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Hyperosmolar Solution Effects in Guinea Pig Airways. II. Epithelial Bioelectric Responses to Relative Changes in Osmolarity

David X.-Y. Wu, Richard A. Johnston, Appavoo Rengasamy, Michael R. Van Scott, and Jeffrey S. Fedan

Pathology and Physiology Research Branch, Health Effects Laboratory Division, National Institute for Occupational Safety and Health, Morgantown, West Virginia (D.X.-Y.W., R.A.J., A.R., J.S.F.); Department of Pharmacology and Toxicology, Robert C. Byrd Health Sciences Center of West Virginia University, Morgantown, West Virginia (J.S.F., R.A.J.); and Department of Physiology, The Brody School of Medicine at East Carolina University, Greenville, North Carolina (M.R.V.S.)

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ABSTRACT

Osmotic challenge of airways alters the bioelectric properties of the airway epithelium and induces the release of factors that modulate smooth muscle tone. Recent studies in our laboratory suggested that methacholine-contracted airways relax in response to incremental increases in osmolarity, rather than from cell shrinkage or absolute solute concentration. In the present study, guinea pig tracheae were mounted in Ussing chambers to elucidate the bioelectric effects of challenge of the epithelium with hyperosmolar and isosmolar solutions. Transepithelial short-circuit current (I_{sc}) across tracheae stimulated with basolateral methacholine was inhibited by apical amiloride, apical 5-nitro-2-(3-phenylpropylamino)benzoic acid, basolateral bumetanide, basolateral ouabain, and Cl^--free solution, but not by basolateral iberiotoxin. Apical hyperosmolar challenge with NaCl variably decreased or increased I_{sc}, but D-mannitol (D-M) always inhibited I_{sc}; bumetanide attenuated decreases in I_{sc}. The effects of the transport blockers depended upon whether I_{sc} was initially decreased or increased. Unique concentration-dependent changes in I_{sc} and transepithelial resistance (R_t) were observed when ionic (NaCl and KCl), nonionic impermeant (D-M and sucrose), and nonionic permeant (urea) osmolytes were added to the apical and basolateral baths. At concentrations that doubled the osmolarity of the apical bath, D-M, urea, and *N*-methyl-D-glucamine-gluconate (NMDG-Glu) decreased I_{sc}. Apical isosmolar NMDG-Glu solution decreased I_{sc}, and additional NMDG-Glu caused a further decrease in I_{sc}. Inclusion of one permeant ion, either Na^+, K^+, or Cl^-, reversed the response to apical isosmolar and hyperosmolar solutions. Thus, bioelectric responses of the airway epithelium to hyperosmolar solution are induced by incremental increases in osmolarity.

Asthmatic patients may experience obstruction at the conclusion of exercise (exercise-induced asthma), whereas nonasthmatic individuals experience bronchodilation during exercise (Kagawa and Kerr, 1970; Godfrey, 1997). The increased minute ventilation during exercise causes evaporative water loss. The ensuing elevation in the osmolarity of the airway surface liquid is thought to stimulate bronchoconstriction (Anderson et al., 1982; Freed and Davis, 1999; Anderson and Daviskas, 2000) resulting from the effects of released mediators, including leukotrienes (Umeno et al., 1990; Makhdum and Pearce, 1993; Freed, 1995; Israel and Drazen, 1999). In addition to reacting to exercise, asthmatic patients are hyperreactive to inhaled hypertonic saline solution (Makker et al., 1994).

Several in vitro studies have suggested that challenge of the epithelium with hyperosmolar solution stimulates bioelectric events. Application of hyperosmolar solution[1] to ferret tracheal epithelium resulted in ion transport, the result of

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[1] Hypertonic solutions are those that cause cell shrinkage. Hyperosmolar solutions have osmolarity greater than that of the physiological extracellular solution. For simplicity, in this report we do not draw distinctions between the two terms when describing general phenomena.

ABBREVIATIONS: D-M, D-mannitol; EpDRF, epithelium-derived relaxing factor; MKHS, modified Krebs-Henseleit solution; I_{sc}, short-circuit current; R_t, transepithelial resistance; SPD, spontaneous potential difference; MCh, methacholine; NMDG, *N*-methyl-D-glucamine; NMDG-Cl, *N*-methyl-D-glucamine-chloride; NMDG-Glu, *N*-methyl-D-glucamine-gluconate; Na-Glu, Na-gluconate; NPPB, 5-nitro-2-(3-phenylpropylamino) benzoic acid; K-Glu, K-gluconate.

which was to restore isoosmolarity to the solution (Price et al., 1993). In cultured human nasal epithelium (Willumsen et al., 1994), hyperosmolar challenge of the apical (but not the basolateral) surface of the cells decreased Na^+ absorption and deactivated basolateral membrane K^+ and apical membrane Cl^- conductance. In dog tracheal epithelium (Yankaskas et al., 1987), hyperosmolar NaCl but not D-mannitol (D-M) increased paracellular permeability. Hyperosmolar solution caused cell shrinkage of human nasal (Willumsen et al., 1994) and guinea pig tracheal epithelial cells (Hjoberg et al., 1999). In dog trachea, hyperosmolar solution caused depolarization of epithelium upon challenge of the apical but not the basolateral membrane; however, the cells were shrunken by basolateral hyperosmolarity, not apical hyperosmolarity (Man et al., 1984).

In the guinea pig isolated perfused trachea preparation, hyperosmolar solutions applied to the mucosal or basolateral surfaces caused relaxation of the smooth muscle (Munakata et al., 1988; Fedan et al., 1990, 1999), which is mediated via the release of an epithelium-derived relaxing factor (EpDRF). EpDRF is released in association with transepithelial depolarization (Dortch-Carnes et al., 1999) and is associated with Na^+ and Cl^- transport, as judged by the inhibitory effects of Na^+ and Cl^- channel blockers (Fedan et al., 1999). EpDRF is neither nitric oxide nor a prostanoid (Munakata et al., 1990; Spina and Page, 1991; Fedan et al., 1999, 2003a; Johnston et al., 2003).

In the guinea pig isolated perfused trachea, permeant and impermeant osmolytes were equieffective in their ability to elicit relaxation, i.e., release EpDRF, when used to raise the osmolarity of the physiological salt solution (Fedan et al., 2003a). Osmolytes differed in their activity as contractile and relaxant agents when applied to the apical[2] and basolateral surfaces of the trachea to raise osmolarity. Exposure of the apical surface of the epithelium to isosmolar solutions, a procedure that stimulates cell shrinkage in neutrophils and ovary cells (Krump et al., 1997; Szászi et al., 1997), caused a diversity of mechanical responses that rarely mimicked the relaxation caused by hyperosmolar solution. Experiments in which the osmolarity of the perfusion solution was increased from isosmolar to hyperosmolar, or from hypoosmolar to isosmolar ("osmolar jump"), suggested that increment in osmolarity, rather than the absolute osmolar concentration or cell shrinkage, is the stimulus to the release of EpDRF (Fedan et al., 2003a).

Here we examined bioelectric responses of the guinea pig tracheal epithelium to challenge with hyperosmolar and isosmolar osmolyte solutions to compare these with mechanical responses (Fedan et al., 2003a). We characterized the effects of ion transport blockers and the effects of different classes of osmolytes, examined polarity of the responses, and investigated the bioelectric responses to osmolar jump.

Materials and Methods

Animals. These studies were conducted in facilities accredited fully by the Association for the Assessment and Accreditation of Laboratory Animal Care International and were approved by the institutional Animal Care and Use Committee. The animals were anesthetized with sodium pentobarbital (65 mg/kg i.p.) and sacrificed by thoracotomy and bleeding before removing the trachea. Other details of animal use have been given previously (Fedan et al., 2003a).

Bioelectric Measurements in Tracheal Segments. The Ussing chamber (WPI, Sarasota, FL) was used to measure changes in transepithelial short-circuit current (I_{sc}) and transepithelial resistance (R_t) in response to various solutions and agents. After sacrifice of the animal, a 4-cm segment of trachea was removed, placed in modified Krebs-Henseleit solution (MKHS), cleaned, and slit along its length through the smooth muscle band. The segment was stretched to its original length, reflected open, and bisected so that the proximal end of the trachea was anchored across an aperture of 0.125 cm^2, thereby separating the two hemi-chambers of the apparatus. Both hemi-chambers were perfused separately with recirculating MKHS (37°C). Two silver/silver chloride-agar bridge voltage electrodes containing 0.9% NaCl, and two silver/silver chloride-agar bridge current electrodes containing 0.9% NaCl, were placed to monitor transepithelial potential difference and to deliver current, respectively. Isotonic NaCl-containing bridge electrodes were used in place of 3 M KCl-containing bridges to prevent possible changes in osmolarity arising from KCl diffusion from the electrodes, inasmuch as the epithelium responds to slight elevations in osmolarity (Johnston et al., 2003). The apical and basolateral baths contained 5 ml of recirculating MKHS (37°C) in each reservoir. The preparations were allowed to equilibrate and the MKHS was changed at 15- to 30-min intervals. I_{sc} was measured with an automatic voltage/current clamp amplifier (DVC 1000 or EVC 4000; WPI). The preparation was continuously short-circuited. In experiments involving an examination of the effects of ion channel blockers and the development of osmolyte concentration-response curves, for 0.5 s every 30 s a constant voltage pulse (dV = 10 mV) was applied to yield a current response (dI). In the experiments in which the effects of hyperosmolar and isosmolar solutions and osmolar jump were being studied, a 1-mV, 5-s pulse was delivered at 50-s intervals. In both cases, R_t was determined from the relation dV/dI. The spontaneous potential difference (SPD) was determined from Ohm's law, i.e., $R_t \times I_{sc}$.

Effect of Ion Transport Blockers on Epithelial Bioelectric Responses to Apical Hyperosmolarity. In these experiments, conditions were used that mimicked those in studies of the relaxant effects of hyperosmolarity (Fedan et al., 2003a). While recording I_{sc} and delivering voltage pulses, the preparations were equilibrated for 3 h in the Ussing chamber. Methacholine (MCh; 3×10^{-7} M) was then added to the basolateral bath. At the plateau of the response, either D-M (120 mosM) or NaCl (120 mosM) was administered to the apical bath to obtain a control response. Volume equivalents of MKHS were added simultaneously to the basolateral bath to equalize hydrostatic pressure. After stabilization of the response, both chambers were washed with fresh MKHS at 15-min intervals over a 90-min period. An ion transport blocker was then added to the apical or basolateral bath, as appropriate, and incubated for 30 min. MCh was again added to the basolateral bath, and the same osmolyte was added to the apical bath to raise osmolarity. Vehicle control preparations (inhibitor not present) were run separately.

Concentration Dependence of Osmolyte-Induced Bioelectric Responses. After equilibration an osmolyte was then added cumulatively to the MKHS of the apical bath to obtain an osmolar concentration-response curve. A volume equivalent of MKHS was added simultaneously to the basolateral bath. At the conclusion of this procedure the preparation was washed bilaterally with fresh MKHS and, after 1 h, the same osmolyte was added in cumulative concentrations to the basolateral bath. Each preparation was used to study one osmolyte. In preliminary experiments, the preparations became unstable after the basolateral concentration-response curve; therefore, the apical curves were obtained before the basolateral curves.

[2] Apical and mucosal are terms that correspond to the intraluminal bath in perfused trachea experiments and the "air side" of the trachea. Basolateral and serosal are terms that correspond to the extraluminal bath in perfused trachea experiments and the "blood side" of the trachea.

Bioelectric Responses of Tracheal Epithelium to Apical Hyperosmolar and Isosmolar Non-MKHSs, and Incremental Osmolar Jump. After equilibration, the preparations were challenged with apical hyperosmolar solution, in an amount needed to double the osmolarity of the MKHS, which was measured in every experiment (Osmette A automatic osmometer; Precision Systems, Inc., Sudbury, MA). In experiments in which the effects of apical isosmolar solutions and incremental osmolar jump were also investigated, at the conclusion of the response both hemi-chambers were washed with MKHS at 15-min intervals for 1 h. The solution in the apical hemi-chamber was then rapidly changed to one containing isosmolar osmolyte dissolved in water. [The pH of these solutions were not adjusted to 7.4 to avoid introduction of transportable ions, and because responses to mechanical responses to isosmolar solutions adjusted to pH 7.4 were not different from those using untitrated solutions (Fedan et al., 2003a).] Upon stabilization of this response, the apical solution was made hyperosmolar by administering additional amounts of the same osmolyte to the isosmolar solution. The osmolytes chosen for study of the bioelectric effects of challenge with isosmolar solution were necessarily ionic. These experiments were conducted both in the absence, or in separate experiments, in the presence of basolateral MCh.

Solution Resistance. Solution resistances of the various solutions were measured in Ussing chambers under current clamp conditions. These solutions were MKHS, MKHS containing hyperosmolar osmolyte, isosmolar solution in water, and twice isosmolar solution in water, for the osmolytes NaCl, KCl, *N*-methyl-D-glucamine gluconate (NMDG-Glu), *N*-methyl-D-glucamine chloride (NMDG-Cl), and Na gluconate (Na-Glu). The resistances (0.0002–0.003 $\Omega \cdot cm^2$) were negligible compared with those recorded when the tracheal segment was present (see *Results*).

Drugs and Reagents. All drugs and reagents were from Sigma-Aldrich (St. Louis, MO).

MKHS. MKHS 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. The osmolarity of the MKHS was 281 ± 5 mosM.

Analysis of Results. The results are expressed as mean ± S.E.; n is the number of separate experiments. The results were analyzed for differences using Student's t test for paired or nonpaired samples, or analysis of variance, as appropriate. $p < 0.05$ was considered significant.

Results

Effects of Ion Transport Blockers on Bioelectric Responses to Hyperosmolarity. In these experiments, basal I_{sc} was 47.8 ± 1.6 $\mu A/cm^2$. This value is very close to that obtained previously with Ussing chambers (37 $\mu A/cm^2$; Stutts and Bromberg, 1987) but differs from those obtained from cable analysis of intact trachea (91 $\mu A/cm^2$; Croxton, 1993) and primary culture of epithelial cells (14 $\mu A/cm^2$; Robison and Kim, 1994).

Apical amiloride (Na^+ channel blocker; 3×10^{-5} M) decreased basal I_{sc} by 35.6%, whereas apical 5-nitro-2-(3-phenylpropylamino)benzoic acid (NPPB; Cl^- channel blocker; 10^{-4} M) decreased I_{sc} by 83.3 ± 4.9%, indicating that the guinea pig tracheal epithelium is a predominantly Cl^--secretory rather than a Na^+-absorptive epithelium. Basolateral bumetanide (Na^+,K^+,$2Cl^-$-cotransport inhibitor; 10^{-5} M) decreased I_{sc} by 8.9 ± 1.6%, but iberiotoxin (Ca^{2+}-activated K^+-channel inhibitor; 10^{-7} M) in both the apical and basolateral baths had no effect on basal I_{sc}. Basolateral ouabain (Na^+,K^+-pump inhibitor; 10^{-5} M) decreased I_{sc} by 94.3 ± 3.3%.

Basolateral MCh increased I_{sc}. This I_{sc} response was inhibited substantially by apical NPPB and basolateral bumetanide, modestly by basolateral ouabain, but was not inhibited by apical amiloride or basolateral iberiotoxin (Table 1). These findings suggest that the bioelectric response to MCh involved activation of Cl^- secretion. In the presence of MCh, apical hyperosmolar D-M (120 mosM) decreased I_{sc} (Figs. 1, 10, and others).

TABLE 1

Effects of transport blockers on I_{sc} responses to basolateral MCh (3×10^{-7} M)

In the controls, two responses were obtained in the absence of any agent. The effects of the blockers were tested on the second of two responses, the first of which was the control (see *Materials and Methods*).

Inhibitor (n)	ΔI_{sc} ($\mu A/cm^2$)	$\%\Delta I_{sc}$
Control (4)		
First response	9.3 ± 1.1	21.4 ± 4.5
Second response	5.9 ± 0.6	14.2 ± 2.1
Amiloride (6)		
Absent	6.8 ± 1.1	13.2 ± 1.7
Present	6.4 ± 1.2	19.2 ± 2.7*
NPPB (4)		
Absent	7.0 ± 0.3	13.5 ± 2.0
Present	0.8 ± 0.2*	58.3 ± 47.2
Bumetanide (6)		
Absent	8.7 ± 1.9	16.9 ± 2.8
Present	2.6 ± 0.9*	5.1 ± 1.3*
Ouabain (4)		
Absent	13.5 ± 1.3	26.1 ± 2.9
Present	10.5 ± 1.3*	16.7 ± 17.7
Iberiotoxin (4)		
Absent	10.6 ± 1.2	24.2 ± 3.4
Present	10.3 ± 0.7	22.5 ± 4.9

*Response in the presence of the inhibitor significantly different from the response in the absence of the inhibitor.

Hyperosmolar NaCl (120 mosM) caused two types of I_{sc} responses. In the first type (Fig. 1), NaCl caused a triphasic I_{sc} response: an abrupt decrease, a transient rise, and a long-lasting decrease. The early transient response could be attributable to asymmetric concentrations of Na^+ and Cl^- across the epithelium and resultant changes in passive Na^+ absorptive and passive Cl^- secretory fluxes. The second phase of the response, however, could only be explained by an active response of the epithelium to osmolar challenge, because this response was also observed with D-M. The second type of response to apical hyperosmolar NaCl, seen in separate experiments, consisted of a monotonic increase in I_{sc}, demonstrative of the preponderance of the passive flux of the ions over the active cellular response (see representative responses in later figures). We attribute the qualitative vari-

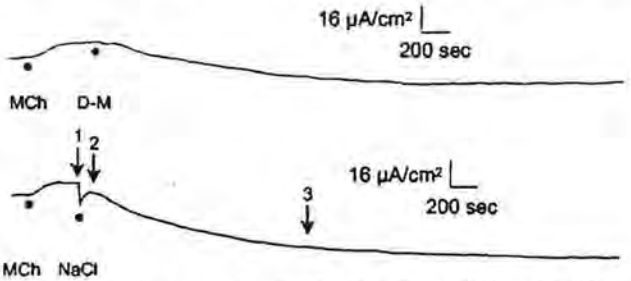

Fig. 1. Representative tracing showing the effects of 120 mosM D-M (top tracing) and 120 mosM NaCl (bottom tracing) on I_{sc} in guinea pig tracheal segments mounted in Ussing chambers. The osmolytes were added after the response to basolaterally applied MCh (3×10^{-7} M) became stabilized. MCh increased I_{sc}. NaCl elicited a triphasic response, whereas D-M caused a monophasic decrease in I_{sc}. It was the sustained decrease in I_{sc} that was quantified to evaluate the effects of blocking drugs (Fig. 2).

ability in the response to apical hyperosmolar NaCl to between-animal variability, which we have noted in mechanical studies (Fedan et al., 2003a). This variability was never observed with D-M, and preparations that responded to apical hyperosmolar NaCl with an increase in I_{sc} responded with a decrease in I_{sc} when challenged with apical D-M. The basis for differences in passive flux behavior between animals is not known.

In preparations in which apical hyperosmolar NaCl decreased I_{sc}, the prolonged decrease in response to NaCl and D-M was smaller in the presence of apical amiloride and basolateral bumetanide (Fig. 2); apical NPPB and basolateral ouabain reversed the polarity of the responses to both solutes; and iberiotoxin had no effect. These effects were not seen in control preparations (blockers omitted) nor were they due to vehicle. The decreases in basal I_{sc} caused by amiloride, NPPB, and bumetanide could have affected the subsequent responses to hyperosmolar challenge. Analysis of the results in terms of the percentage change of I_{sc} caused by the osmolyte from the value in the presence of the inhibitors revealed that only bumetanide inhibited the I_{sc} responses (Fig. 2). These findings suggest that Na^+,K^+,$2Cl^-$-cotransport was activated during hyperosmolar challenge.

In preparations in which apical hyperosmolar NaCl increased I_{sc}, the blockers did not inhibit the I_{sc} response (ΔI_{sc} or $\%\Delta I_{sc}$) to NaCl but, in most cases, potentiated the responses (Fig. 3). The different pharmacological effects of the blockers suggest that passive ion fluxes were preponderant in preparations in which hyperosmolar NaCl increased I_{sc}.

Effect of Cl^--Free MKHS on the Bioelectric Response to D-M. In the absence of Cl^- (gluconate was substituted for Cl^- in the apical and basolateral baths), the reduc-

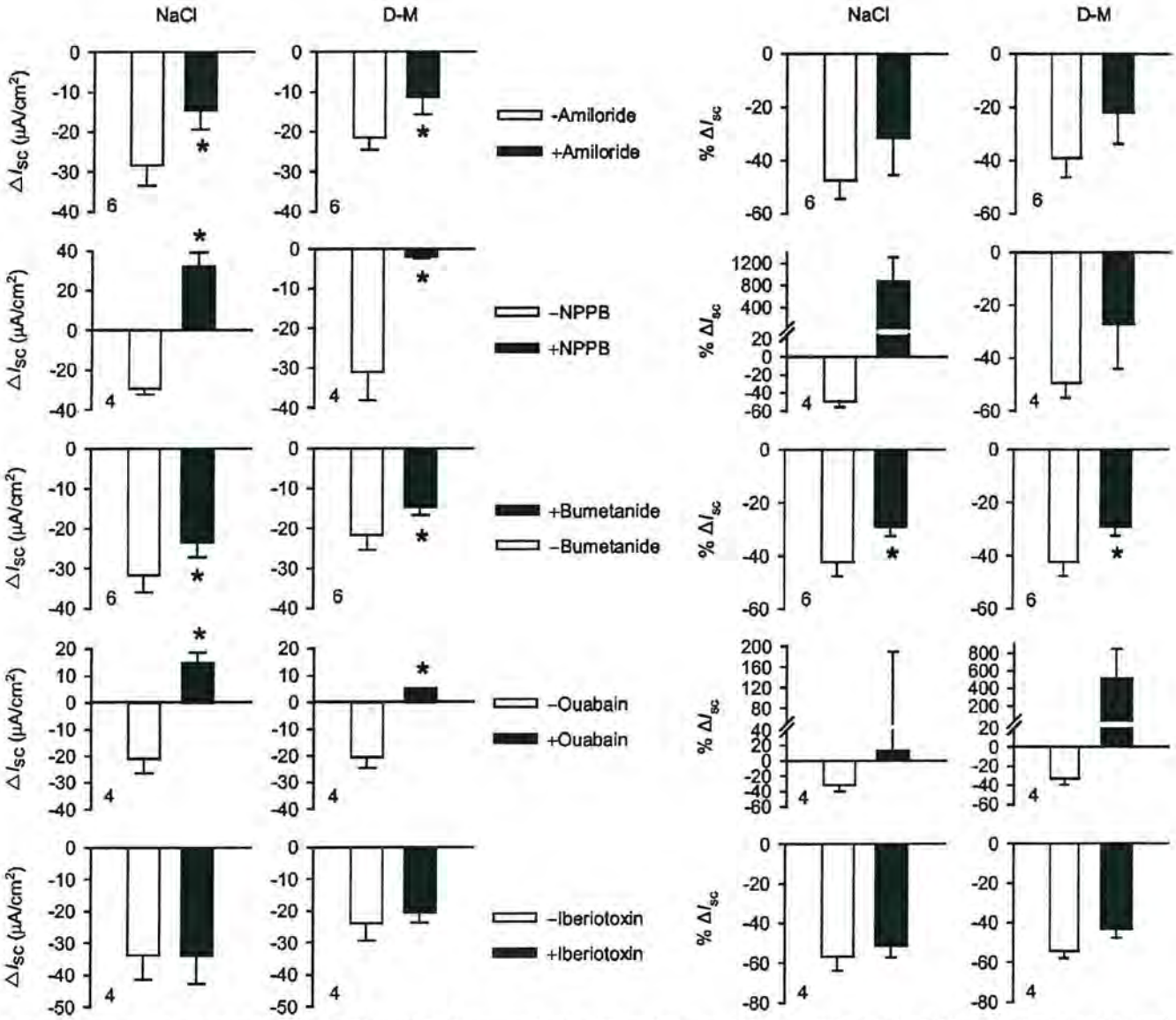

Fig. 2. Effects of amiloride (3×10^{-5} M), NPPB (10^{-4} M), bumetanide (10^{-5} M), ouabain (10^{-5} M), and iberiotoxin (10^{-7} M) on I_{sc} responses of tracheal segments to 120 mosM NaCl and 120 mosM D-M. This figure depicts results obtained in preparations in which NaCl decreased I_{sc}. NaCl and D-M were added in the presence of basolaterally administered MCh (3×10^{-7} M). The number of experiments performed is indicated by the number in each panel. The graphs to the left of the legend depict responses in terms of ΔI_{sc}; the graphs to the right of the legend depict the results normalized as a percentage of the baseline I_{sc}, i.e., $\%\Delta I_{sc}$. Some of the transport inhibitors affected the baseline (see text).

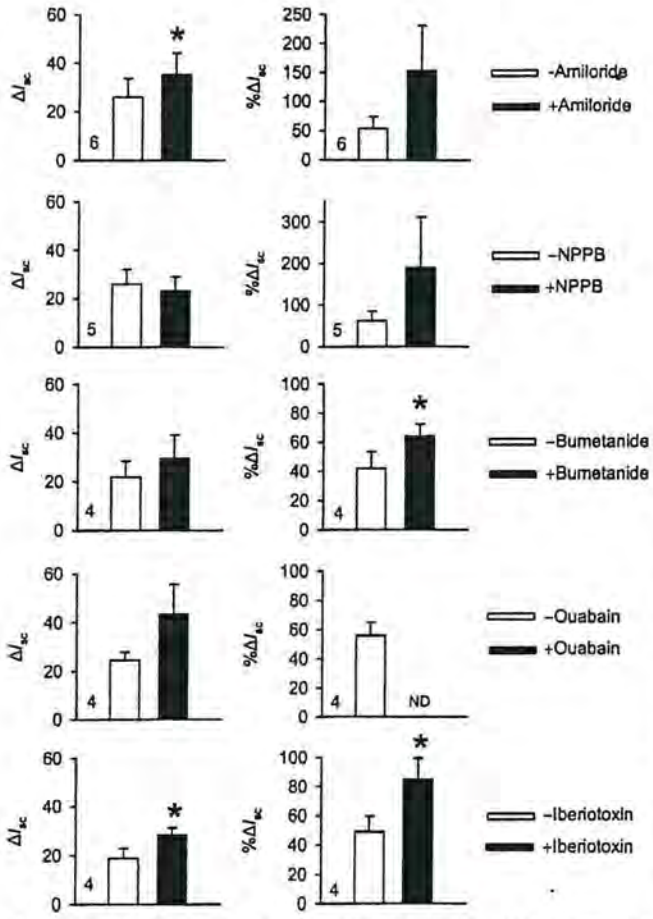

Fig. 3. Effects of amiloride (3×10^{-5} M), NPPB (10^{-4} M), bumetanide (10^{-5} M), ouabain (10^{-5} M), and iberiotoxin (10^{-7} M) on I_{sc} responses of tracheal segments to 120 mosM NaCl. This figure depicts results obtained in preparations in which NaCl increased I_{sc}. See Fig. 2 legend for other details.

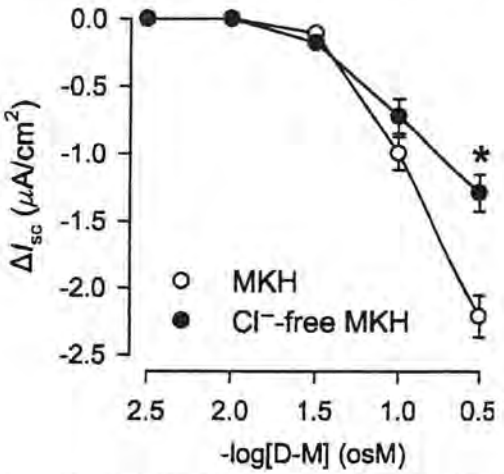

Fig. 4. Comparison of bioelectric responses to hyperosmolarity in normal and Cl^--free MKH solutions. In this experiment gluconate replaced Cl^- in the MKHS of the apical and basolateral chambers and the tracheal segment was incubated with gluconate-containing MKHS before D-M was administered to the apical chamber to increase osmolarity. *, the decrease in I_{sc} in response to D-M was significantly inhibited in the absence of Cl^-. $n = 4$.

tion in I_{sc} in response to apical 266.8 mosM D-M was attenuated. (Fig. 4). Thus, the decrease in I_{sc} in response to D-M reflects an active response of the epithelium to hyperosmolar challenge.

Comparison of Bioelectric Responses to Apically and Basolaterally Applied Osmolytes: Cumulative Concentration-Response Curves. In these experiments, basal I_{sc} was 45.3 ± 2.4 $\mu A/cm^2$. Figures 5 to 8 illustrate the bioelectric effects of cumulative additions of NaCl, KCl, urea, D-M, and sucrose to the MKHS in the apical and basolateral baths. MCh was not present in these experiments. It should be noted that asymmetrical ion concentrations across the epithelium were induced by addition of NaCl and KCl but not urea, D-M, or sucrose. There was a clear delineation in the type of responses obtained that depended on the osmolyte used and the bath to which it was added. The agents could be grouped into one of three categories of response profile. Apically applied NaCl and KCl increased I_{sc} (Figs. 5 and 6). The increases in I_{sc} in lower concentrations of the salts were small, but beginning at 502 mosM large increases occurred. R_t was decreased, as has been reported previously (Yankaskas et al., 1987), and the changes in R_t were observed at lower concentrations than those that altered I_{sc} appreciably. The large changes in I_{sc} observed at higher osmolar concentrations could be attributed to induction of passive nonspecific cation absorption, but changes in R_t in the absence of changes in I_{sc} was evidence of an active compensation of the epithelium to apical challenge. Basolaterally-applied NaCl and KCl decreased I_{sc} and R_t. The epithelium was more reactive to basolateral than apical hyperosmolarity. Given the known differences in channel distribution between the apical and basolateral membranes in airway epithelium, it was interesting that apical and basolateral addition of NaCl and KCl resulted in identical osmolarity-response curves.

Urea decreased I_{sc} after addition to the apical and basolateral chambers (Fig. 7); the responses in both baths were essentially the same, although two points on the curves exhibited small, significant differences. Basolateral urea decreased R_t, but apical urea increased R_t. The difference in R_t responses could not be an artifact of voltage clamping because the SPD also showed a separation of the two curves that was consistent with the differences in R_t.

The third I_{sc} response profile, exhibited by the impermeant osmolytes D-M and sucrose, was characterized by a greater decrease in I_{sc} in the apical bath than in the basolateral bath (Fig. 8). D-M and sucrose had minimal effects on R_t. Thus, all five osmolytes decreased I_{sc} when applied to the basolateral bath and decreased SPD in both baths. Only the salts increased I_{sc} when applied apically, but the increase was not linear or as large as would be expected for a pure Nernstian relationship. The remaining solutes decreased I_{sc} after apical bath application. The results demonstrate polarity in the reactivity of the epithelium to increases in osmolarity. The bioelectric responses to increases in osmolarity due to permeant ions reflect both passive and active responses, and the latter can be studied using nonionic, impermeant solutes.

Bioelectric Effects of Hyperosmolar MKHS. Exposure of the apical membrane to twice-strength MKHS, which relaxed the perfused trachea (Fedan et al., 2003a), elicited an increase in I_{sc} (Fig. 9); R_t was slightly but significantly decreased. These changes emulated those caused by the higher osmolar concentrations of apically applied NaCl and KCl (Fig. 5).

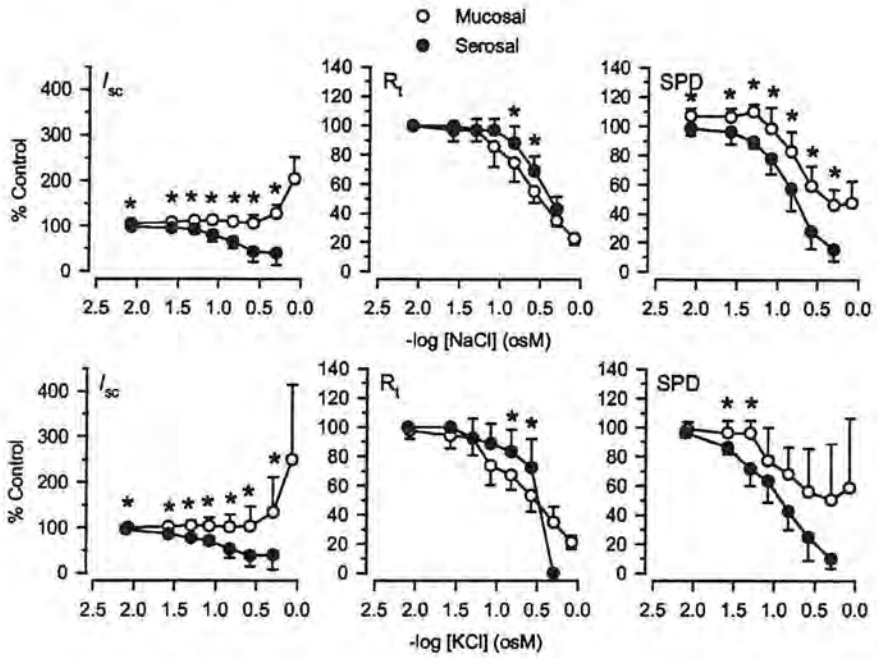

Fig. 5. Bioelectric effects of hyperosmolar NaCl and KCl in tracheal epithelium. NaCl and KCl were added cumulatively to the apical or basolateral baths. The apical concentration-response curves were obtained first. The apical administration of NaCl and KCl increased I_{sc}, whereas the basolateral administration of NaCl and KCl decreased I_{sc}. R_t and SPD were decreased by apical and basolateral NaCl and KCl. There is no data point at the highest concentration of basolateral NaCl and KCl, because the salts caused marked, deleterious and irreversible alterations in the epithelium. Note that the ordinate for I_{sc} responses (left-hand panels) is compressed compared with those shown in Figs. 5 to 7. *, apical versus basolateral, $p < 0.05$. $n = 9$ and 7 for NaCl and KCl, respectively.

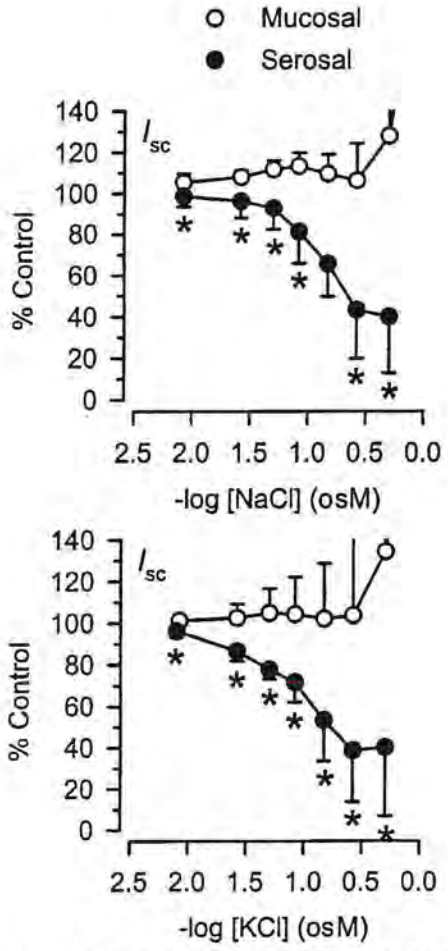

Fig. 6. Bioelectric effects of hyperosmolar NaCl and KCl in tracheal epithelium. This figure illustrates the effects of NaCl and KCl shown in Fig. 5 in greater detail so that the results can be compared with those in Figs. 6 and 7.

Bioelectric Effects of Hyperosmolar D-M and Urea. Addition of D-M to the apical chamber to double the osmolarity decreased I_{sc} without changing R_t (Fig. 10). In the same preparations, hyperosmolar urea decreased I_{sc} in most experiments, but in some preparations complex responses were obtained; in one experiment urea increased I_{sc}. Identical results were obtained in the absence or presence ($n = 4$; data not shown) of basolateral MCh.

Bioelectric Effects of Apical Hyperosmolar and Isosmolar Solutions and Incremental Osmolar Jump. Permeant and nonpermeant solutes have distinct effects on relaxation responses of the guinea pig perfused trachea (Fedan et al., 2003a). Initially, the osmolarity of the apical bath was elevated by adding NMDG-Glu to the MKHS, thereby maintaining the concentrations of the ions in MKHS. This manipulation decreased I_{sc}, in the manner of D-M, and decreased R_t (Fig. 11). In contrast, elevation of apical bath osmolarity with NaCl (Fig. 12) increased I_{sc} and decreased R_t, as before (Fig. 5). Hyperosmolar KCl increased I_{sc} but did not affect R_t (Fig. 13).

Isosmolar NMDG-Glu solution decreased I_{sc} and increased R_t (Fig. 11); in fact, the polarity of the epithelium was reversed. These results were consistent with termination of transepithelial Na^+ absorption and induction of transepithelial cation secretion. Subsequent doubling of the osmolarity of the apical bathing solution by addition of NMDG-Glu resulted in a further decrease in I_{sc}.

To elucidate further the roles of permeant and nonpermeant ions in the bioelectric responses, Cl^- and Na^+ were substituted for NMDG and gluconate. Elevation of apical osmolarity by addition of NMDG-Cl to MKHS increased I_{sc} and decreased R_t (Fig. 14). In one-half of the preparations, the increase in I_{sc} was sustained; the other one-half exhibited a transient increase in I_{sc} followed by a sustained decrease. The responses were not random, but were consistent for a preparation, as evidenced by subsequent exposure to isosmolar NMDG-Cl and rechallenge with hyperosmolar NMDG-Cl

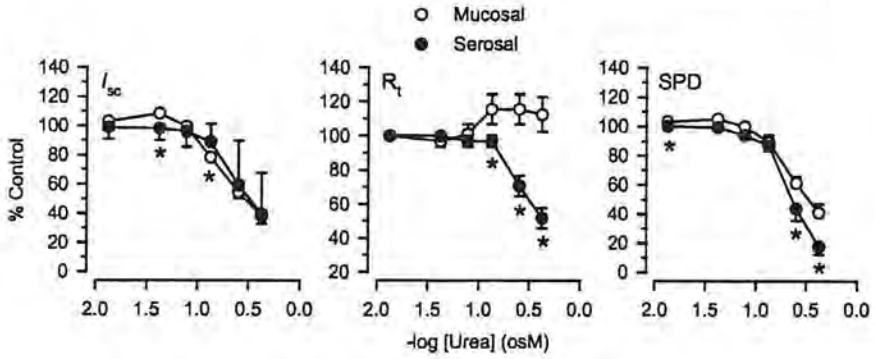

Fig. 7. Bioelectric effects of hyperosmolar urea in tracheal epithelium. Urea was added cumulatively to the apical or basolateral baths. The apical concentration-response curves were obtained first. The apical and basolateral administration of urea decreased I_{sc} and SPD. Apical urea increased R_t slightly, whereas R_t was decreased by basolateral urea. *, apical versus basolateral, $p < 0.05$. $n = 5$.

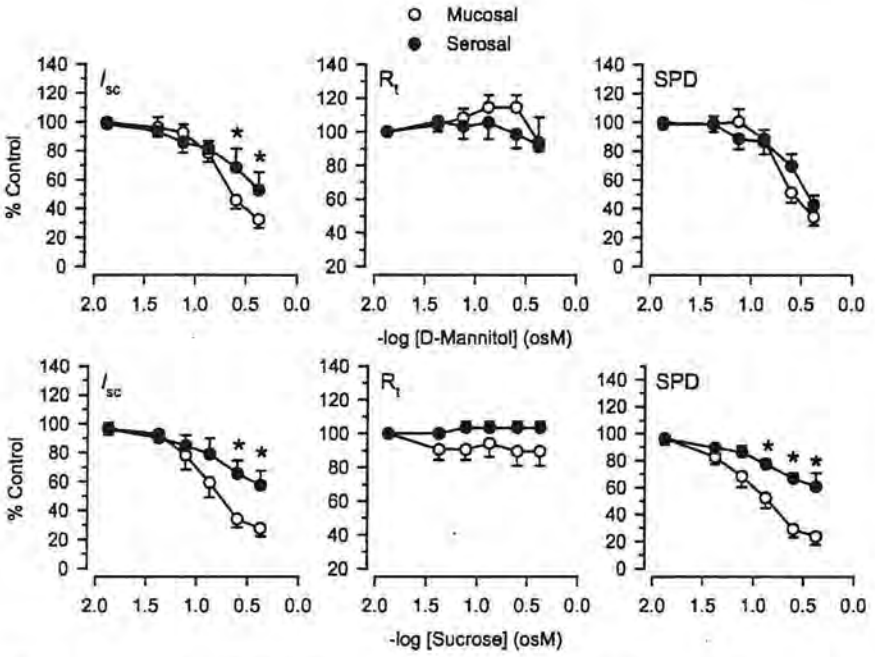

Fig. 8. Bioelectric effects of hyperosmolar D-M and sucrose on tracheal epithelium. D-M and sucrose were added cumulatively to the apical or basolateral baths. The apical concentration-response curves were obtained first. When applied to the apical bath D-M decreased I_{sc} and decreased SPD. Apically added D-M evoked larger I_{sc} responses than basolaterally applied D-M. A similar pattern of responses was obtained with sucrose, except that SPD decreases were significantly larger in the apical bath than in the basolateral bath. Neither sugar had an appreciable effect on R_t. $n = 5$ and 4 for D-M and sucrose, respectively.

(Fig. 14). Isosmolar NMDG-Cl caused a large decrease in I_{sc} in all preparations, resulting in a reversal of polarity; R_t was increased. It should be noted that under these conditions, there was minimal difference between apical and basolateral concentrations of Cl^-, yet I_{sc} was greatly affected in all preparations, and reversal of the I_{sc} was observed in 50% of the preparations. Hence, induction of passive anion absorption is unable to explain the effects of isosmolar NMDG-Cl. In contrast to NMDG-Glu, addition of hyperosmolar NMDG-Cl increased I_{sc} back toward the original baseline and decreased R_t (Fig. 14). Thus, the presence of Cl^- in the apical bath is linked to the active increase in I_{sc} observed in response to hyperosmolar solutions. It should also be noted that the preparations that had exhibited biphasic responses to addition of NMDG-Glu to MKHS also exhibited biphasic responses to addition of NMDG-Cl to isosmolar NMDG-Cl, and preparations that exhibited monotonic responses to addition of NMDG-Cl to MKHS exhibited monotonic responses to addition of NMDG-Cl to isosmolar NMDG-Cl. A possible explanation for these findings is that the response to hyperosmolar NMDG-Cl has several potential components: absorption of Cl^- due to an elevated concentration in the apical bath, secretion of Na^+ and HCO_3^- due to asymmetrical concentrations across the epithelium, and an active, electrogenic cellular response involving anion secretion. The balance between these processes within a given preparation could determine the response pattern for that preparation under a variety of conditions. It is also possible that a paracellular conductance of NMDG leads to an electrodiffusive NMDG absorption. This is unlikely, however, because the transepithelial driving force for NMDG is infinite under both isosmolar and hyperosmolar conditions.

Elevation of osmolarity of the apical bath via addition of Na-Glu to MKHS increased I_{sc} (Fig. 15). In 18 of the 20 preparations, the increase in I_{sc} was sustained above baseline, and in the remaining two preparations I_{sc} eventually decreased to below baseline. The increase in I_{sc} was consistent with induction of a passive absorption of Na^+ due to asymmetrical concentrations. Hyperosmolar Na-Glu decreased R_t. Isosmolar Na-Glu induced a large biphasic increase in I_{sc} consistent with induction of passive Cl^- secretion. Subsequent addition of Na-Glu to isosmolar Na-Glu decreased R_t, but had little additional effect on I_{sc} compared with isosmolar Na-Glu. This observation provides further evidence that the increase in I_{sc} in response to isosmolar Na-Glu was due to transepithelial secretion of anions, because the chemical driving force would be increased by isos-

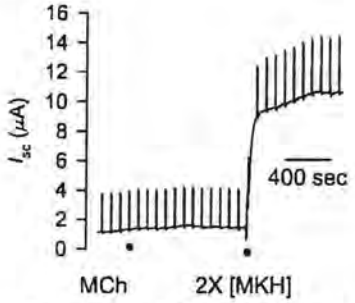

Fig. 9. Representative experiment showing the effects of twice-strength MKHS (2X [MKH]) in the apical bath on I_{sc}. Two times [MKH] increased I_{sc} and decreased R_t ($p < 0.05$). $n = 4$.

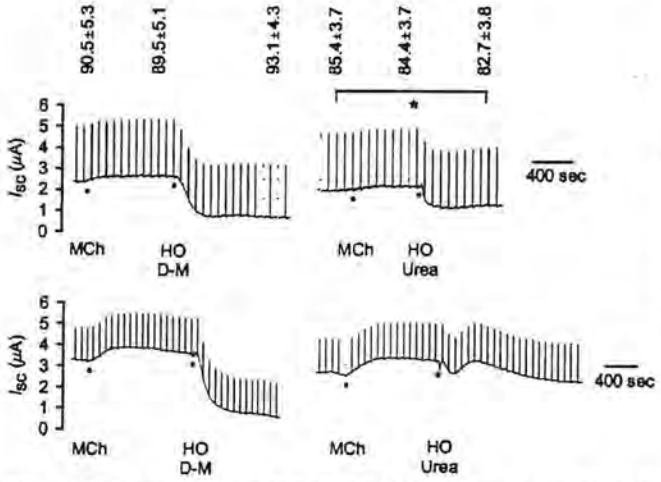

Fig. 10. Effect of hyperosmolar (HO) D-M and urea on I_{sc} and R_t of tracheal epithelium. The osmolytes were applied to the apical bath after the response to MCh (3×10^{-7} M; basolateral bath) was stabilized. D-M and urea were added in amounts needed to double the osmolarity of the MKHS, which was measured before every experiment. Hyperosmolar D-M decreased I_{sc} but had no effect on R_t. The I_{sc} responses to hyperosmolar urea were diverse (see text); shown here are two preparations, one (top tracing) in which I_{sc} was reduced and another (bottom tracing) in which the response to urea was multiphasic. The numbers above the tracings in this figure and in Figs. 10 to 15 are R_t values ($\Omega \cdot cm^2$) and reflect mean values obtained from all experiments of a given type. *, hyperosmolarity with urea produced a small but significant decrease in R_t compared with the value before MCh addition. These results are typical of those seen in 12 separate experiments.

molar Na-Glu but would not be increased further by increasing the concentration of Na-Glu.

This set of experiments was concluded by repeating the protocol with NaCl, KCl, and K-gluconate (K-Glu). As predicted from the osmolarity-response curves, increasing the osmolarity of the apical bathing solution by addition of NaCl or KCl induced similar increases in I_{sc} (Figs. 12 and 13). Isosmolar NaCl (Fig. 12) and KCl (Fig. 13) caused small decreases in I_{sc}, and addition of NaCl or KCl to the respective isosmolar solutions yielded increases in I_{sc} consistent with the induction of driving forces for passive transepithelial cation absorption. Consistency in responses to Na^+ and K^+ was also seen in response to hyperosmolar K-Glu (Fig. 16).

When applied to preparations already incubated in apical isosmolar solution, the administration of the same osmolyte to cause hyperosmolarity elicited I_{sc} and R_t responses that were generally similar to those obtained when the osmolyte was added to MKHS to create hyperosmolar conditions (Figs. 11–16). Together, these experiments indicate that responses

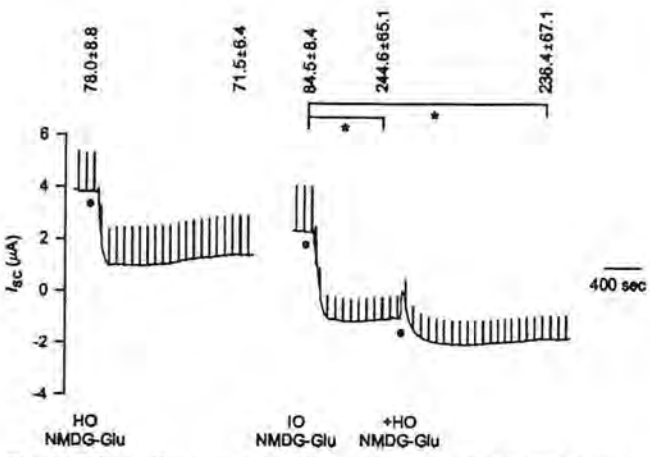

Fig. 11. Effect of hyperosmolar (HO) and isosmolar (IO) NMDG-Glu and osmolar jump from isosmolar solution, on I_{sc} and R_t of tracheal epithelium. Hyperosmolar solution was obtained by adding NMDG-Glu in amounts needed to double the osmolarity of the MKHS, which was measured before every experiment; this protocol applies to the osmolytes the results of which are shown in Figs. 11 to 15. Hyperosmolar NMDG-Glu decreased I_{sc} but had no effect on R_t. After washout, perfusion with isosmolar NMDG-Glu also decreased I_{sc}; R_t was increased significantly (*, $p < 0.05$) compared with the value before isosmolar solution challenge. Osmolar jump through the administration of added NMDG-Glu to the isosmolar solution resulted in another decrease in I_{sc} or complex responses (see text), but R_t was not affected further. These results are typical of those seen in 14 separate experiments.

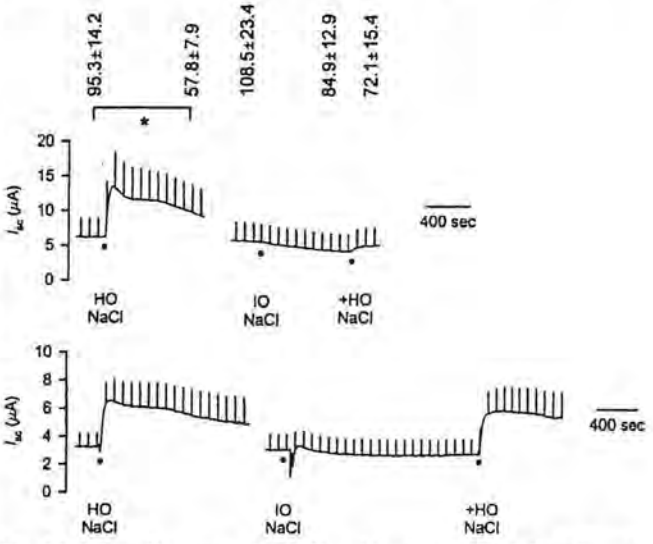

Fig. 12. Effect of hyperosmolar (HO) and isosmolar (IO) NaCl and osmolar jump from isosmolar solution, on I_{sc} and R_t of tracheal epithelium. The protocol used in this experiment and the remaining figures was the same as in Fig. 10. Hyperosmolar NaCl increased I_{sc} and decreased R_t significantly (*, $p < 0.05$). Two response types occurred during isosmolar challenge with NaCl: a decrease in I_{sc} (top tracing) or no change (bottom tracing). Osmolar jump through the administration of added NaCl to the isosmolar solution resulted in an increase in I_{sc} but R_t was not affected. These results are typical of those seen in 12 separate experiments.

to incremental increases in osmolarity (i.e., osmolar jump) were similar, whether by elevation of osmolarity in MKHS or in isosmolar solution of osmolytes, regardless of the direction of the I_{sc} response. Solutes containing Na^+, K^+, and Cl^- increased I_{sc} when added in hyperosmolar amounts to MKHS or isosmolar solution (osmolar jump). The impermeant osmolytes, in contrast, decreased I_{sc} under both conditions.

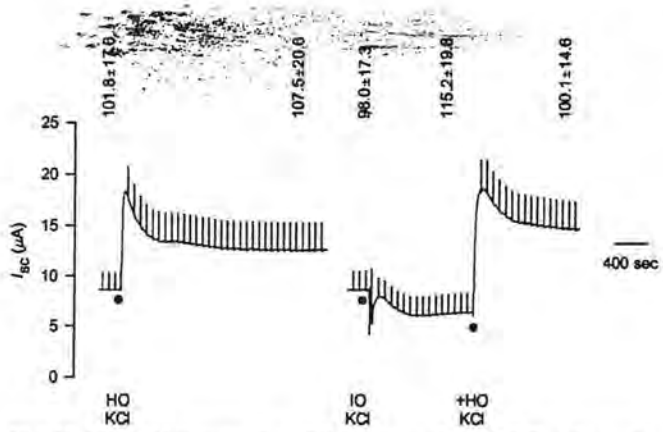

Fig. 13. Effect of hyperosmolar (HO) and isosmolar (IO) KCl and osmolar jump from isosmolar solution, on I_{sc} and R_t of tracheal epithelium. The protocol used in this experiment was the same as in Fig. 10. Hyperosmolar KCl increased I_{sc} but did not affect R_t. Isosmolar challenge with KCl decreased I_{sc} without affecting R_t. Osmolar jump through the administration of added KCl to the isosmolar solution resulted in an increase in I_{sc}; R_t was not affected. These results are typical of those seen in 16 separate experiments.

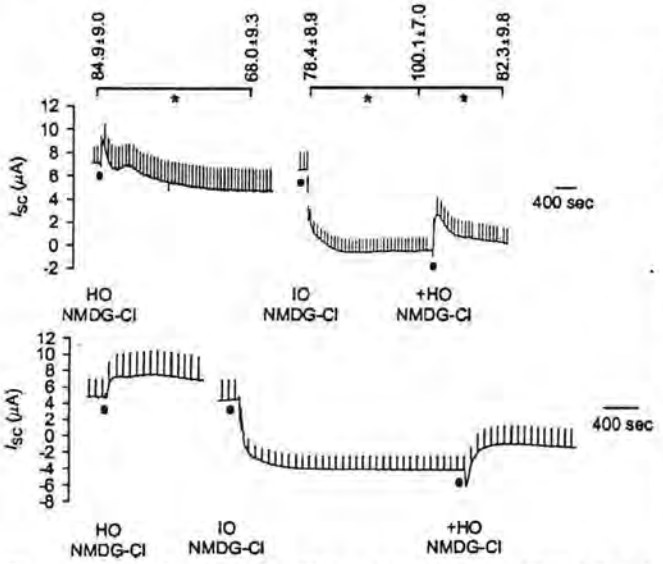

Fig. 14. Effect of hyperosmolar (HO) and isosmolar (IO) NMDG-Cl and osmolar jump from isosmolar solution, on I_{sc} and R_t of tracheal epithelium. The protocol used in this experiment was the same as in Fig. 10. The two traces show that responses to hyperosmolar NMDG-Glu had complex shapes, with some beginning with a transient increase in I_{sc} and ending in a net decrease in I_{sc}, whereas in others only an increase in I_{sc} occurred; R_t was decreased significantly by hyperosmolar NMDG-Cl (*, $p < 0.05$). During isosmolar challenge with NMDG-Glu a decrease in I_{sc} was elicited, most often causing a change in epithelial polarity (top tracing); R_t was increased significantly (*, $p < 0.05$). Osmolar jump through the administration of added NMDG-Glu to the isosmolar solution resulted in an increase in I_{sc}, sometimes preceded by a transient decrease, and a significant decrease in R_t. These results are typical of those seen in 16 separate experiments.

Curiously, hyperosmolar urea also decreased I_{sc}, and the reason why this permeant substance should do so is not understood.

Bioelectric Effects of Apical Hyperosmolar and Isosmolar Solutions and Hyperosmolar Jump in the Presence of MCh. To examine whether MCh could affect the bioelectric responses to apical hyperosmolar and isosmolar solutions and incremental osmolar jump, the experiments

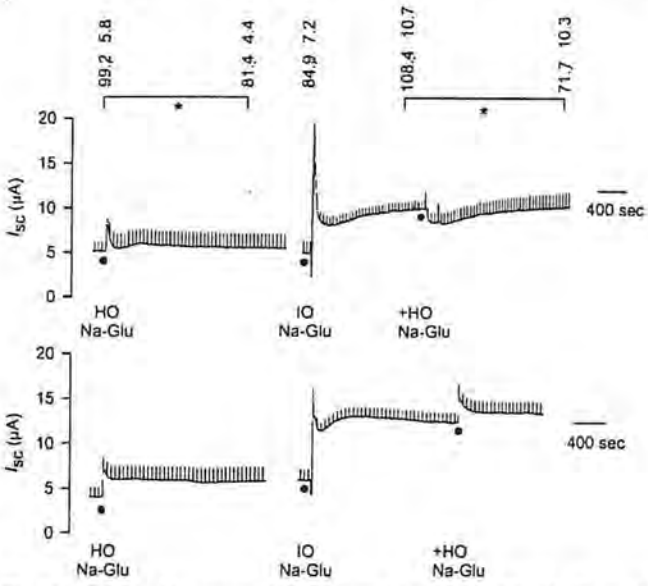

Fig. 15. Effect of hyperosmolar (HO) and isosmolar (IO) Na-Glu and osmolar jump from isosmolar solution, on I_{sc} and R_t of tracheal epithelium. The protocol used in this experiment was the same as in Fig. 10. The two traces depict the various types of responses that were elicited. Hyperosmolar Na-Glu caused a transient or sustained increase in I_{sc} and decreased R_t significantly (*, $p < 0.05$). Isosmolar Na-Glu evoked a transient increase in I_{sc} that decayed to a plateau. In a minority of cases the "trough" between phases decreased to below the prestimulation level, or a net decrease in I_{sc} resulted. Isosmolar Na-Glu solution did not affect R_t. Osmolar jump through the administration of added NMDG-Glu to the isosmolar solution resulted in an increase in I_{sc} in a majority of preparations; in two of the 20 preparations examined hyperosmolar jump led to a decrease in I_{sc}. Osmolar jump from isosmolar solution caused a decrease in R_t (*, $p < 0.05$). Thus, in several respects, there was some preparation to preparation variability in the bioelectric responses to the various Na-Glu solutions.

with ionic osmolytes were repeated using the same protocols but in the presence of basolateral 3×10^{-7} M MCh. MCh had no appreciable effect on bioelectric responses to isosmolar and incremental osmolar jump (NMDG-Glu, NMDG-Cl, NaCl, KCl, Na-Glu, and K-Glu; $n = 4$ separate experiments for each osmolyte; data not shown).

Solution Resistance. Changes in R_t caused by non-MKHS osmolyte solutions could have involved a change in the fluid resistance of the apical solution. Therefore, the electrical resistance of the various solutions was measured (see *Materials and Methods*), and it was observed that the solution resistances were negligible compared to these developed by the epithelium. These findings indicate that the fluid resistance of the solutions cannot explain the I_{sc} and R_t responses, both their direction and magnitude. In addition, the effects of changed fluid resistance would have been instantaneous, whereas the responses measured throughout this study were slow-developing.

Discussion

The airway epithelium modulates the reactivity of the underlying smooth muscle via EpDRF, which is released in response to hyperosmolar challenge. In this study, we sought information about the bioelectric effects of hyperosmolar solutions on airway epithelium to gain insight into the mechanisms that could be involved in exercise-induced obstruction and obstruction after inhalation of hyperosmolar solutions,

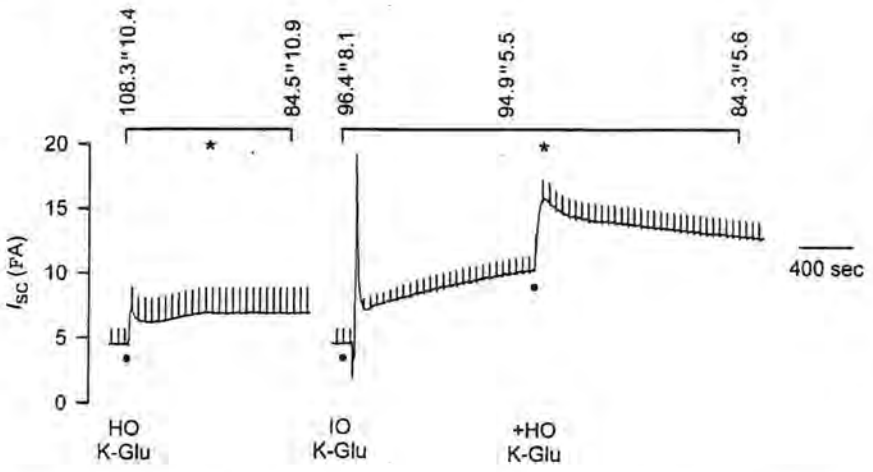

Fig. 16. Effect of hyperosmolar (HO) and isosmolar (IO) K-Glu and osmolar jump from isosmolar solution, on I_{sc} and R_t of tracheal epithelium. The protocol used in this experiment was the same as in Fig. 10. The various K-Glu solutions all increased I_{sc}, and in many respects the responses mimicked those to the Na-Glu solutions (Fig. 14). Osmolar jump, whether from MKHS or from isosmolar K-Glu solution, increased I_{sc} and decreased R_t (*, $p < 0.05$). These results are typical of those seen in eight separate experiments.

in asthmatic patients. The results demonstrated that the airway epithelium is an osmolarity sensor that responds to alterations in the osmolarity of the milieu with passive and active changes in electrogenic ion transport. The effects of hyperosmolarity on electrophysiological responses of the epithelium are complex, osmolyte-specific, and polarized across the airway wall. In addition, our studies suggest that the bioelectric responses of the tracheal epithelium to hyperosmolar challenge result from the incremental change in osmolarity rather than from shrinkage of the epithelium or the absolute osmolarity of the medium per se, in agreement with a parallel study of mechanical responses of the perfused trachea (Fedan et al., 2003a).

The bioelectric responses to hyperosmolarity induced with Na^+-, K^+-, and/or Cl^--containing osmolytes seems to involve passive and active responses in the epithelium, giving rise to increases or decreases, respectively, in I_{sc}. The preponderance of these two processes varied apparently between animals when these ions were used as solutes. However, in all preparations hyperosmolar D-M decreased I_{sc}; this response involves Cl^- transport.

The effects of the ion transport blockers on bioelectric responses to hyperosmolarity using D-M and NaCl (in those preparations in which the active, cellular response prevailed) were similar to the effects of the same or similar blockers on relaxation responses (Fedan et al., 1999, 2003b). Both amiloride and NPPB inhibited relaxation and attenuated the decrease in I_{sc}; Cl^--free MKHS also blunted bioelectric responses to hyperosmolarity. Likewise, K^+-channel blockade did not affect relaxation (glibenclamide) and had no effect on I_{sc} responses (iberiotoxin). Such correlations buttress the hypothesis that apical membrane Na^+ and Cl^- transport and associated bioelectric events are linked to and may initiate the release of EpDRF (Dortch-Carnes et al., 1999; Fedan et al., 1999). This parallelism diverged in the case of bumetanide and ouabain: both of these agents were silent in relaxation studies but inhibited the decrease in I_{sc} in response to D-M. Analysis of the effects of the blockers on I_{sc} with their initial baseline actions in mind revealed that only bumetanide antagonized bioelectric responses to hyperosmolar challenge. It is, therefore, difficult at present to model the ion transport changes that occur in the epithelium during hyperosmolar challenge in the context of EpDRF release and airway smooth muscle relaxation. Moreover, the effects of amiloride and NPPB are not absolutely restricted to Na^+ and Cl^- channels, and it is conceivable that the agents could interfere with Na^+,H^+- and/or Cl^-,HCO_3^- exchange, respectively, to alter ion transport secondarily.

In those preparations in which hyperosmolar NaCl elicited an increase in I_{sc}, none of the ion transport blockers inhibited the responses. This evidence suggests that passive ion absorption stimulated by the asymmetrical distribution of the salt was responsible for the bioelectric events in these tracheal segments. In addition, the absence of any effect of the blockers on these responses would suggest that passive ion absorption is not associated with EpDRF release.

As in the mechanical studies (Fedan et al., 2003a), there was substantial diversity in the bioelectric responses when the epithelium was challenged with hyperosmolar and isosmolar solutions. So long as an osmolyte was impermeant (D-M, sucrose and NMDG-Glu) the bioelectric response to elevation of osmolarity in MKHS was a decrease in I_{sc} without a change in R_t. We interpret these results as indicative of the active response of the cells to hyperosmolarity unmitigated by passive diffusion of the osmolyte as a component of the response, such as would be the case for the other ionic osmolytes. Urea also decreased I_{sc} when applied cumulatively (and caused multiphasic responses in some tissues when added in a single concentration). Thus, with respect to their ability to relax the perfused trachea and elicit bioelectric responses, D-M, NMDG-Glu, and urea were pharmacologically equivalent when they were used to elevate osmolarity. Inasmuch as urea would not induce long-term shrinkage of the epithelial cells, these findings, by themselves, could suggest that the stimulus to the relaxant and bioelectric responses to these four osmolytes is the osmolar concentration of the perfusing MKHS rather than shrinkage of the cells. However, the hyperosmolar jump experiments indicated that increment in osmolarity rather than absolute osmolarity is a far greater stimulus of the cells.

The results indicate that osmolytes containing one permeant ion, when added to increase the osmolarity of MKHS, stimulated a transient or sustained increase in I_{sc}. Interestingly, the charge of the permeant ion is irrelevant, because NaCl, KCl, NMDG-Cl, Na-Glu, and K-Glu exhibit approximately the same effect, i.e., to increase I_{sc} (left tracings of Figs. 12–16). This is in contrast to the decrease in I_{sc} caused by D-M, NMDG-Glu, sucrose, and urea. Yet, all of the os-

molytes elicited relaxation when added to normosmolar solutions (Fedan et al., 2003a).

In conclusion, guinea pig tracheal epithelium is sensitive to alterations in extracellular osmolarity at the apical and basolateral membranes, although bioelectric responses to osmolytes differ among permeant and nonpermeant solutes, and the responses are polarized. As with the mechanical responses, bioelectric responses to hyperosmolarity seem to be stimulated by an incremental increase in osmolarity rather than by solute concentration or cell shrinkage per se. However, a direct link between the mechanical and bioelectric responses to osmolar challenge is not evident.

Acknowledgments

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References

Anderson SD and Daviskas E (2000) The mechanism of exercise-induced asthma is. *J Allergy Clin Immunol* **106**:453–459.

Anderson SD, Schoeffel RE, Follet R, Perry CP, Daviskas E, and Kendall M (1982) Sensitivity to heat and water loss at rest and during exercise in asthmatic patients. *Eur J Respir Dis* **63**:459–471.

Croxton TL (1993) Electrophysiological properties of guinea pig tracheal epithelium determined by cable analysis. *Am J Physiol* **265**:L38–L44.

Dortch-Carnes J, Van Scott MR, and Fedan JS (1999) Changes in smooth muscle tone during osmotic challenge in relation to epithelial bioelectric events in guinea-pig isolated trachea. *J Pharmacol Exp Ther* **289**:911–917.

Fedan JS, Nutt ME, and Frazer DG (1990) Reactivity of guinea-pig isolated trachea to methacholine, histamine and isoproterenol applied serosally vs. mucosally. *Eur J Pharmacol* **190**:337–345.

Fedan JS, Dowdy JA, Johnston RA, and Van Scott MR (2003a) Hyperosmolar solution effects in guinea pig airways. I. Mechanical responses to relative changes in osmolarity. *J Pharmacol Exp Ther* **308**:10–18.

Fedan JS, Dowdy JA, Van Scott MR, Wu DX-Y, and Johnston RA (2003b) Hyperosmolar solution effects in guinea pig airways. III. Studies on the identity of epithelium-derived relaxing factor in isolated perfused trachea using pharmacological agents. *J Pharmacol Exp Ther* **308**:30–36.

Fedan JS, Yuan LX, Chang VC, Viola JO, Cutler D, and Pettit LL (1999) Osmotic regulation of airway reactivity by epithelium. *J Pharmacol Exp Ther* **289**:901–910.

Freed AN (1995) Models and mechanisms of exercise-induced asthma. *Eur Respir J* **8**:1770–1785.

Freed AN and Davis MS (1999) Hyperventilation with dry air increases airway surface fluid osmolality in canine peripheral airways. *Am J Respir Crit Care Med* **159**:1101–1107.

Godfrey S (1997) Exercise-induced asthma, in *Asthma* (Barnes PJ, Grunstein MM, Leff AR, and Woolcock AJ eds) pp 1105–1119, Lippincott-Raven Publishers, New York.

Hjoberg J, Högman M, and Hedenstierna G (1999) *The Hyperosmolar Airway. Mechanisms of Reduced Response to Nitric Oxide*. Comprehensive Summaries of Uppsala Dissertations from the Faculty of Medicine, University Hospital, Uppsala University, Uppsala, Sweden.

Israel E and Drazen JM (1999) Role of 5-lipoxygenase metabolites of arachidonic acid in exercise-induced asthma, in *Exercise-Induced Asthma* (McFadden ER ed) pp 167–180, Marcel Dekker, Inc., New York.

Johnston RA, Van Scott MR, Kommineni C, Millecchia LL, Dortch-Carnes J, and Fedan JS (2003) Hyperosmolar solution effects in guinea-pig airways. IV. Lipopolysaccharide-induced alterations in airway reactivity and epithelial bioelectric responses to methacholine and hyperosmolarity. *J Pharmacol Exp Ther* **308**:xxx–xxx.

Kagawa J and Kerr HD (1970) Effects of brief graded exercise on specific airway conductance in normal subjects. *J Appl Physiol* **28**:138–144.

Krump E, Nikitas K, and Grinstein S (1997) Induction of tyrosine phosphorylation and Na^+/H^+ exchanger activation during shrinkage of human neutrophils. *J Biol Chem* **272**:17303–17311.

Makhdum A and Pearce FL (1993) Hyperosmolar induced histamine release from mast cells: a mechanism for the pathogenesis of exercise-induced asthma? *Agents Actions* **38**:C191–C193.

Makker HK, Walls AF, Goulding D, Monteforte S, Varley JJ, Carroll M, Howarth PH, and Holgate ST (1994) Airway effects of local challenge with hypertonic saline in exercise-induced asthma. *Am J Respir Crit Care Med* **149**:1012–1019.

Man SF, Hulbert W, Park DS, Thomson AB, and Hogg JC (1984) Asymmetry of canine tracheal epithelium: osmotically induced changes. *J Appl Physiol* **57**:1338–1346.

Munakata M, Masaki Y, Sakuma I, Ukita H, Otsuka Y, Homma Y, and Kawakami Y (1990) Pharmacological differentiation of epithelium-derived relaxing factor from nitric oxide. *J Appl Physiol* **69**:665–670.

Munakata M, Mitzner W, and Menkes H (1988) Osmotic stimuli induce epithelial-dependent relaxation in the guinea pig trachea. *J Appl Physiol* **64**:466–471.

Price AM, Webber SE, and Widdicombe JG (1993) Osmolality affects ion and water fluxes and secretion in the ferret trachea. *J Appl Physiol* **74**:2788–2794.

Robison TW and Kim K-J (1994) Air-interface cultures of guinea pig airway epithelial cells: effects of active sodium and chloride transport inhibitors on bioelectric properties. *Exp Lung Res* **20**:101–117.

Spina D and Page CP (1991) The release of a non-prostanoid inhibitory factor from rabbit bronchus detected by co-axial bioassay. *Br J Pharmacol* **102**:896–903.

Stutts MJ and Bromberg PA (1987) Effects of ozone on airway epithelial permeability and ion transport. *Toxicol Lett* **35**:315–319.

Szászi K, Buday L, and Kapus A (1997) Shrinkage-induced protein tyrosine phosphorylation in Chinese hamster ovary cells. *J Biol Chem* **272**:16670–16678.

Umeno E, McDonald DM and Nadel JA (1990) Hypertonic saline increases vascular permeability in the rat trachea by producing neurogenic inflammation. *J Clin Investig* **85**:1905–1908.

Willumsen NJ, Davis CW, and Boucher RC (1994) Selective response of human airway epithelia to luminal but not serosal solution hypertonicity. Possible role for proximal airway epithelia as an osmolality transducer. *J Clin Investig* **94**:779–787.

Yankaskas JR, Gatzy JT, and Boucher RC (1987) Effects of raised osmolarity on canine tracheal epithelial ion transport function. *J Appl Physiol* **62**:2241–2245.

Address correspondence to: Dr. Jeffrey S. Fedan, Health Effects Laboratory Division, National Institute for Occupational Safety and Health, 1095 Willowdale Rd., Morgantown, WV 26505-2888. E-mail: jsf2@cdc.gov