European Journal of Pharmacology 598 (2008) 98–103

Contents lists available at ScienceDirect

European Journal of Pharmacology

journal homepage: www.elsevier.com/locate/ejphar

Pulmonary, Gastrointestinal and Urogenital Pharmacology

Simultaneous measurement of mechanical responses and transepithelial potential difference and resistance, in guinea-pig isolated, perfused trachea using a novel apparatus: Pharmacological characterization☆,☆☆

Yi Jing [a], Janet A. Dowdy [b], Michael R. Van Scott [c], Jeffrey S. Fedan [b,*]

[a] Department of Biochemistry and Molecular Pharmacology, Robert C. Byrd Health Sciences Center, West Virginia University, Morgantown, West Virginia, USA
[b] Pathology and Physiology Research Branch, Health Effects Laboratory Division, National Institute for Occupational Safety and Health, Morgantown, West Virginia, USA
[c] Department of Physiology, The Brody School of Medicine, East Carolina University, Greenville, North Carolina, USA

ARTICLE INFO

Article history:
Received 5 June 2008
Received in revised form 28 August 2008
Accepted 9 September 2008
Available online 22 September 2008

Keywords:
Perfused trachea
Transepithelial potential difference
Transepithelial resistance
Airway smooth muscle
Epithelium
Guinea pig

ABSTRACT

The isolated, perfused trachea preparation has been used to compare reactivity of the intact airway in response to differential exposure of the mucosal (intraluminal) and serosal (extraluminal) surfaces to contractile and relaxant agonists and other agents, and to gain insight into the modulatory role of the epithelium and the pathways involved. The apparatus has also been configured for simultaneous measurement of transepithelial potential difference and changes in tracheal diameter, thereby providing parallel observations of epithelial and smooth muscle function and reactivity to drugs. The transepithelial potential difference is a product of transepithelial resistance and short circuit current, and the present study describes a novel isolated, perfused tracheal apparatus which allows simultaneous measurement of transepithelial potential difference, transepithelial resistance and mechanical responses of the smooth muscle. The apparatus was validated using well-known ion transport inhibitors [intraluminal amiloride and 5-nitro-2-(3-phenylpropyl-amino) benzoic acid (NPPB), extraluminal ouabain and bumetanide], bronchoactive agonists (extraluminal methacholine, histamine and terbutaline), and osmolytes (intraluminal D-mannitol and NaCl) to induce epithelium-derived relaxing factor-mediated relaxations. This apparatus will facilitate investigation of interactions between the epithelium and smooth muscle in airways that retain their in situ structure, and signaling mechanisms potentially involved in the regulation of airway smooth muscle tone.

Published by Elsevier B.V.

1. Introduction

Regulation of bronchomotor tone, airway resistance to airflow and airway reactivity involves interaction between the surface epithelium and the underlying airway smooth muscle, and the response of airways often depends on which side of the airway is exposed to the agent. A number of methods have been employed to measure smooth muscle reactivity to contractile and relaxant drugs and neurotransmitters (Fedan et al., 2001). Strips of trachea or smaller airways, which are widely used, are a mainstay of pharmacological investigation, but in an organ bath agents have access to both sides of the airway wall. Farmer and Coleman (1970 and others cited in references therein) described a method using fluid-filled tracheas for measuring pressure responses of trachea to drugs applied to the serosal surface or induced by electrical field stimulation. A major methodological advance arrived with the description of the isolated, perfused trachea preparation (Munakata et al., 1988, 1989, 1990), used subsequently by our laboratory (Fedan et al., 1990; Jing et al., 2008) and others (de Boer et al., 1996; Folkerts et al., 1988, 1995; Fortner et al., 2001; Hjoberg et al., 1999; Pavlovic et al., 1989) (space prevents listing many others who employed the technique). With this method, fluid is perfused through the airway lumen, unlike the earlier intact airway method of Farmer and Coleman, where fluid in the lumen is static. This apparatus allows selective challenge of the mucosal surface, i.e., epithelium, by adding agents to the intraluminal perfusate or the serosal surface, i.e., smooth muscle, by adding drugs and agents to the extraluminal bath. By separating the mucosal and serosal baths, the perfused airway preparation allows investigation of the epithelium as a diffusion barrier and modulator of the reactivity of the underlying smooth muscle.

An integrated view of airway physiology and pharmacology also requires knowledge of the effects of diseases and drugs on ion transport by airway epithelium. The ion transport parameters, transepithelial potential difference, transepithelial resistance and short circuit current, are readily measurable from epithelium in culture (Grubb et al., 2006) or adherent to the airway wall (Wu et al., 2004; Yasuda et al., 2007) using the Ussing chamber, in which cylindrical tracheal segments are flattened

☆ This work was supported, in part, by a Pre-Doctoral Fellowship from the Ohio Valley Affiliate of the American Heart Association (to Y. J.).
☆☆ The findings and conclusions in this report are those of the authors and do not necessarily represent the views of the National Institute for Occupational Safety and Health. Mention of brand name does not constitute product endorsement.

* Corresponding author. Pathology and Physiology Research Branch, National Institute for Occupational Safety and Health, 1095 Willowdale Road, Morgantown, WV 26505, USA. Tel.: +1 304 285 5766; fax: +1 304 285 5938.
E-mail address: jsf2@cdc.gov (J.S. Fedan).

0014-2999/$ – see front matter. Published by Elsevier B.V.
doi:10.1016/j.ejphar.2008.09.009

and rigidly clamped between hemi-chambers. The Ussing chamber does not allow for simultaneous evaluation of epithelial bioelectric and muscle mechanical responses. In addition, distortion and edge damage associated with mounting the tissue between the hemi-chambers can affect baseline bioelectric properties, reducing transepithelial potential difference by two thirds or more compared to the level measured in the intact airways (Croxton, 1993; Dortch-Carnes et al., 1999; Wu et al., 2004) (present study). Croxton (1993) developed a cable analysis model to measure short circuit current, transepithelial resistance and tracheal diameter in intact trachea, but the system, while elegant, is not amenable to high throughput or complex experimental protocols.

In response to the limitations of earlier methods, we modified the perfused trachea apparatus to allow simultaneous measurement of transepithelial potential difference and contractile and relaxant responses of the perfused guinea-pig trachea (Dortch-Carnes et al., 1999) and used it to gain insight into the bioelectric and mechanical effects of hyperosmolar challenge of the epithelium (Johnston et al., 2004) and the role of kinases in these responses (Jing et al., 2008). However, transepithelial potential difference responses could reflect alterations in ion transport as well as in electrical resistance of the epithelium owing to changes in paracellular permeability. Therefore, in the present study, the perfusion system was modified further to detect changes in transepithelial resistance in parallel with changes in transepithelial potential difference and lumen diameter in response to agents. The apparatus makes it possible to correlate changes in transepithelial potential difference and mechanical responses, and to determine if the response is caused by changes in electrogenic transport or transepithelial resistance.

To evaluate the performance of this apparatus, selected agents were tested for their bioelectric and mechanical effects on guinea-pig trachea. The agents included ion channel blockers; ion pump and transporter inhibitors; bronchoactive agonists; and organic and non-organic osmolytes. The results demonstrate the utility of this novel apparatus for studying airway physiology and pharmacology.

2. Materials and methods

2.1. 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. Male guinea pigs (550–700 g), HsdPoc:DH, from Harlan (Indianapolis, IN), monitored free of endogenous viral pathogens, parasites, and bacteria were used in all experiments. The animals were acclimated before use and were housed in filtered ventilated cages on Alpha-Dri virgin cellulose chips and hardwood Beta-chips as bedding, provided HEPA-filtered air, Teklad 7006 diet and tap water ad libitum, under controlled light cycle (12 h light) and temperature (22–25 °C) conditions. The animals were anesthetized with sodium pentobarbital (65 mg/kg, i.p.) and sacrificed by thoracotomy and bleeding before removing the trachea.

2.2. Perfused trachea preparation for simultaneous measurement of transepithelial potential difference, transepithelial resistance, and changes in tracheal diameter

The tracheal perfusion apparatus that was used previously to measure simultaneously transepithelial potential difference and tracheal diameter (Dortch-Carnes et al., 1999; Johnston et al., 2004) was modified to deliver current pulses for transepithelial resistance concomitant measurement. This preparation also allows agents to be added separately to the mucosal (intraluminal bath) and serosal (extraluminal bath) surfaces of the trachea while measuring changes in these parameters.

A 4.2-cm segment of trachea was removed, cleaned and mounted at its natural length on a holder for the recording of inlet minus outlet perfusion pressure difference (ΔP, cm H_2O), a direct readout of tracheal diameter changes (Munakata et al., 1989). Once mounted, indwelling cannulae with side-holes were inserted into the lumen from either end of the trachea. Current electrodes were connected to the intraluminal and extraluminal baths, i.e., on either side of the tracheal wall, for delivery of transmural current pulses used for the calculation of transepithelial resistance. Likewise, voltage electrodes were placed in the intraluminal and extraluminal baths for the measurement of transepithelial potential difference. The inner voltage electrode was in continuity with the intraluminal bath through the side-hole apertures of the indwelling cannula inserted into the proximal end of the trachea, which was connected to a silver/AgCl electrode (via a 4% agar/saline bridge); the opening of the aperture was 6 mm from the point where the modified Krebs–Henseleit (MKH) solution entered the trachea. The outer voltage electrode was placed in the extraluminal bath and consisted of an MKH solution-filled glass tube, placed ~1 cm from the tracheal wall, that was in continuity with a silver/AgCl electrode (via a 4% agar/saline bridge). The position of the outer voltage electrode was directly across the tracheal wall from the inner voltage electrode. The inner current electrode was in continuity with the intraluminal bath at the point of entry of MKH into the trachea, i.e., proximal to the voltage electrode. The outer current electrode in the extraluminal bath consisted of a platinum mesh cylinder, which encircled the trachea and was placed outside the outer voltage electrode. Both voltage and current electrodes were connected to a voltage/current clamp amplifier (DVC-1000; World Precision Instruments, Inc., Sarasota, FL). The holder was made of plastic, and the inner cannulae were made from stainless steel tubing that had been coated with nail polish for insulation. Fluid resistance was compensated electronically before the trachea was mounted on to the holder. Square-wave current pulses (20 μA, 5 s duration, 50 s interval) were delivered transmurally through the current electrodes. Transepithelial potential difference (mV) and the voltage deflections caused by the pulses were recorded under open-circuit conditions. Thus, the apparatus emulates the Ussing chamber configuration but also permits simultaneous assessment of changes in tracheal diameter.

In contrast to the conventional Ussing chamber, in which current is delivered across the epithelium in a virtually constant field and transepithelial resistance and short circuit current may be corrected for area, in this apparatus current pulses were delivered across the tracheal wall between an internal point source and an outer cylindrical electrode. Consequently, the field of current was not uniformly distributed over the tracheal surface. Therefore, transepithelial resistance could not be corrected for area, and no attempt was made to quantify short circuit current. Current would fall exponentially with distance from the point source, with an unknown space constant reflecting cable properties of the epithelium (Croxton, 1993). In addition, since the side-holes of the proximal inner cannula used for ΔP measurement as well as serving as the inner voltage electrode were oriented distal from the intraluminal current electrode, voltage changes between the current electrode and the tracheal wall were not sampled. Acknowledging the geometric limitations of the device, transepithelial resistance was uncorrected for surface area, and it is understood that transepithelial resistance under these conditions is a useful index but inexact parameter. This consideration is of minor importance, as we sought to investigate the changes in transepithelial resistance caused by drugs, rather than the absolute values of transepithelial resistance. Thus, the effects of the pharmacological agents on the transepithelial potential difference and transepithelial resistance were expressed as mV and Ohms, and also normalized in terms of percent change. The method permits determination of changes in transepithelial potential difference, transepithelial resistance and tracheal diameter in real time.

2.3. Effects of ion transport inhibitors, bronchoactive agonists and osmolytes on transepithelial potential difference, transepithelial resistance and ΔP

After mounting, the preparations were equilibrated for a 2.5–3 h period to allow stabilization of transepithelial potential difference and ΔP

while perfusing (20 ml/min) MKH solution through the lumen with a pump. Current pulses were then delivered. Agents were added to the intraluminal or extraluminal baths, as appropriate. Ion transport inhibitors and histamine were applied to unstimulated preparations. Relaxation responses to terbutaline, added NaCl, and D-mannitol were elicited after the tracheas were first contracted with extraluminal methacholine (3×10^{-7} M; ~EC_{50}). In previous studies 240 mosM NaCl and D-mannitol added to the perfusing MKH solution elicited depolarization and epithelium-derived relaxing factor-mediated relaxation (Dortch-Carnes et al., 1999), and added 294 mosM NaCl caused an increase of tight junction permeability in airway epithelium (Hogman et al., 2002). Therefore, NaCl and D-mannitol were added in a concentration of 300 mosM in this component of the study for comparison purposes.

2.4. Materials

MKH solution contained 113 mM NaCl, 4.8 mM KCl, 2.5 mM $CaCl_2$, 1.2 mM KH_2PO_4, 1.2 mM $MgSO_4$, 25 mM $NaHCO_3$ and 5.7 mM glucose and was saturated with 95% O_2 and 5% CO_2, pH 7.4, 37 °C.

Acetyl-β-methylcholine chloride (methacholine), amiloride, 5-nitro-2-(3-phenylpropyl-amino) benzoic acid (NPPB), ouabain, bumetanide, histamine, terbutaline, NaCl and D-mannitol were purchased from Sigma-Aldrich (St. Louis, MO). Methacholine and D-mannitol solutions were prepared in saline. Amiloride, NPPB and bumetanide were dissolved in DMSO. Hyperosmolar solutions were prepared by adding osmolytes (D-mannitol or NaCl) to the MKH solution.

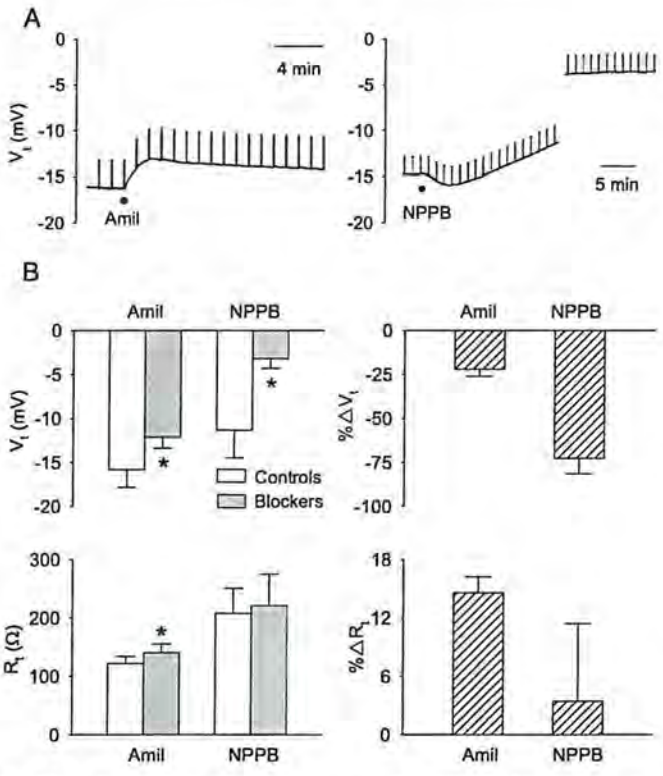

Fig. 1. Effects of amiloride (Amil, 3×10^{-5} M, intraluminal) and NPPB (10^{-4} M, intraluminal) on transepithelial potential difference (V_t), transepithelial resistance (R_t) and ΔP. (A) Representative tracings. Amiloride elicited an immediate depolarization. NPPB caused a gradual depolarization that was preceded by a small, transient hyperpolarization. The gap in the NPPB tracing represents 20 min, roughly the time required for the effect of the drug to reach a steady state. (B) Summary of the effects of amiloride and NPPB on transepithelial potential difference and transepithelial resistance. The open bars (Controls) represent values obtained before the addition of the blockers; the gray bars (Blockers) represent values obtained upon stabilization of the responses to the blockers. The hatched bars represent the responses as percentage change from the control values. The depolarization caused by amiloride was accompanied by a small increase in transepithelial resistance, while NPPB had no effect on transepithelial resistance. Neither agent affected ΔP (data not shown). $n=4$, $^*P<0.05$.

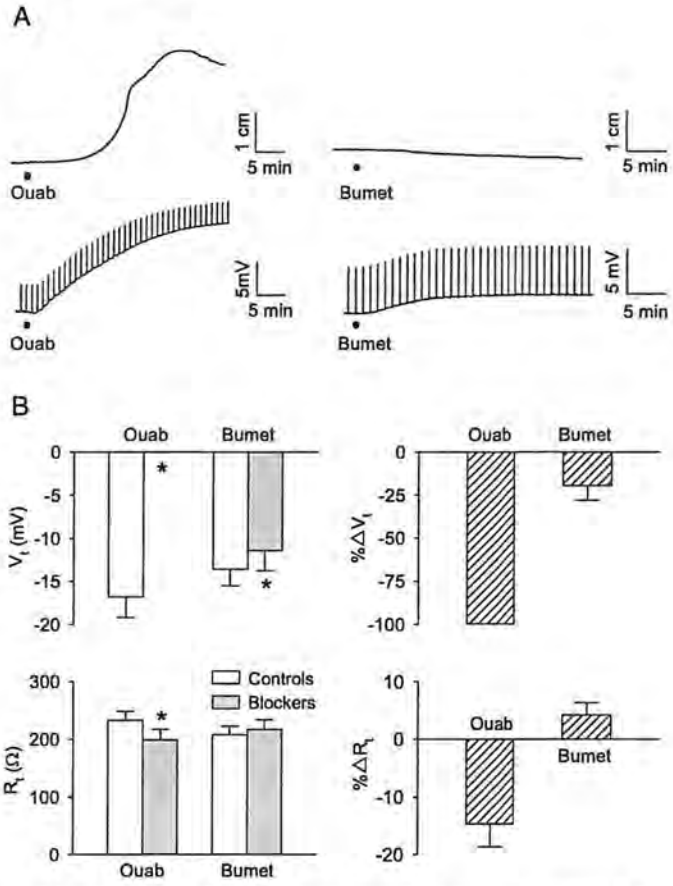

Fig. 2. Effects of ouabain (Ouab, 10^{-5} M, extraluminal) and bumetanide (Bumet, 10^{-5} M, extraluminal) on transepithelial potential difference (V_t), transepithelial resistance (R_t) and ΔP. (A) Representative tracings. Ouabain caused an immediate depolarization and a delayed contraction; bumetanide caused depolarization of slow onset and a slight relaxation. (B) Summary of the effects of ouabain and bumetanide on transepithelial potential difference and transepithelial resistance. The open bars (Controls) represent values obtained before the addition of the blockers; the gray bars (Blockers) represent values obtained upon stabilization of the responses to the blockers. The hatched bars represent the responses as percentage change from the control values. Ouabain abolished transepithelial potential difference and decreased transepithelial resistance. Bumetanide caused a small depolarization and had no effect on transepithelial resistance. $n=4$, $^*P<0.05$.

2.5. Data analysis

Transepithelial potential difference was quantified in mV; transepithelial resistance was quantified in Ohms. Transepithelial potential difference values are presented in mV and as percent change in transepithelial potential difference ($\%\Delta V_t$) before and after exposure to the agents. Transepithelial resistance was calculated from values measured before and after agents were applied and is presented as Ohms and as percent change in transepithelial resistance ($\%\Delta R_t$). Airway contraction and relaxation responses were measured as changes in ΔP, in cm H_2O. The results are presented as means±S.E.M. Differences were analyzed statistically using Student's paired t-test. $P<0.05$ was considered significant.

3. Results

3.1. Basal transepithelial potential difference

At the conclusion of the 2.5–3 h equilibration period, transepithelial potential difference was −14.8±0.8 mV ($n=32$). This value is greater than that obtained in Ussing preparations (ca. −3 to −5 mV)(Wu et al., 2004), comparable to earlier tracheal perfusion experiments in our laboratory (Dortch-Carnes et al., 1999; Jing et al., 2008), but less than that (−38 mV) obtained using cable analysis (Croxton, 1993).

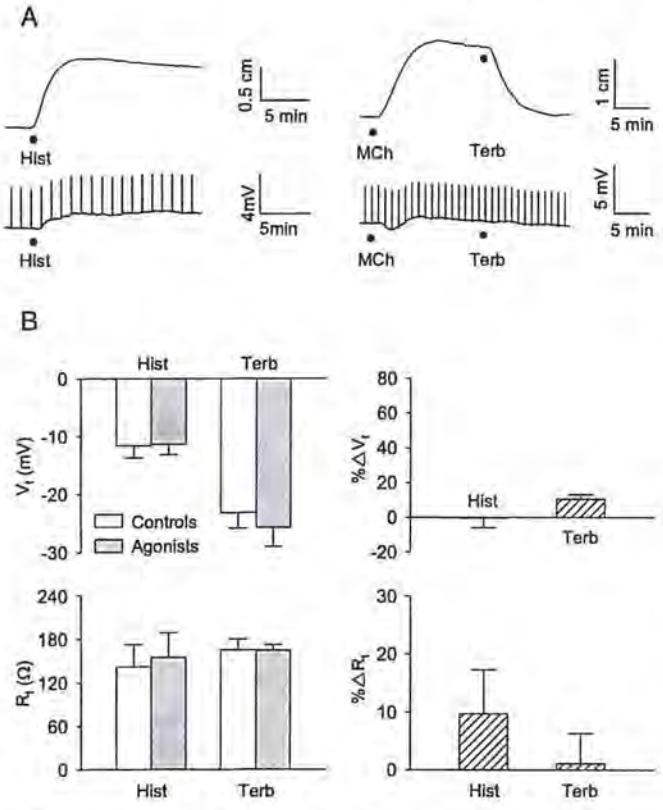

Fig. 3. Effects of histamine (Hist, 7.4×10^{-5} M, extraluminal), methacholine (MCh, 3.7×10^{-5} M, extraluminal), and terbutaline (Terb, 10^{-7} M, extraluminal) on transepithelial potential difference (V_t), transepithelial resistance (R_t) and ΔP. (A) Representative tracings. Histamine caused strong contractions and variable transepithelial potential difference responses; shown in this tracing is a depolarization but, on average, histamine did not affect transepithelial potential difference. Methacholine caused a strong contraction and increased transepithelial potential difference (see also Fig. 4), but did not affect transepithelial resistance (not shown). Terbutaline caused a strong relaxation in all tracheas and a transient hyperpolarization in 3 out of 4 preparations, followed by slow depolarization. (B) Summary of the effects of histamine and terbutaline on transepithelial potential difference and transepithelial resistance. The open bars (Controls) represent values obtained before the addition of the agonists; the gray bars (Agonists) represent responses obtained upon stabilization of the responses to the agonists. In the case of histamine, the control values were basal values; in the case of terbutaline the control values were those obtained at the plateau of the response to methacholine before terbutaline was added. The hatched bars represent the responses as percentage change from the control values. Neither agonist affected transepithelial resistance. $n=4$, $^*P<0.05$.

3.2. Effects of amiloride and NPPB on transepithelial potential difference, transepithelial resistance and ΔP

The apical membrane Na^+-channel blocker, amiloride (3×10^{-5} M), and the apical membrane Cl^--channel blocker, NPPB (10^{-4} M), decreased transepithelial potential difference when applied to the intraluminal bath. The effect of amiloride was immediate, while the response to NPPB took over 20 min to develop and involved a larger depolarization (Fig. 1). Amiloride caused a 15% increase in transepithelial resistance, while NPPB had no effect. Neither amiloride nor NPPB altered ΔP ($n=4$, data not shown).

3.3. Effects of ouabain and bumetanide on transepithelial potential difference, transepithelial resistance and ΔP

Added to the extraluminal bath, the basolateral Na^+,K^+-pump inhibitor, ouabain (10^{-5} M), and the basolateral Na^+,K^+,$2Cl^-$-cotransporter inhibitor, bumetanide (10^{-5} M), decreased transepithelial potential difference (Fig. 2). Ouabain completely depolarized the epithelium over a 30 min period, decreased transepithelial resistance, and induced a delayed contraction. Bumetanide caused a slight relaxation. The depolarization caused by bumetanide was not accompanied by a change in transepithelial resistance.

3.4. Effects of histamine and terbutaline on transepithelial potential difference, transepithelial resistance and ΔP

Mechanical responses to contractile and relaxant agonists were investigated. After addition to the extraluminal bath, methacholine (3×10^{-7} M) and histamine (7.4×10^{-5} M) elicited contraction (Fig. 3). Terbutaline (10^{-7} M) evoked relaxation in methacholine-contracted tracheas. The concentrations chosen were 10-fold higher than the extraluminal EC_{50} for histamine-induced contraction reported previously (Fedan and Frazer, 1992) and approximately twice the extraluminal EC_{50} (5.4×10^{-8} M) of terbutaline for relaxant responses derived from separate experiments ($n=4$; data not shown).

Both histamine and terbutaline caused small changes in transepithelial potential difference, even though they provoked large responses in the smooth muscle. β-Adrenoceptor stimulation is well known to stimulate Cl^- secretion in airway epithelium, so the lack of a bioelectric response to terbutaline was unexpected. This might reflect differences in the EC_{50}s for the agonist's effects between smooth muscle and epithelium, or to a species difference. Croxton (1993) also

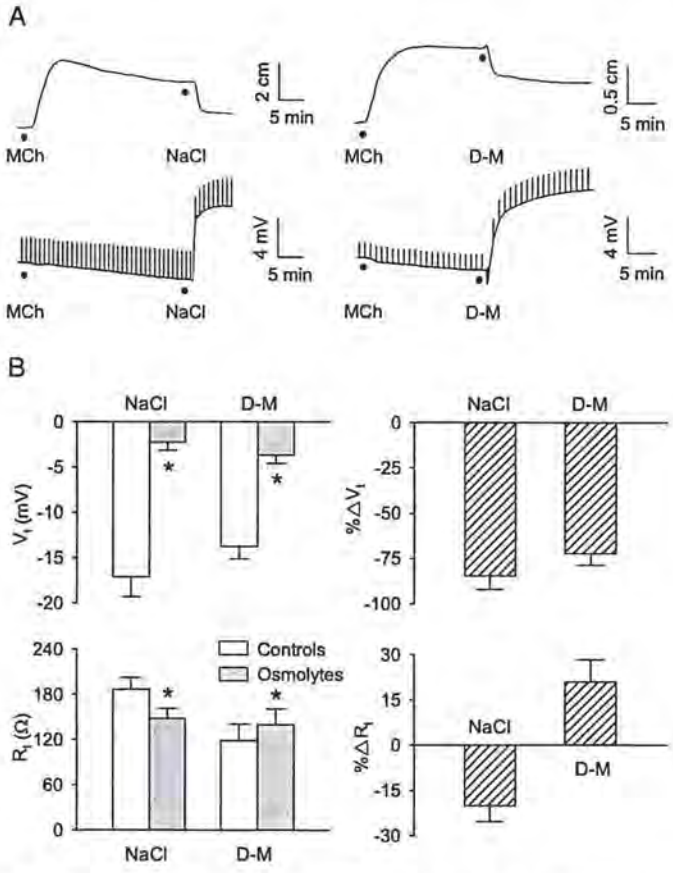

Fig. 4. Effects of hyperosmolar NaCl and D-mannitol (D-M)(each 300 mosM, intraluminal) on transepithelial potential difference (V_t), transepithelial resistance (R_t) and ΔP. (A) Representative tracings. NaCl and D-mannitol caused substantial depolarization and relaxation in the presence of methacholine. (B) Summary of the effects of NaCl and D-mannitol on transepithelial potential difference and transepithelial resistance. The open bars (Controls) represent values obtained in the presence of methacholine before the addition of the osmolytes; the gray bars (Osmolytes) represent responses obtained upon stabilization of the responses to the osmolytes. The hatched bars represent the responses as percentage change from the control values. Though both osmolytes caused depolarization and relaxation, NaCl decreased transepithelial resistance while D-mannitol increased transepithelial resistance. $n=4$, $^*P<0.05$.

reported that extraluminal isoproterenol (10^{-6} M), a non-selective β-adrenoceptor agonist, caused only a slight hyperpolarization of guinea-pig tracheal epithelium. Neither histamine nor terbutaline affected transepithelial resistance.

3.5. Effects of hyperosmolarity on transepithelial potential difference, transepithelial resistance and ΔP

Raising intraluminal osmolarity with NaCl and D-mannitol (300 mosM) caused relaxation and depolarization (Fig. 4). NaCl decreased transepithelial resistance, in agreement with morphological findings and Ussing chamber bioelectric measurements, and D-mannitol increased transepithelial resistance, in general agreement with previous observations (Hogman et al., 2002; Wu et al., 2004).

4. Discussion

Our investigation described the feasibility of measuring simultaneously mechanical (ΔP) and bioelectric (transepithelial potential difference and transepithelial resistance) responses of isolated trachea to a variety of ion transport blockers and agonist drugs and agents.

Amiloride and NPPB decreased basal transepithelial potential difference. The ~25% decrease by amiloride was smaller than the ~50% decrease determined in cable analysis experiments on guinea-pig trachea (Croxton, 1993). This difference could be related to the greater basal transepithelial potential difference (~38 mV) recorded in the cable experiments compared to that (~15 mV) measured in our experiments, but the basis for the different basal transepithelial potential difference values is not apparent. An increase in transepithelial resistance by amiloride, observed here, also has been reported for rabbit airways (Poulsen et al., 2006). The larger depolarization elicited by NPPB observed here and reported previously (Wu et al., 2004) indicates that guinea-pig tracheal epithelium is primarily a Cl^--secreting rather than a Na^+-absorbing epithelium.

Ouabain and bumetanide induced epithelial depolarization. Although ouabain also decreased transepithelial resistance, it is not difficult to conclude that the abolition of transepithelial potential difference was primarily due to the inhibition of the Na^+,K^+-pump. The activity of the Na^+,K^+,$2Cl^-$-cotransporter in guinea-pig tracheal epithelium supports ~10% of short circuit current in the Ussing apparatus (Wu et al., 2004). Bumetanide, which had no effect on transepithelial resistance, caused a ~20% decrease in transepithelial potential difference. This effect is comparable with that obtained in cultured guinea-pig tracheal epithelium (Robison and Kim, 1994). The ouabain-induced muscle contraction is well known to be due to Na^+ loading and Ca^{2+} accumulation in the smooth muscle (Chideckel et al., 1987). Extraluminally-added bumetanide-induced relaxation can be attributed, in part, to its inhibition of the smooth muscle Na^+,K^+, $2Cl^-$-cotransporter (Lavallee et al., 1997).

Histamine and terbutaline caused, as expected, strong contraction and relaxation responses, respectively. However, they had modest effects on transepithelial potential difference and transepithelial resistance. Histamine has been reported to increase transepithelial potential difference and short circuit current in canine tracheas (Yamada et al., 1994), but weak transepithelial potential difference responses were observed in the present study. In guinea-pig cultured epithelium, terbutaline, in a 10-fold higher concentration (10^{-6} M) than used here, increased short circuit current identically after basolateral and apical administrations (Robison and Kim, 1994). The disparity in reactivity between the fresh and cultured cells may manifest differences in the concentrations used or phenotypic adaptation to cell culture (Fedan et al., 2007).

Hyperosmolar NaCl and D-mannitol in the high concentration (300 mosM) elicited depolarization and relaxation, as reported earlier (Dortch-Carnes et al., 1999). In agreement with observations made with the Ussing apparatus (Poulsen et al., 2006; Wu et al., 2004), and microelectrode and microscopy studies (Hogman et al., 2002; Willumsen et al., 1994), NaCl decreased transepithelial resistance but D-mannitol increased transepithelial resistance. Because the NaCl-induced $\%\Delta R_t$ (20.0%) was less ($P<0.05$) than the $\%\Delta V_t$ (84.7%), the depolarization induced by NaCl probably reflected decreased transcellular ion transport. In contrast, D-mannitol increased transepithelial resistance in the face of depolarization. Decreased ion transport in response to hypertonic NaCl and D-mannitol was also observed in previous studies (Willumsen et al., 1994; Wu et al., 2004).

This novel preparation offers many advantages over the available techniques (see Introduction) for studying airway epithelium and smooth muscle in an organ that, except for blood supply and intact afferent and efferent innervation, is identical in configuration to the in situ situation and avoids many of the shortcomings of other in vitro methods. It may be a suitable surrogate for certain types of in vivo physiological and pharmacological investigations of the actions of, for example, bronchoconstrictor, bronchodilator, and anti-inflammatory drugs. A great deal of information is made available from one trachea regarding the effects of these and other types of agents on epithelium and smooth muscle, and the synchronization between epithelial and smooth muscle events is evident. The degree to which the properties we observed in trachea would be shared by smaller airways is unknown at present, and requires further methodological development and investigation. However, the technique could easily substitute for other methods already using trachea.

In conclusion, a method was described for the study of airway physiology and pharmacology using tracheas that retain their in situ configuration. The effects of the agents used in the investigation recapitulated their predicted behavior. This method made it possible to examine epithelial airway bioelectric and mechanical responses simultaneously and provided insight into the contribution of changes in transepithelial resistance to bioelectric responses. The method will facilitate examination of epithelial bioelectric phenomena that may influence the reactivity of the smooth muscle.

References

Chideckel, E.W., Frost, J.L., Mike, P., Fedan, J.S., 1987. The effect of ouabain on tension in isolated respiratory tract smooth muscle of humans and other species. Br. J. Pharmacol. 92, 609–614.

Croxton, T.L., 1993. Electrophysiological properties of guinea pig tracheal epithelium determined by cable analysis. Am. J. Physiol. 265, L38–L44.

de Boer, J., Meurs, H., Coers, W., Koopal, M., Bottone, A.E., Visser, A.C., Timens, W., Zaagsma, J., 1996. Deficiency of nitric oxide in allergen-induced airway hyperreactivity to contractile agonists after the early asthmatic reaction: an ex vivo study. Br. J. Pharmacol. 119, 1109–1116.

Dortch-Carnes, J., Van Scott, M.R., Fedan, J.S., 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.

Farmer, J.B., Coleman, R.A., 1970. A new preparation of the isolated intact trachea of the guinea-pig. J. Pharm. Pharmacol. 22, 46–50.

Fedan, J.S., Frazer, D.G., 1992. Influence of epithelium on the reactivity of guinea-pig isolated, perfused trachea to bronchoactive drugs. J. Pharmacol. Exp. Ther. 262, 741–750.

Fedan, J.S., Nutt, M.E., Frazer, D.G., 1990. Reactivity of guinea-pig isolated trachea to methacholine, histamine and isoproterenol applied serosally versus mucosally. Eur. J. Pharmacol. 190, 337–345.

Fedan, J.S., Van Scott, M.R., Johnston, R.A., 2001. Pharmacological techniques for the in vitro study of airways. J. Pharmacol. Toxicol. Methods 45, 159–174.

Fedan, J.S., Wu, D.X., Van Scott, M.R., 2007. Altered ion transport and responsiveness to methacholine and hyperosmolarity in air interface-cultured guinea-pig tracheal epithelium. J. Pharmacol. Toxicol. Methods 55, 135–143.

Folkerts, G., Henricks, P.A., Slootweg, P.J., Nijkamp, F.P., 1988. Endotoxin-induced inflammation and injury of the guinea pig respiratory airways cause bronchial hyporeactivity. Am. Rev. Respir. Dis. 137, 1441–1448.

Folkerts, G., van der Linde, H., Verheyen, K.C.P., Nijkamp, F.P., 1995. Endogenous nitric oxide modulation of potassium-induced changes in guinea-pig airway tone. Br. J. Pharmacol. 115, 1194–1198.

Fortner, C.N., Lorenz, J.N., Paul, R.J., 2001. Chloride channel function is linked to epithelium-dependent airway relaxation. Am. J. Physiol., Lung Cell. Mol. Physiol. 280, L334–L341.

Grubb, B.R., Rogers, T.D., Diggs, P.C., Boucher, R.C., Ostrowski, L.E., 2006. Culture of murine nasal epithelia: model for cystic fibrosis. Am. J. Physiol., Lung Cell. Mol. Physiol. 290, L270–277.

Hjoberg, J., Högman, M., Hedenstierna, G., 1999. Hyperosmolarity reduces the relaxing potency of nitric oxide donors in guinea-pig trachea. Br. J. Pharmacol. 127, 391–396.

Hogman, M., Mork, A.C., Roomans, G.M., 2002. Hypertonic saline increases tight junction permeability in airway epithelium. Eur. Respir. J. 20, 1444–1448.
Jing, Y., Dowdy, J.A., Van Scott, M.R., Fedan, J.S., 2008. Hyperosmolarity-induced dilation and epithelial bioelectric responses of guinea-pig trachea in vitro. Role of kinase signaling. J. Pharmacol. Exp. Ther. 326, 186–195.
Johnston, R.A., Van Scott, M.R., Kommineni, C., Millecchia, L.L., Dortch-Carnes, J., Fedan, J.S., 2004. 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, 37–46.
Lavallee, S.L., Iwamoto, L.M., Claybaugh, J.R., Dressel, M.V., Sato, A.K., Nakamura, K.T., 1997. Furosemide-induced airway relaxation in guinea pigs: relation to Na–K–2Cl cotransporter function. Am. J. Physiol. 273, L211–216.
Munakata, M., Mitzner, W., Menkes, H., 1988. Osmotic stimuli induce epithelial-dependent relaxation in the guinea pig trachea. J. Appl. Physiol. 64, 466–471.
Munakata, M., Huang, I., Mitzner, W., Menkes, H., 1989. Protective role of epithelium in the guinea pig airway. J. Appl. Physiol. 66, 1547–1552.
Munakata, M., Masaki, Y., Sakuma, I., Ukita, H., Otsuka, Y., Homma, Y., Kawakami, Y., 1990. Pharmacological differentiation of epithelium-derived relaxing factor from nitric oxide. J. Appl. Physiol. 69, 665–670.
Pavlovic, D., Fournier, M., Aubier, M., Pariente, R., 1989. Epithelial vs. serosal stimulation of tracheal muscle: role of epithelium. J. Appl. Physiol. 67, 2522–2526.
Poulsen, A.N., Klausen, T.L., Pedersen, P.S., Willumsen, N.J., Frederiksen, O., 2006. Nucleotide regulation of paracellular Cl^- permeability in natural rabbit airway epithelium. Pflugers Arch. 452, 188–198.
Robison, T.W., 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.
Willumsen, N.J., Davis, C.W., Boucher, R.C., 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. Invest. 94, 779–787.
Wu, D.X., Johnston, R.A., Rengasamy, A., Van Scott, M.R., Fedan, J.S., 2004. Hyperosmolar solution effects in guinea pig airways. II. Epithelial bioelectric responses to relative changes in osmolarity. J. Pharmacol. Exp. Ther. 308, 19–29.
Yamada, K., Shimura, S., Satoh, M., Sasaki, T., Yamauchi, K., Takishima, T., 1994. HMT regulates histamine-induced Cl^- secretion across the canine tracheal epithelium. Respir. Physiol. 97, 105–109.
Yasuda, M., Niisato, N., Miyazaki, H., Hama, T., Dejima, K., Hisa, Y., Marunaka, Y., 2007. Epithelial ion transport of human nasal polyp and paranasal sinus mucosa. Am. J. Respir. Cell Mol. Biol. 36, 466–472.