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Original article

Altered ion transport and responsiveness to methacholine and hyperosmolarity in air interface-cultured guinea-pig tracheal epithelium

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Abstract

Introduction: Challenge of guinea-pig tracheal epithelium with hyperosmolar solution alters ion transport and evokes the release of epithelium-derived relaxing factor (EpDRF). Cultured tracheal epithelial cells (CE) offer the potential to examine biochemical pathways related to EpDRF release, but whether the bioelectric properties and responses of fresh, adherent epithelial cells (FE) are modeled by CE has not been established. **Methods:** Tracheal epithelial cells grown in air-interface culture and fresh tracheal segments were mounted in Ussing chambers to determine short circuit current (I_{sc}) and transepithelial resistance (R_t) and to compare responses to transport inhibitors, methacholine and hyperosmolarity. **Results:** Significant differences in basal I_{sc} and R_t between FE and CE were observed (I_{sc}, 41.3±3.5 and 8.5±0.8 μA/cm^2, $P<0.05$; R_t, 106±7 and 422±4 Ω cm^2, $P<0.05$; respectively); basal spontaneous potential difference values were not different (4.2±0.3 and 3.4±0.3 mV, respectively). Amiloride (mucosal, 3×10^{-5} M), bumetanide (basolateral, 10^{-5} M) and ouabain (basolateral, 10^{-5} M) reduced I_{sc} equally in FE and CE. In contrast, NPPB (10^{-5} M) in the presence of amiloride had a differential effect, decreasing I_{sc} by 11% in FE and 71% in CE ($P<0.05$). Iberiotoxin (basolateral, 10^{-7} M) was without effect in either preparation. In FE, serosal methacholine (3×10^{-5} M) elicited an NPPB-insensitive monotonic increase in I_{sc}, but in CE caused a large, transient, NPPB-inhibitable increase which was followed by an NPPB-resistant plateau. Addition of apical D-mannitol (0.3–267 mosM) to increase osmolarity decreased I_{sc} in FE, whereas in CE D-mannitol initially increased (0.3–84.3 mosM) and then decreased (84.3–267 mosM) I_{sc}. **Discussion:** Cell culture causes substantial changes in the bioelectric and pharmacological properties of respiratory epithelium. Caution should be exercised when using CE as a substitute for FE in studies of ion transport- and cell volume-dependent processes.

Keywords: Airway; Bioelectric responses; Guinea pig; Epithelium; Cell culture effects; Hyperosmolarity; D-Mannitol; Methacholine; Methods; Ussing chamber

1. Introduction

The epithelium in the respiratory tract has many complex biological roles. It serves as a protective barrier that limits the movement of inhaled chemicals, particulates and other agents into the body and maintains the composition of the airway surface liquid (ASL) through regulated transepithelial ion transport, thereby allowing efficient propulsion of substances up the mucociliary escalator by ciliated epithelial cells. In addition, the epithelium metabolizes drugs, neurotransmitters and inflammatory mediators (Goldie & Hay, 1997) and secretes an array of inflammatory mediators (Churchill et al., 1989; Churchill, Friedman, Schleimer, & Proud, 1992; Diamond, Legarda, & Ryan, 2000; Ge et al., 2004; Guo et al., 1997; Holtzman, Ferdman, Bohrer, & Turk, 1991; Kwon et al., 1994; Lilly et al., 1997; Marini, Vittori, Hollemborg, & Mattoli, 1992; Sousa et al., 1994; Watkins, Garlepp, & Thompson, 1997). Epithelial cells are involved in host

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defense, innate immunity (Bals & Hiemtra, 2004) and resolution of inflammation (Sexton, Al-Rabia, Blaylock, & Walsh, 2004). In addition, the epithelium may be involved in airway remodeling observed in asthma through release of growth factors such as transforming growth factor-β1 (Kumar, Herbert, & Foster, 2004).

The respiratory epithelium is also involved in the regulation of the reactivity of airway smooth muscle through the release of epithelium-derived relaxing factor (EpDRF)(Fedan, Hay, Farmer, & Raeburn, 1988; Flavahan, Aarhus, Rimele, & Vanhoutte, 1985; Folkerts & Nijkamp, 1998; Goldie & Hay, 1997), which can diffuse to the smooth muscle and inhibit contraction or induce relaxation. EpDRF is released following exposure of the epithelium to hyperosmolar solution (Fedan et al., 1999, 2000; Munakata, Mitzner, & Menkes, 1988). The significance of this phenomenon is that the ASL is thought to become hyperosmolar during the hyperventilation that occurs during exercise, causing bronchodilation in normal individuals but obstruction in asthmatic patients (Anderson and Daviskas, 1992). The ability of inhaled D-mannitol (D-M) to elicit obstruction in asthmatics is thought to be initiated by a rise in ASL osmolarity (Anderson and Brannan, 2003). Release of EpDRF following hyperosmolar challenge is associated with bioelectric events in the epithelium that are linked to changes in $Na^+$and Cl^- transport (Dortch-Carnes, Van-Scott, & Fedan, 1999; Fedan et al., 1999; Wu, Johnston, Rengasamy, Van Scott, & Fedan, 2004). The identity of EpDRF has not been established unequivocally, but evidence has been obtained to suggest that it resembles carbon monoxide in some respects (Fedan, Dowdy, Johnston, & Van Scott, 2004; Fedan, Dowdy, Van Scott, Wu, & Johnston, 2004).

Elucidation of the mechanisms involved in EpDRF release could be facilitated by the use of cultured epithelial cells. Previous work on EpDRF has been performed using the adherent epithelium in the guinea-pig trachea. Confirmation that the physiological and pharmacological properties of this tissue are retained in cell culture is particularly required before signaling pathways are explored biochemically. Therefore, in this report we compared the bioelectric properties and responses to muscarinic stimulation and hyperosmolar solutions in guinea-pig air-interface-cultured epithelial cells (CE) and fresh cells (FE) attached to the tracheal wall. Our results indicate that basal bioelectric properties and responses to hyperosmolar challenge and methacholine are altered in the cultured cells.

2. 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 (300–350 g; Crl:HA) from Charles River (Wilmington, MA), 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. Preparation of CE

The cells were isolated from guinea-pig tracheal epithelium and cultured using published methods (Adler, Cheng, & Kim, 1990; Robison & Kim, 1994) for air-interface culture, with modifications. After anesthesia, a 4-cm length section of trachea was removed under sterile conditions and placed in Hank's balanced salt solution (HBSS) containing amphotericin B (1 μg/ml), gentamycin (100 μg/ml) and nystatin (40 U/ml). The trachea was cleaned of extraneous tissue under a dissection microscope, cut open longitudinally through the smooth muscle band, and incubated with 0.2% protease in minimum essential medium (MEM) at 37 °C in 95% air–5% CO_2 for 1 h. Epithelial cells were gently scraped off with a scalpel, pooled from different animals, suspended with gentle trituration in MEM containing 10% fetal bovine serum (FBS) and 1 mg/ml DNase, and centrifuged for 5 min at 250 *g* at room temperature. The supernatant was discarded and the pellet was suspended in 10 ml MEM containing 10% FBS. Cells were suspended and centrifuged for a second time in 10 ml MEM containing 10% FBS. Then, cells were filtered through a 70 μm filter and centrifuged a third time. Finally, cells were suspended in 1 ml PC-1 medium containing L-glutamine (2 mM), penicillin (100 U/ml), streptomycin (100 μg/ml), and retinoic acid (10^{-7} M). (A significant improvement to the published methods was the addition of retinoic acid to the culture medium. Retinoic acid increased the pseudostratification of the epithelial cells, and led to an increase in the prevalence of ciliated cells.) Cells were plated on permeable matrix, Costar 7 12-mm Snapwells (Corning, Inc.), 10^6 cells per well. All culture wells had been coated previously with type-1 rat-tail collagen. A stock solution (50 μg/ml) of collagen was prepared in 0.02 N glacial acetic acid. The solution had been sterilized by passage through a 0.22 μm filter and added to the wells in an amount of 60 μg/cm^2. The wells were incubated at room temperature overnight and were allowed to dry. The coated wells were rinsed with HBSS and PC-1 media before the cells were seeded into the wells.

The cells were initially cultured in immersion with PC-1 medium containing L-glutamine (2 mM), penicillin (100 U/ml), streptomycin (100 μg/ml), and retinoic acid (10^{-7} M) at 37 °C in 95% air–5% CO_2 to allow adherence to the collagen-coated matrix. After 24 h, non-adherent cells were rinsed away with PC-1 medium, and the cells were cultured in air-interface at 37 °C in 95% air–5% CO_2. The PC-1 medium in the bottom chamber was changed daily.

2.3. Monitoring CE confluence and transepithelial resistance (R_t)

The cells were observed daily using a phase-contrast microscope (Zeiss Axiovert 100TV). Upon reaching confluence (2 to 3 days), R_t was measured daily by placing the Snapwell into an Endohm−24 Snap which was connected to a current-passing electrometer (EVOM, World Precision Instruments, Inc.).