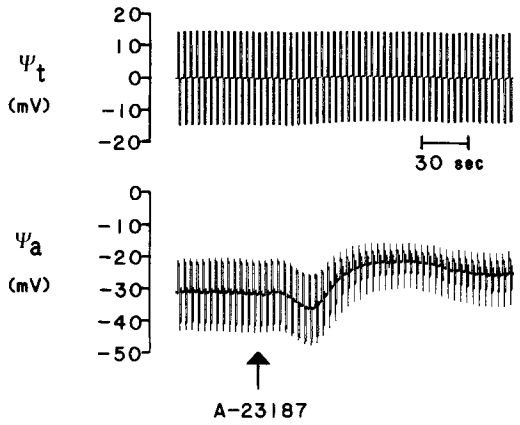

Fig. 7. Effect of adding the calcium ionophore A23187 (10^{-6} M) to the mucosal bathing solution

this tracing, Ψ_t also hyperpolarized and R_t decreased slightly; however, these were inconsistent findings. Because these changes in Ψ_t and R_t might possibly result from some residual β-adrenergic effect of phenylephrine (Al-Bazzaz & Cheng, 1979), the monolayers were preteated with propranolol (10^{-5} M), a β-adrenergic antagonist. Incubation with propranolol minimized the changes in Ψ_t, but did not always completely abolish them. However, we should also note that the changes in Ψ_t and R_t were much less than those observed with β-adrenergic stimulation. These findings are consistent with previous observations (Al-Bazzaz & Cheng, 1979; Smith et al., 1982) that α adrenergic agonists do not alter Cl transport by the intact epithelium. Table 2 shows the results obtained following addition of phenylephrine in nine monolayers; phenylephrine consistently hyperpolarized Ψ_a and increased f_R but had minimal effects on transmonolayer electrical properties.

One further way of demonstrating the α adrenergic response of the epithelium is shown in Fig. 6. Epinephrine was first added to the monolayer, followed by propranolol to block the β adrenergic effects. Following addition of propranolol, Ψ_a hyperpolarized to a value greater than that observed before addition of epinephrine and f_R increased above baseline values. Coincident with these changes Ψ_t decreased and R_t increased back toward baseline values. In six monolayers undergoing the same treatment as that shown in Fig. 6, baseline Ψ_a was −40 ± 4 mV. Ψ_a hyperpolarized to −48 ± 4 mV following addition of epinephrine and then reached a stable value of −21 ± 2 mV. Following addition of propranolol (10^{-5} M), Ψ_a hyperpolarized to −46 ± 5 mV.

Effect of A23187 and Exogenous cAMP

In tracheal epithelium (Al-Bazzaz & Cheng, 1979; Al-Bazzaz, 1981; Smith et al., 1982) as well as a variety of other tissues (Lefkowitz et al., 1983), the effect of β adrenergic agonists is thought to be mediated by an increase in cellular levels of cAMP. In contrast, in many tissues the response to α_1 adrenergic agonists is mediated, at least in part, by an increase in cell Ca (Exton, 1981). To determine if the effect of α adrenergic stimulation might be mimicked by an increase in cell Ca, we examined the electrophysiologic response to the Ca ionophore, A23187 (10^{-6} M). Figure 7 shows that addition of A23187 initially hyperpolarized Ψ_a and increased f_R, a response similar to that observed with α adrenergic stimulation alone or in combination with β stimulation. Table 3 shows the mean initial responses obtained from six monolayers; A23187 produced changes similar to those observed with phenylephrine (Table 2) or acutely with epinephrine (Figs. 1 and 2). The similarity suggests that the acute effects of α adrenergic stimulation may result from an increase in cell Ca.

We also examined the electrical response to addition of 8-bromo-cAMP (10^{-4} M) and theophylline (2.5×10^{-3} M); Fig. 8 shows a representative example. Following addition of cAMP and the phosphodiesterase inhibitor, theophylline, the changes in electrical properties resembled those observed with

Table 3. Acute response to A23187 (10^{-6} M) added to the mucosal solution[a]

	Ψ_t (mV)	Ψ_a (mV)	R_t (Ω cm^2)	f_R
Baseline	1.4	−32	280	0.59
	±0.6	± 2	± 72	±0.08
A23187	1.7	−38*	274*	0.64*
	±0.6	± 3	± 71	±0.09

[a] Values represent the mean ± SEM from six monolayers. Values were taken at the time of the acute hyperpolarization of Ψ_a.
* $P < 0.05$ compared to baseline by paired analysis.

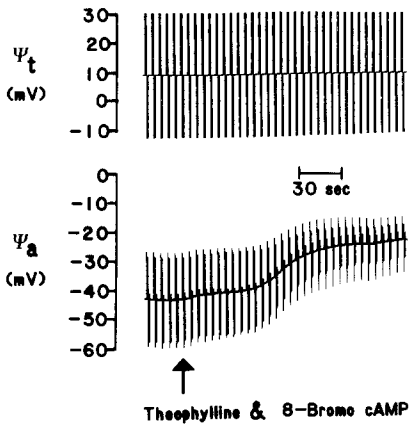

Fig. 8. Effect of adding 8-bromo-cAMP (10^{-4} M) and theophylline (2.5×10^{-3} M) to the mucosal bathing solution

β adrenergic stimulation alone (e.g. compare to Fig. 1). Table 4 shows the mean values of electrical properties obtained in eight monolayers during the baseline period and following addition of cAMP and theophylline. These agents consistently depolarized Ψ_a and f_R; the acute hyperpolarization seen with α adrenergic agents or A23187 was never seen.

Effect of Isoproterenol in the Intact Epithelium

The results presented above demonstrate both α and β adrenergic effects on the cell membrane ion transport processes. However, the pattern of response differs from that observed in the intact (or native) tracheal epithelium (Welsh et al., 1982, 1983). In the cultured epithelium epinephrine first increases G_b^K and then increases in G_a^{Cl}. In contrast, in the intact epithelium, epinephrine produces an acute increase in G_a^{Cl} followed by a rapid increase in G_b^K. To determine if the rapid increase in G_b^K and thus the marked biphasic changes in Ψ_a and f_R observed in the intact epithelium result from an α-adrenergic effect, we examined the electrical response to addition of isoproterenol (5×10^{-6} M) in the intact epithelium. A representative tracing is shown in Fig. 9. We observed a similar response in five other tissues; we never observed the rapid biphasic changes observed with epinephrine in the intact epithelium (*see* Fig. 8, Welsh et al., 1982 and Fig. 3, Welsh et al., 1983). These results suggest that in the intact epithelium, as well as in the cultured epithelium, there is an effect of α adrenergic stimulation that increases basolateral K conductance.

Discussion

The results of this study indicate that both β and α adrenergic agents regulate cell membrane ion transport processes in canine tracheal epithelium. The use of intracellular microelectrode and cell culture techniques allowed us to demonstrate an α adrenergic effect on the ion transport properties of the surface epithelial cells that had not previously been appreciated from the measurement of transepithelial electrical properties in intact epithelia (Al-Bazzaz & Cheng, 1979; Smith et al., 1982). The α adrenergic effect appeared to be mediated via an α_1 adrenergic receptor, because the α_1 adrenergic antagonist, prazosin blocked the effects of epinephrine. The nonspecific α blocker phentolamine also blocked epinephrine's effects, while the α_2 antagonist, yohimbine, did not. These findings suggest that the α_1 adrenergic receptors previously localized to the epithelium with autoradiographic techniques (Barnes & Basbaum, 1983), may function in controlling ion transport. Thus α adrenergic nerves may influence tracheal epithelial cells as well as the submucosal glands (Phipps et al, 1980; Ueki et al., 1980).

We observed that addition of epinephrine produce a different pattern of cellular electrophysiologic response in cultured and intact tracheal epithelium. In the cultured epithelium G_b^K increases and then G_a^{Cl} increases. In the intact epithelium the sequence is reversed. However, in the intact epithelium comparison of the response to isoproterenol, a pure β agonist (Fig. 9), with that to epinephrine, a combined β and α agonist (Welsh et al., 1982, 1983) suggests that the intact epithelium also exhibits an effect of α adrenergic stimulation. The observation that prostaglandin E_1 increases cellular cAMP levels (Al-Bazzaz et al., 1981; Smith et al., 1982; Lazarus et al., 1984) and produces an electrophysiologic response (Welsh et al., 1982) similar to that seen with isoproterenol is consistent with this conclusion. The difference between the intact and cultured

Table 4. Effect of mucosal 8-bromo-cAMP (10^{-4} M) and theophylline (2.5×10^{-3} M) on cellular electrical properties[a]

	Ψ_t (mV)	Ψ_a (mV)	R_t (Ω cm^2)	f_R
Baseline	2.4	−45	201	0.76
	±0.7	± 2	± 43	±0.03
8-bromo cAMP	4.2*	−31*	183	0.42*
and theophylline	±0.7	± 3	± 35	±0.07

[a] Values represent mean ± SEM from eight monolayers.
* $P < 0.002$ compared to baseline by paired analysis.

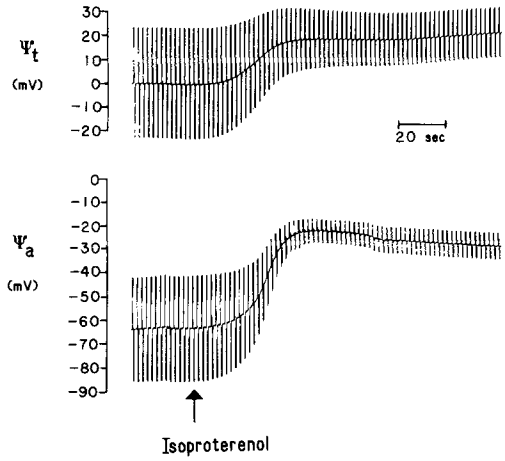

Fig. 9. Effect of isoproterenol (5×10^{-6} M) on the cellular electrical properties of intact canine tracheal epithelium

epithelium may relate to a difference in the number of receptors, the shape of the cells (the intact epithelium has columnar cells while the cultured cells are flatter), the relative density of Cl and K channels on the two cell membranes, or some unknown factor. However, this difference clearly does not alter or detract from our conclusions.

The main conclusion of this work is that there is a substantial difference between the effect of β adrenergic stimulation and that of α adrenergic stimulation on the cell membrane conductive transport processes. First, consider the response to β adrenergic stimulation.

1) β stimulation is sufficient to stimulate Cl secretion in the cultured epithelium, as evidenced by the increase in Ψ_t and fall in R_t (Fig. 1). These results are similar to previous observations that isoproterenol stimulates Cl secretion in cultured monolayers (Welsh, 1985). They are also consistent with transepithelial studies in the intact epithelium: Al-Bazzaz and Cheng (1979) observed that isoproterenol produced a maximal rate of Cl secretion and Davis et al. (1979) made a similar observation using the β-selective agonist, terbutaline.

2) The cellular electrophysiologic effects of β-adrenergic stimulation are the same as those observed with exogenous addition of cAMP and theophylline (*compare* Figs. 1 and 8). These results extend previous observations based on transepithelial electrical and flux measurements in the intact epithelium that exogenous cAMP stimulates Cl secretion (Al-Bazzaz, 1981).

3) β adrenergic agents increase cellular levels of cAMP in the surface epithelium (Lazarus et al., 1982; Smith et al., 1982) and in the cultured cells (*unpublished observation*).

4) β adrenergic stimulation produces an increase in the Cl conductance of the apical cell membrane, G_a^{Cl}. The first effect of adding β adrenergic agents or cAMP is a depolarization of Ψ_a, and decrease in f_R, consistent with an increase in G_a^{Cl}. In a previous study (Welsh, 1985) we demonstrated that, following stimulation with isoproterenol, the apical membrane is predominately Cl conductive.

Next consider the response to α adrenergic stimulation.

1) α adrenergic stimulation alone is not sufficient to stimulate transepithelial Cl secretion. Phenylephrine alone did not produce substantial increases in Ψ_t nor reduce R_t, as would be expected for an increase in Cl secretion. For example, compare the effect of phenylephrine with that of isoproterenol or cAMP (Fig. 5 and Table 2 *vs.* Fig. 1 and Table 4 or Tables 1–3 from Welsh, 1985). These results are similar to those previously made in the intact epithelium (Al-Bazzaz & Cheng, 1979; Smith et al., 1982).

2) The cellular electrophysiologic effects of α-adrenergic stimulation are the same as the acute effects observed following addition of the calcium ionophore. For example, compare Figs. 5 and 7 and Tables 2 and 3. Moreover, they are different from the response to cAMP and theophylline (Fig. 8 and Table 4).

3) The α adrenergic agent phenylephrine does not increase cellular levels of cAMP in the surface epithelium (Smith et al., 1982).

4) α adrenergic stimulation produces an increase in the K conductance of the basolateral cell membrane G_b^K. The first effect of addition of α agonists, either alone or in combination with β agonists, is a hyperpolarization of the cell, an increase in f_R, and at times a small decrease in R_t (Figs. 1, 2, 3, 5 and Table 2). The only reasonable interpretation of these findings is an increase in G_b^K. Support for this interpretation is our previous finding that the basolateral membrane of cultured cells is predominately K conductive under both secreting and nonstimulated conditions (Welsh, 1985), an obser-

vation similar to that made in the intact epithelium (Welsh, 1983; Smith & Frizzell, 1984).

In summary, the results indicate that β and α adrenergic agents have strikingly different effects on apical and basolateral conductances. The primary or first effect of β adrenergic stimulation (probably mediated by intracellular cAMP) may be to increase G_a^{Cl}. The primary effect of α adrenergic stimulation (possibly mediated by intracellular Ca) may be to increase G_b^K. Consistent with the latter notion is our recent finding that Ca regulates the basolateral K channel in excised patches of membrane (Welsh & McCann, 1985). In addition α_1 adrenergic stimulation is known to increase cell Ca in many different types of cells (Exton, 1981). However, it is also clear that the two membranes do not function independently. For example, β stimulation (cAMP) does not result solely in an increase in G_a^{Cl}; G_b^K also increases. The cellular mechanisms involved in this phenomenon are as yet uncertain.

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