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Changes in Smooth Muscle Tone During Osmotic Challenge in Relation to Epithelial Bioelectric Events in Guinea Pig Isolated Trachea[1]

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ABSTRACT

The relationship between epithelial bioelectric events and epithelium-dependent relaxant and contractile responses of airway smooth muscle in response to hyperosmolar and hypo-osmolar solutions was investigated in guinea pig isolated trachea. Tracheae were perfused with normal or nonisosmotic modified Krebs-Henseleit solution while simultaneously monitoring transepithelial potential difference (V_T) and contractile and relaxant responses of the muscle. Baseline V_T was −10.1 to −13.3 mV (distal and proximal ends, respectively). Intraluminal amiloride (10^{-4} M) induced a 3.7-mV depolarization, verifying that the V_T was of epithelial origin. Extraluminal methacholine (3×10^{-7} M; EC_{50}) caused hyperpolarization and smooth muscle contraction; intraluminal methacholine had very little effect. Increasing intraluminal bath osmolarity via addition of 240 mOsM NaCl or KCl caused an immediate and prolonged depolarization and epithelium-dependent relaxation. Increasing intraluminal bath osmolarity with sucrose evoked similar responses, except that an immediate, transient hyperpolarization and contraction preceded the depolarization and relaxation. Increasing extraluminal bath osmolarity with 240 mOsM NaCl induced depolarization and a longer lasting epithelium-dependent relaxation, whereas extraluminally added 240 mOsM KCl induced a complex smooth muscle response (i.e., transient relaxation followed by contraction), which was accompanied by prolonged depolarization. Intraluminal hypo-osmolarity produced a transient hyperpolarization followed by depolarization along with contraction of the smooth muscle. Bioelectric responses always preceded smooth muscle responses. These results suggest that bioelectric events in the epithelium triggered by nonisosmotic solutions are associated with epithelium-dependent responses in tracheal smooth muscle.

Interest in modulation of airway smooth muscle function by the epithelium has been stimulated by observations that loss of, or damage to, the airway epithelium is a common feature of respiratory diseases that are characterized by increased airway responsiveness, e.g., asthma and bronchopulmonary dysplasia (Laitinen et al., 1988; Lee and O'Brodovich, 1988; Jeffery et al., 1989; Coalson et al., 1992; Montefort et al., 1992). Epithelial damage and loss could affect the responsiveness of the underlying smooth muscle by perturbing several of its important functions (Fedan et al., 1988; Goldie and Hay, 1997): 1) the epithelium acts as a protective and selective barrier that restricts access of environmental agents to the smooth muscle and nerves; 2) the epithelium contains enzymes that degrade contractile and relaxant agonists and mediators; and 3) the epithelium releases substances that modulate the activity of the muscle. Eicosanoids, cytokines, nitric oxide, and the epithelium-derived relaxing factor (EpDRF; Flavahan et al., 1985; Hay et al., 1986; Fernandes and Goldie, 1991; Spina and Page, 1991) are examples of substances that originate from the epithelium and modulate smooth muscle tone.

The guinea pig isolated, perfused trachea has been useful for identifying the modulatory role of the epithelium in large airways because agents can be applied selectively to either side of the tracheal wall (Munakata et al., 1988; Fedan et al., 1990; Fedan and Frazer, 1992). Munakata et al. (1988) used the preparation to define the epithelium-dependent effects of intraluminal hyperosmolarity on carbachol-induced smooth muscle contraction. Addition of KCl to the mucosal surface caused an epithelium-dependent relaxation, even though KCl applied directly to the muscle on the serosal surface of the trachea or to the perfusate of epithelium-denuded tracheae caused contraction of the muscle (Munakata et al., 1988). The epithelial-dependent relaxation, mediated by Ep-

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ABBREVIATIONS: EpDRF, epithelium-derived relaxing factor; MKH, modified Krebs-Henseleit solution; V_T, transepithelial potential difference; EL, extraluminal; IL, intraluminal; MCh, methacholine.

DRF, was independent of the solute used to elevate osmolarity. Thus, elevated intraluminal osmolarity was demonstrated to stimulate epithelial-dependent relaxation.

Because many cells demonstrate volume-related regulation of ion transport processes when exposed to anisosmotic media, it is reasonable to think that the release of EpDRF in response to hyperosmolarity is somehow associated with electrical activity in the cell from which it is derived. It is well known that solution osmolarity can have important effects on transepithelial Na^+ transport across epithelia. Ussing (1965) demonstrated that hyperosmolar serosal solutions decreased Na^+ transport (as measured by amiloride-sensitive short-circuit current, I_{sc}) and caused cell shrinkage in isolated frog skin. Conversely, hypo-osmolar serosal solutions resulted in increased I_{sc} and cell swelling. More recently, hyperosmolar solutions have been reported to cause a decrease in basolateral membrane K^+ conductance (Lewis and Donaldson, 1990). Using human airway epithelium, Willumsen et al. (1994) demonstrated that luminal hyperosmolarity decreased Na^+ absorption and caused cell shrinkage, whereas basolateral hyperosmolarity did not elicit such changes.

Electrogenic Na^+,K^+-pumping, electroneutral Na^+-K^+-$2Cl^-$ cotransport and electrodiffusion of Na^+, K^+, and Cl^- play important roles in compensatory cell volume regulation, and the roles of these mechanisms in the release and/or actions of EpDRF have been investigated (Raeburn and Fedan, 1989; Lamport and Fedan, 1990; companion article, Fedan et al., 1999). The effects of ion transport inhibitors on mechanical responses of isolated, perfused guinea pig tracheae indicated that Na^+ and Cl^- channels are involved in EpDRF activity in response to application of hyperosmolar solutions to the mucosal surface, but that the Na^+, K^+-pump and the Na^+-K^+-$2Cl^-$ cotransporter have little role (companion article, Fedan et al., 1999).

In the present study, we used a novel in vitro method for evaluating the bioelectrical events in guinea pig tracheal epithelium associated with stimulation by nonisotonic solutions, while simultaneously monitoring smooth muscle responses, to examine the hypothesis that epithelial bioelectric effects and epithelium-dependent smooth muscle mechanical responses are functionally linked.

Materials and Methods

Guinea Pig Isolated, Perfused Trachea Preparation. Male English short-hair guinea pigs (482–816 g, Harlan Sprague-Dawley; Indianapolis, IN) were anesthetized by i.p. injection of pentobarbital sodium (65 mg/kg) and sacrificed by opening the chest and puncturing the heart. A 4.2-cm length of the trachea was removed, placed in modified Krebs-Henseleit (MKH) solution, and cleaned. The isolated trachea was attached at its upper and lower ends to a plastic perfusion holder (modeled after Fedan and Frazer, 1992) that contained indwelling side-hole catheters that were connected to the positive (inlet) and negative (outlet) sides of a differential pressure transducer. Once mounted, the tracheal segment was stretched to its original in situ length and placed in an organ bath at 37°C containing 25 ml MKH solution, which is referred to as the extraluminal bath. The trachea was perfused (22 ml/min) with recirculating MKH solution from a separate, 30-ml reservoir, which is referred to as the intraluminal bath. Responses were measured as changes in the inlet minus outlet pressure difference (ΔP), in cm of H_2O. A 2.5-h equilibration period was allowed before experiments were begun while changing the MKH solution in both baths at 15-min intervals. MKH solution contained: 113.0 mM NaCl, 4.8 mM KCl, 2.5 mM $CaCl_2$, 1.2 mM KH_2PO_4, 1.2 mM $MgSO_4$, 25.0 mM $NaHCO_3$, and 5.7 mM glucose, pH 7.4 (37°C), gassed with 95% O_2-5% CO_2.

Measurement of Transepithelial Potential Difference (V_T). A voltage/current clamp amplifier was used to record V_T at the proximal and distal ends of the trachea. V_T was recorded by placing voltage electrodes (filled with 3 M KCl in 2% agar) at the basolateral (V_b) and apical (V_a) surfaces of the trachea. The electrode at the basolateral surface was placed in the extraluminal bath while the apical electrode was positioned in the intraluminal perfusion line. The potentials (lumen negative) were each equal to the sum of an apical and basolateral potential ($V_a + V_b = V_T$) and an offset potential. Calomel half-cells were matched to less than 2-mV offsets, and the offset potentials were adjusted to zero before mounting the tracheae.

Removal of Epithelium. For experiments in which the effects of epithelium removal on V_T were assessed, a 5-cm long piece of pipe cleaner was advanced into and withdrawn from the tracheal lumen to remove the epithelium (Fedan and Frazer, 1992).

Addition of Agents. After an appropriate equilibration period, methacholine (MCh) was added to the extraluminal bath in a concentration (3×10^{-7} M), which approximates the EC_{50} for contraction in that bath (Fedan and Frazer, 1992). Responses to intra- and extraluminally applied KCl, NaCl, or sucrose, which were added to the MKH solution to elevate osmolarity, were generated after having obtained a stable contraction to MCh. When amiloride (10^{-4} M) was used, it was added to the intraluminal bath. The concentrations of NaCl, KCl, and sucrose given in *Results* refer to the concentrations added to the MKH solution. When it was added to the intraluminal bath, the switch from normal MKH solution to hyperosmolar or hypo-osmolar MKH solution was done in such a way as to present the new perfusing solution to the trachea abruptly. For experiments examining the effect of reduced osmolarity the MKH solution was made hypo-osmolar either by halving the NaCl concentration in MKH solution or by diluting the MKH solution with distilled water.

Drugs. MCh (acetyl-β-methylcholine) chloride and amiloride were obtained from Sigma Chemical Co. (St. Louis, MO). MCh was prepared in saline, and amiloride was prepared in distilled H_2O.

Data Analysis. The results shown are means ± S.E.M. The data were analyzed for differences using Student's t test for paired or nonpaired comparisons, repeated measures ANOVA, and correlation analysis, as appropriate. The 0.05 level of probability was considered significant.

Results

Validation of Model. Basal V_T was recorded for extended periods to determine an appropriate equilibration time necessary to achieve stable V_T. There was a progressive hyperpolarization during the first hour of equilibration, which reached a stable level by approximately 2 h (data not shown). Therefore, all experiments were conducted after tracheae were allowed to equilibrate for 2.5 h.

A previous study (Fedan and Frazer, 1992) demonstrated that age-related differences in tracheal diameter affected the measurement of ΔP, but tracheae excised from 600- to 800-g animals yielded consistent readings. An evaluation of the relationship between animal size and basal V_T showed no correlation (Fig. 1, $p = .38$). Animals weighing 600 to 800 g were therefore used in all subsequent experiments.

The perfusion holder allowed comparison of V_T from two points in the trachea, i.e., at the proximal (inflow) and distal (outflow) ends of the trachea. V_T recorded at the proximal end (−13.3 ± 1.3 mV) was slightly but not significantly larger than that recorded at the distal end (−10.1 ± 1.0 mV, $n = 19$). The V_T values presented below were obtained from the proximal end.

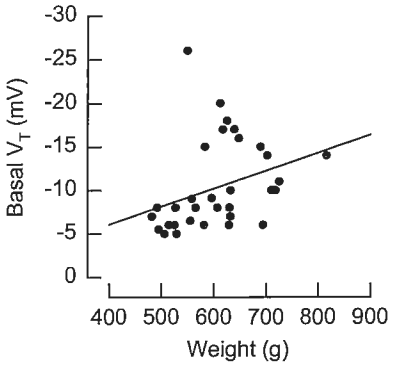

Fig. 1. Scatter plot of basal V_T versus animal weight. The plot revealed no correlation between animal weight and basal V_T.

To confirm that V_T represented a transepithelial potential difference, V_T was measured before and after denudation of the tracheae. After V_T was recorded from the intact tracheae, the organs were removed from the holders. The epithelium was removed and the tracheae were remounted on the holders, equilibrated for 2.5-h, and V_T was measured again. In the absence of the epithelium, V_T (-0.1 ± 0.1 mV; $n = 4$) was not significantly different from zero mV.

Amiloride (10^{-4} M) was added unilaterally to the bathing solutions to verify integrity and responsiveness of the epithelium. When added to the luminal bath, amiloride depolarized the epithelium (Fig. 2). Amiloride had a negligible effect in the extraluminal bath (Fig. 2), nor did it affect ΔP during the 5-min exposure in either bath (not shown).

MCh (3×10^{-7} M) had little effect on either V_T or smooth muscle tone when added to the intraluminal bath, but induced hyperpolarization and contraction when added to the extraluminal bath (Fig. 3). The MCh-induced hyperpolarization of the epithelium was abolished upon removal of the epithelium (data not shown). These results verified that the tracheal epithelium and smooth muscle responded normally to pharmacologic agents.

Hyperosmolar Solutions in Lumen Induce Epithelial Depolarization Followed by Smooth Muscle Relaxation. After the 2.5-h incubation period, tracheae were contracted with 3×10^{-7} M MCh in the extraluminal bath. NaCl or KCl (240 mOsM) added to intraluminal bath caused depolarization of the epithelium. The depolarization of the epithelium was accompanied by relaxation of the smooth muscle (Figs. 4 and 5). Repeated additions and washout of 120 mM KCl or 120 mM NaCl at 1-h intervals led to reproducible depolarization responses.

To examine the possibility that the effects of NaCl and KCl

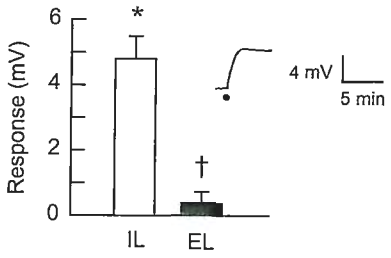

Fig. 2. Effect of intraluminal (IL) and extraluminal (EL) amiloride (10^{-4} M) on V_T. V_T was measured after 5 min of exposure. Inset tracing shows time course of response to intraluminally applied amiloride. *Significant depolarization by amiloride. †Significantly less than intraluminal application. $n = 3$.

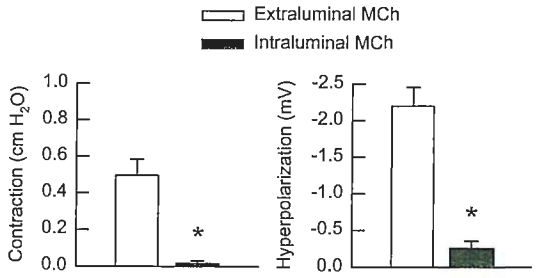

Fig. 3. Effect of extraluminal and intraluminal administration of MCh (3×10^{-7} M) on V_T and ΔP in guinea pig trachea. Left, effects of MCh on V_T; right, effects of MCh on ΔP. $n = 7$.

on V_T and ΔP reflected the effects of osmolarity per se as opposed to specific ionic interactions, sucrose was used as an alternative means of raising osmolarity. In contrast to NaCl and KCl, sucrose (240 mOsM) produced biphasic effects on V_T and ΔP (Fig. 4). There was a transient hyperpolarization followed by prolonged depolarization of the epithelium occurring concomitantly with a transient contraction followed by relaxation of the smooth muscle. The responses to repeated additions of sucrose also were reproducible (data not shown).

In all cases, the epithelial bioelectric response preceded the mechanical response. The application of hyperosmolar NaCl to the intraluminal perfusate produced an immediate depolarization with the onset of relaxation occurring after several seconds, i.e., ca. 14-, 6-, and 15-s later for NaCl, KCl, and sucrose, respectively (Table 1). The reason(s) for the differences in these times and its possible biological significance are not understood. However, taken together, these results indicate that mucosal hyperosmolar solution induces parallel changes in epithelial electrogenic transport and smooth muscle tone, with the predominant effects being depolarization of the epithelium and smooth muscle relaxation.

Extraluminal Hyperosmolar Solutions Depolarize Epithelium. It was of interest to ascertain whether extraluminal hyperosmolarity could initiate responses comparable with those elicited with intraluminal hyperosmolarity. In precontracted tracheae, raising the osmolarity of the extraluminal MKH solution by the addition of NaCl (240 mOsM) produced immediate depolarization of the epithelium followed by transient relaxation of the smooth muscle (Fig. 6). Addition of hyperosmolar KCl (240 mOsM) to the extraluminal bath likewise decreased V_T and induced a biphasic change in smooth muscle tone, which consisted of transient relaxation followed by sustained contraction (Fig. 6).

When added to the extraluminal bath, hyperosmolar NaCl and KCl also triggered an immediate depolarization. The depolarization preceded the mechanical response by approximately 29 and 17 s for NaCl and KCl, respectively (Table 1). It is important to note that the delays observed after the extraluminal additions were significantly larger than those seen after the intraluminal additions.

The transient changes in V_T and ΔP were consistent with rapid depolarization of the epithelium and relaxation of the precontracted smooth muscle. The later increases in ΔP produced by intraluminal NaCl and KCl were consistent with a more slowly developing stimulation of smooth muscle contraction that overcame the early inhibitory activity.

Depolarization-Associated Relaxation Is Epithelial-Dependent. The epithelial dependence of responses to extraluminal and intraluminal increases in osmolarity were

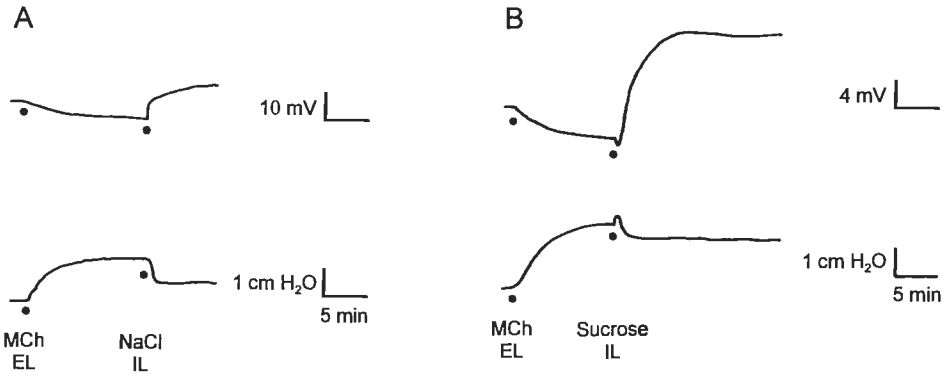

Fig. 4. Typical recording of effects of intraluminally (IL) applied (240 mOsM) NaCl and sucrose on V_T (top tracings) and ΔP (bottom tracings) in perfused trachea. A, effects of NaCl on V_T and ΔP; B, effects of sucrose on V_T and ΔP. Electrical and pressure responses were recorded simultaneously from same preparation. Tissues were precontracted with extraluminal (EL) MCh (3×10^{-7} M). Similar results were obtained with KCl (not shown).

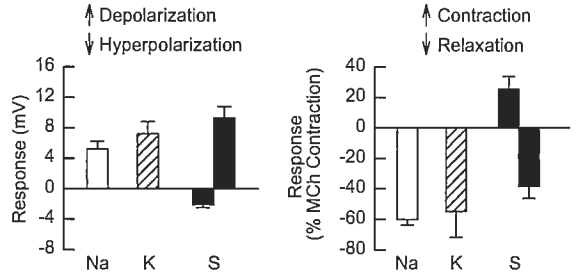

Fig. 5. Summary of effects of intraluminal NaCl, KCl, and sucrose (240 mOsM) on V_T (left) and ΔP responses (right) of MCh (3×10^{-7} M)-contracted tracheae. NaCl, KCl, and sucrose were added separately. ↓ denotes a hyperpolarization and relaxation response; ↑ denotes a depolarization and contractile response. On the left, transient hyperpolarization caused by sucrose is shown by the downward-directed bar and sustained depolarization is shown by the upward-directed bar; on the right, transient contraction to sucrose is shown by the upward-directed bar and sustained relaxation is shown by the downward-directed bar. ΔP responses are expressed as a percentage of MCh-induced contraction. n = 3 to 8.

TABLE 1

Time delay between onset of depolarization of epithelium and relaxation of smooth muscle after intra- and extraluminal additions of 240 mOsM NaCl, KCl, or sucrose

Agent	Delay
	s
Intraluminal	
NaCl	13.7 ± 2.2
KCl	6.4 ± 1.4**
Sucrose	14.8 ± 2.6
Extraluminal	
NaCl	28.7 ± 1.8*
KCl	17.0 ± 1.7*,**

* Significantly longer than after intraluminal addition of the same salt.
** Significantly shorter than after NaCl administration; n = 6–8 for NaCl and KCl; n = 3 for sucrose.

investigated in MCh-contracted tracheae denuded of their epithelium (Fig. 7). The relaxations initiated by intra- and extraluminal application of elevated NaCl and KCl concentration (240 mOsM) were inhibited by removal of the epithelium. These results provide evidence that epithelial depolarization is associated with release of EpDRF during response to hyperosmolar solutions.

Depolarization-Associated Relaxation Is Independent of MCh. In tracheae that were not precontracted with MCh, addition of NaCl to the extraluminal or intraluminal baths decreased V_T and basal tone (if any was present; Fig. 8). Addition of KCl to the intra- or extraluminal baths both resulted in a decrease in V_T (Fig. 8); intraluminal KCl caused a decrease in basal tone (if present), whereas extraluminal KCl caused contraction (Fig. 8). These results confirm that relaxation associated with epithelial depolarization occurs both in the presence and absence of MCh and is not dependent upon smooth muscle contraction.

Effect of Intraluminal Hypo-osmolarity on V_T and ΔP. We observed (companion article, Fedan et al., 1999) that the trachea responds to decreases as well as increases in luminal osmolarity. Perfusion of the tracheal lumen with hypo-osmolar MKH solution, which had been prepared with half the normal osmolar concentrations of its constituents (162.9 mOsM), induced a rapid, biphasic change in V_T along with contraction of the smooth muscle (Figs. 9 and 10). The biphasic change in V_T consisted of a rapid, transient hyperpolarization followed by sustained depolarization. The same response pattern was observed when mucosal osmolarity was comparably reduced by perfusing with MKH that had been prepared to contain half the normal amount of NaCl (i.e., a reduction of 113 mOsM; Figs. 9 and 10). In addition, the contraction occurred 15.5 ± 2.0 s (diluted MKH) or 16.0 ± 1.8 s (reduced Na^+) after the onset of the hyperpolarization (data not shown, n = 4).

Discussion

We used a novel method to simultaneously measure V_T changes and mechanical responses of the airway smooth muscle to examine the association between epithelial bioelectric events and the release and effects of EpDRF and a putative contractile substance. The results suggest that there is a causal relationship between epithelial bioelectric events and epithelium-dependent mechanical responses of the underlying smooth muscle.

The basal V_T value obtained in this study was smaller than that reported previously for a guinea pig trachea preparation (Croxton, 1993); the reason(s) for the difference is not clear. In contrast, the basal V_T value we measured is similar to that obtained from in vivo studies in dog airway epithelium (Boucher et al., 1980). Confirmation of the epithelial origin of V_T was given by experiments in which V_T was measured before and after the epithelium was removed. Additional evidence was provided by the depolarization of the epithelium by the apical application of the Na^+-channel blocker, amiloride, as observed in rabbit (Takemura et al., 1995) and dog (Al-Bazzaz and Zevin, 1984) tracheae.

Addition of MCh to the basolateral surface of the guinea pig trachea hyperpolarized the epithelium and caused contraction. These responses were significantly less after application of the same concentration of MCh to the mucosal

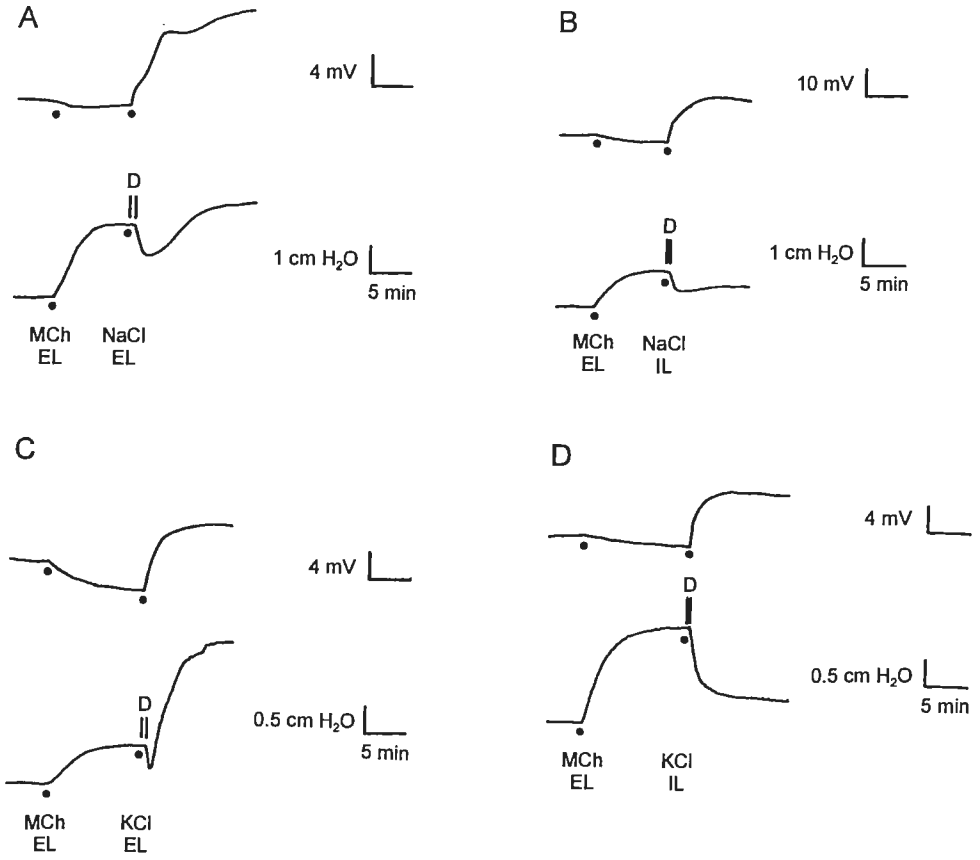

Fig. 6. Typical tracings showing effects of extraluminal (EL) and intraluminal (IL) additions of 240 mOsM NaCl and KCl on V_T (upper tracings) and ΔP (lower tracings) in MCh (3×10^{-7} M)-contracted tracheae. A, extraluminal NaCl; B, intraluminal NaCl; C, extraluminal KCl; D, intraluminal KCl. "D" and vertical lines between tracings indicate time delay between onset of the bioelectric response after addition of salt and the ensuing mechanical response.

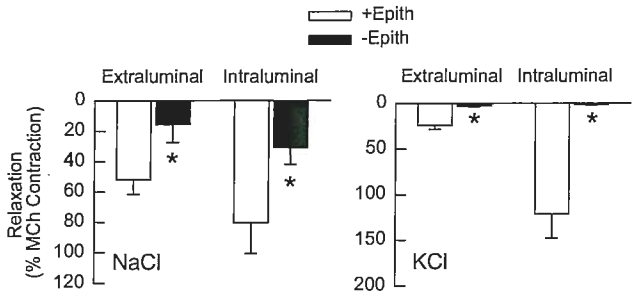

Fig. 7. Effect of epithelium (Epith) removal on relaxation responses elicited with extra- and intraluminal administration of hypertonic NaCl (left) and KCl (right) in guinea pig trachea; $n = 5$ and 6 for NaCl and KCl, respectively. *Significantly less than response before epithelium was removed.

surface. This finding manifests the diffusion barrier effect of the epithelium, i.e., the concentration of MCh at the basolateral surface was reduced upon its addition to the intraluminal bath. It also signifies that there is polarity in the localization or type of muscarinic receptors across the epithelium. The mechanism of the extraluminal MCh-induced hyperpolarization is not known at present. Nevertheless, the effect of MCh on V_T seen in this study is consistent with the observations of Tamaoki et al. (1996), who showed that exogenously applied acetylcholine increased V_T in the rabbit trachea whereas atropine caused it to decline. In addition, Sato (1984) found that MCh hyperpolarized the secretory coil of human ecrine sweat glands.

Application of hyperosmolar solution to the mucosal surface of guinea pig perfused trachea causes an epithelium-dependent relaxation, which is mediated by the release of

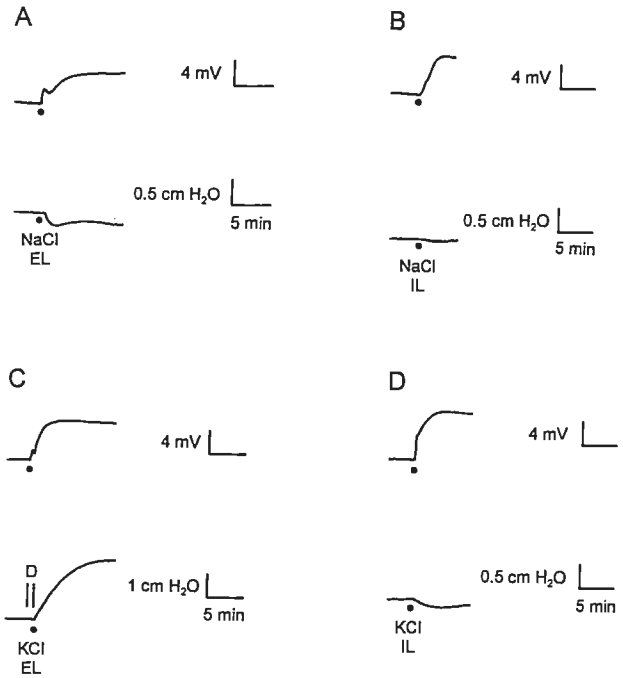

Fig. 8. Typical tracings showing effects of extraluminal (EL) and intraluminal (IL) additions of 240 mOsM NaCl and KCl on V_T (upper tracings) and ΔP (lower tracings) in unstimulated tracheae. A, extraluminal NaCl; B, intraluminal NaCl; C, extraluminal KCl; and D, intraluminal KCl.

EpDRF (Munakata et al., 1988; Fedan et al., 1990, 1999 companion article; Fedan and Frazer, 1992). In this study, we were able to identify electrophysiological events associated with the release and/or effect of EpDRF triggered with hy-

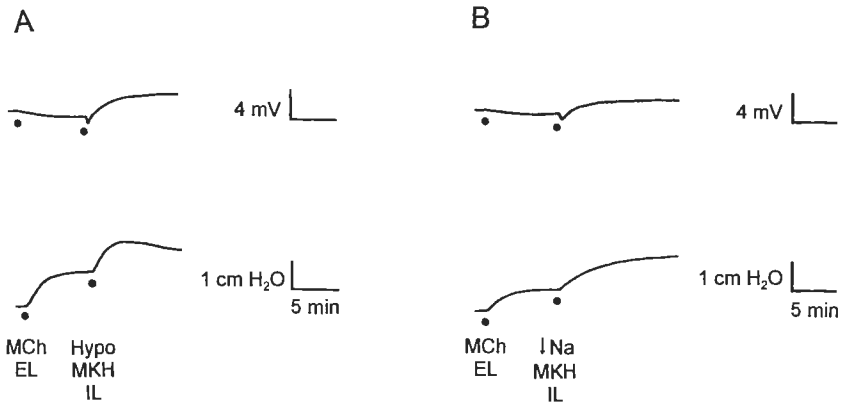

Fig. 9. Typical tracings showing effects of intraluminal (IL) hypotonic MKH on V_T (top traces) and ΔP (bottom traces). Tracheae were contracted with extraluminal (EL) MCh (3×10^{-7} M). A, tonicity of intraluminal bath was reduced by halving the solute concentration of intraluminal MKH solution. B, mucosal solution was made hypotonic by halving the NaCl concentration in MKH solution. Both methods of reducing tonicity of the intraluminal bath yielded similar response patterns.

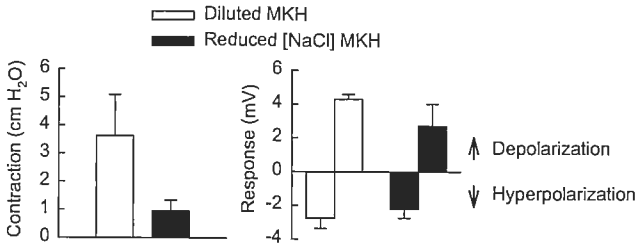

Fig. 10. Effects of intraluminal hypo-osmolarity on ΔP and V_T of extraluminal MCh (3×10^{-7} M)-contracted tracheae. Osmolarity was reduced by halving solute concentration of MKH solution (diluted MKH) or by halving NaCl concentration in MKH solution (reduced NaCl MKH). Left, contractile responses; right, bioelectric responses. On the right, transient hyperpolarization responses are shown by downward-directed bars; sustained depolarization responses are shown by upward-directed bars. n = 4 to 7.

perosmolar KCl and NaCl, and correlated them with mechanical responses of unstimulated or MCh-contracted preparations. Surprisingly, hyperosmolar sucrose applied to the mucosal surface also elicited biphasic V_T and mechanical responses, whereas the responses to intraluminal hyperosmolar KCl and NaCl were monophasic. That is, sucrose produced an initial hyperpolarization followed by prolonged depolarization, and the corresponding effects in the muscle consisted of a transient contraction followed by sustained relaxation. KCl and NaCl caused only depolarization of the epithelium and relaxation of the smooth muscle when applied to the mucosal surface.

EpDRF release by hyperosmolar solution is independent of the agent, i.e., ionic or nonionic, used to raise osmolarity (Munakata et al., 1988; companion article, Fedan et al., 1999). Sucrose was used in this study to examine EpDRF release in response to a hyperosmolar stimulus while circumventing direct interactions of Na^+ and K^+ with specific ion channels, transporters, and pumps. We do not know the mediator or mechanism of the transient contraction produced by sucrose, but it could involve a putative epithelium-derived contracting factor. Previous studies have provided evidence for such a substance. Farmer et al. (1987) observed that arachidonic acid, in the presence of 4-nordihydroguaiaretic acid, an inhibitor of 5′-lipoxygenase, elicited epithelium-dependent contractions of guinea pig tracheal strips. Although removal of the epithelium increased reactivity of guinea pig tracheal strips at 37°C (Hay et al., 1986, 1987), Lamport and Fedan (1990) found at 22°C that concentration-response curves were shifted rightward, not leftward as is seen at 37°C, upon removal of the epithelium. Based on this observation, a putative epithelium-derived excitatory substance was postulated to exist.

The finding that elevated osmolarity at the serosal surface also caused depolarization and relaxation of the trachea is consistent with the idea that the airway epithelium acts as an osmotic sensor transducing information about luminal and extraluminal solution osmolarity to the airway smooth muscle. Munakata et al. (1988) did not report relaxation to extraluminal hyperosmolar KCl, and viewed the effects of hyperosmolarity to stem from mucosal surface effects. The differences in the effects of extraluminal hyperosmolarity in Munakata's study and our study are easily explainable in terms of differences in experimental design. In Munakata's study, the trachea was not precontracted before being exposed to extraluminal KCl, and the initial relaxing effect of cumulatively added extraluminal KCl was not therefore observed before the contractile response occurred. In the present study, however, contraction of the trachea with MCh before exposure to KCl established the condition that allowed the initial, transient relaxation to be evident. It is important to note that the relaxation responses to both mucosally and serosally applied hyperosmolar solution were associated with depolarization and were epithelium dependent. This suggests that hyperosmolarity at either pole of the epithelial cell may initiate comparable bioelectric events, which are linked to EpDRF production and/or release. In contrast, in human airway epithelium only mucosal hyperosmolarity decreased transepithelial potential difference (Willumsen et al., 1994). It is difficult to reconcile our findings with those of Willumsen et al. (1994). Species or regional airway differences might account for the differing results.

Extraluminally added KCl induced contraction of the tracheal smooth muscle after the relaxation phase had ended. This effect was not evident when NaCl was used to raise osmolarity. In other words, the KCl-induced contraction occurred in the face of EpDRF release from the basolateral surface. The differences between the effects seen with NaCl and KCl after extraluminal addition is clearly due to differences in each agent's effect on smooth muscle.

Evidence was obtained that indicates that the release/effects of EpDRF on the smooth muscle follows the bioelectric events. This evidence was generated from the measurable lag between the onset of depolarization and the beginning of relaxation of the smooth muscle after intraluminal or extraluminal additions of KCl or NaCl. The lag is taken to

reflect the time between stimulation of EpDRF synthesis and release, and its diffusion through the airway wall to the level of the smooth muscle. In the case of NaCl, the relaxing effect of EpDRF occurred 14 s after depolarization initiated by intraluminally added hyperosmolarity but 29 s after extraluminal addition; qualitative similar results were seen with KCl. That is, the larger delay after the extraluminal bath addition reflects the additional time required for the hyperosmolar solute to penetrate the airway wall from the serosal site of entry to the epithelium. Thus, it may take 14 s for EpDRF to be released and diffuse to the smooth muscle after intraluminal addition of salt. These findings also argue strongly against the possibility that any effect of hypertonic NaCl in these experiments reflected a direct effect of the salt on the smooth muscle. Had this occurred, the relaxation effect of extraluminally applied NaCl would have preceded the epithelial depolarization.

This study also demonstrated that the effect of intraluminal hypo-osmolarity on the tracheal smooth muscle occurred following bioelectric events in the epithelium. The delay, approximately 16 s, was comparable with the delay between the onset of bioelectric and mechanical changes in response to hyperosmolarity. When the mucosal surface of the guinea pig trachea was exposed to hypo-osmolar MKH, an initial, transient hyperpolarization followed by a sustained depolarization was associated with contraction of the smooth muscle. Two methods for reducing the osmolarity of the intraluminal MKH solution brought about similar bioelectric and mechanical effects. In the preceding report, Fedan et al. (1999) found that addition of hypo-osmolar MKH to the mucosal surface caused contraction of the perfused trachea, and it was suggested that the epithelium mediates the response, at least in part. The mechanism of the contraction and the nature of the putative substance released by hypo-osmolarity are unknown. Because the contraction was associated with hyperpolarization, as was that when sucrose was added to the intraluminal bath, we speculate that it is somehow linked to the generation of the putative contractile factor.

The preceding study (companion article, Fedan et al., 1999) showed that Na^+ and Cl^- the channel blockers inhibited relaxation of MCh contracted tracheae to intraluminal hyperosmolarity, whereas inhibition of the Na^+-K^+-$2Cl^-$ cotransporter produced a modest inhibition of the responses. The involvement of these pathways in osmotically induced bioelectric responses is currently under investigation.

In summary, mucosal hyperosmolarity achieved with KCl and NaCl induced relaxation of the perfused trachea that was associated with depolarization of the epithelium. Relaxation occurred after extraluminal addition of hyperosmotic solutions as well, and we provisionally conclude that this response also is due to EpDRF release. Responses to sucrose appear to involve an additional contractile, hyperpolarizing component. Epithelial electrophysiologic responses to nonisotonic solutions always preceded the mechanical responses of the muscle. These findings suggest that electrophysiological events in the epithelium are responsible for or are associated with osmolarity-induced release of epithelium-derived relaxant and contractile substances.

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