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

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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.

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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

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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