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Detection of Cl^--HCO_3^- and Na^+-H^+ Exchangers in Human Airways Epithelium

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Summary

Molecular species of the Na^+-H^+ exchanger (NHE) and anion exchanger (AE) gene families and their relative abundance in the human airway regions were assessed utilizing RT-PCR and the RNase protection assay, respectively. Organ donor lung epithelia from various bronchial regions (small, medium, and large bronchi and trachea) were harvested for RNA extraction. Gene-specific primers for the human NHE and AE isoforms were utilized for RT-PCR. Our results demonstrated that NHE1, AE2, and brain AE3 isoforms were expressed in all regions of the human airway, whereas NHE2, NHE3, AE1, and cardiac AE3 were not detected. RNase protection studies for NHE1 and AE2, utilizing glyceraldehyde-3-phosphate dehydrogenase as an internal standard, demonstrated that there were regional differences in the NHE1 mRNA levels in human airways. In contrast, the levels of AE2 mRNA remained unchanged. Differential regional expression of NHE1 isoform may be related to a higher acid load in the tracheal epithelial cells than in epithelia of distal airways. Fluctuations in PCO_2 during inspiration and expiration are probably larger in the tracheal lumen than in the lumen of distal airways with associated larger swings in intracellular pH with each respiratory cycle. Immunohistochemical staining for AE2 protein demonstrated localization to the epithelial cells of human bronchial mucosa.

Introduction

The polarized epithelia of the mammalian tracheobronchial mucosa and of alveolar sacs have various mechanisms involving translocation of Na^+ and Cl^- across cell membranes. These include electrogenic processes, such as Na^+ and Cl^- channels [1, 2, 3, 4, 5], and electroneutral processes, such as Na^+-H^+ exchange and Cl^--HCO_3^- exchange [2, 3, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15]. These and other mechanisms, such as electrogenic anion exchangers [16, 17] play a vital role in the regulation of intracellular pH and volume, vectorial transport of these ions, and proton or HCO_3^- secretion in various fluids, such as gastric, intestinal, exocrine pancreatic, and renal tubular transport [17, 18, 19, 20, 21, 22]. Gene families for both the NHE [22, 23, 24, 25] and AEs have been identified [26, 27, 28].

The NHE gene family has been shown to include different isoforms (NHE1 to NHE6), and NHE1, NHE2, and NHE3 isoforms are the most characterized members of this gene family [23, 25]. NHE1 is considered to be the ubiquitous isoform localized to the basolateral

membranes of the polarized epithelial cells and is involved in housekeeping functions, whereas NHE2 and NHE3 isoforms have been considered to be the epithelial isoforms localized to the apical membranes of the polarized epithelial cells. NHE3 has been shown to be an important apical isoform involved in the vectorial Na^+ transport in the kidney and intestinal epithelium. The AE gene family includes four structurally and functionally related anion exchangers, AE1, AE2, and AE3 [26] and AE4 [28]. The AE2 isoform has been suggested to be the epithelial isoform, whereas AE1 has been suggested to be erythroid and AE3 has been suggested to be a neuronal homologue [19, 26, 27]. AE4 is apically located in beta-intercalated cells of the kidney [28].

In the respiratory system, several studies have indicated the presence of Na^+-H^+ and Cl^--HCO_3^- exchange activities in lung alveolar and tracheal epithelia of various species [3, 6, 7, 8, 10, 11, 13, 15, 24]. In this report, we review the molecular species of the NHE and AE gene families and their relative abundance in the human airway regions.

Methods

Details of methods were described previously [29].

Harvesting of the Bronchial Epithelial Cells Ion transport characteristics in a number of organs, e.g., kidney and intestine, exhibit marked regional variations, therefore, epithelial cells were collected from various regions of the human bronchial tree. Whole human lungs collected from organ donors were packed on ice and transported to the laboratory. The airway lumen was opened longitudinally and rinsed with Krebs bicarbonate solution. The mucosa was then gently scraped with a scalpel, and epithelial pellets from each bronchial region were immediately placed in separate vials containing chilled RNazol reagent. Bronchial regions were designated broadly as large, medium and small bronchi. The average age of the donors was 45±0.2 years, and of the 19 donors, 14 were men and 5 were women.

RT-PCR and RNase Protection Assay Methods

The gene-specific PCR primer sets for the human NHE1, NHE2, NHE3, AE1, AE2, bAE3 and cAE3 were designed to detect the isoform-specific mRNA in the human airways. Human NHE1, NHE2, NHE3, AE1, AE2, bAE3 and cAE3 were retrieved from the GenBank CD-ROM supplied with Gene Works software. Human NHE2 primers were designed from the cDNA sequence for this isoform cloned in our laboratory [20].

Total RNA was extracted from the tracheobronchial epithelial cells and used for RT with gene-specific primers and Superscript II RT enzyme utilizing the Superscript kit for first-strand cDNA synthesis. The PCR reaction was carried out using standard step-cycling conditions with 30 cycles of amplification utilizing *Taq* DNA polymerase. RT-PCR products were separated by electophoresis. Bands of expected sized were visualized under ultraviolet light.

RT-PCR products cloned into pGEM vectors or into PCRII vectors were sequenced with universal primer and reverse sequencing primer.

For quantitation of mRNA level, the RNase protection assay was utilized due to its high sensitivity and feasibility with small quantities of RNA isolated from scraped cells of even distal airways. The RNase protection assay was performed as previously described [30].

Immunohistochemistry For detection of AE2, a sample slide as well as a negative control slide were deparaffinized, hydrated (EtOH) and then permeabilized with Triton. Endogenous peroxidases were blocked with 3% H_2O_2 in method and then 1% SDS was applied to each slide. The AE2 antibody was applied to the sample slide in a 1:100 dilution (were non-

immune serum was used on the negative control). Staining was performed with the DAKO LSAB 2 Kit. Detection was performed with a very small amount of DAB (3,3'-Diaminobenzidine tetrahydrochloride) applied briefly to the slides. Finally, Gill's hematoxylin was added, slides were dehydrated and then permanently fixed with permount.

Results

NHE1 isoform was expressed in all the regions of the human airways, whereas NHE2 and NHE3 were not detected. RNase protection studies for NHE1 utilizing glyceraldehyde 3-phosphate dehydrogenase as an internal standard, demonstrated that there were regional differences in the NHE1 mRNA levels in the human airways. NHE1 mRNA levels were significantly higher in the trachea compared with those in the distal bronchial regions ($P<0.05$). NHE1 mRNA levels were the highest in the trachea and progressively decreased from large, to medium, and small bronchi (Figure 1).

To detect AE isoforms in the human tracheobronchial tree, we used gene specific primers for human AE isoforms. We detected AE2 and brain-AE3 isoforms, but we did not detect AE1 or cardiac-AE3 isoforms. The levels of AE2 mRNA were similar in large as well as small bronchi.

Immunohistochemistry analysis indicated staining for AE2 in the bronchial epithelial cells only, no staining was detected in non-epithelial cells. Membrane localization and detailed functional roles of the AE2 and brain-AE3 isoforms expressed in the human airways remain to be defined.

Discussion

Our data demonstrates the detection of NHE1, AE2 and bAE3 along various regions of the human airways. Additionally, we demonstrated the regional variation in the expression of the NHE1 mRNA in the human airways. The NHE1 isoform, which is usually expressed in the basolateral membrane domain has previously been suggested to be involved in a number of 'housekeeping' functions, including maintenance of intracellular pH and regulation of cell volume and cell proliferation [8, 14, 23, 25].

We detected a progressive increase in NHE1 and mRNA expression as airways branching system converged from lung peripheral to proximal airways. This suggests that proximal airways, exemplified by the tracheal epithelium, are subjected to a higher acid load than the epithelia of peripheral airways. Such increased acid load could conceivably result from periodic swings in intracellular pH due to large fluxes of CO_2 across the epithelial cell membrane associated with the respiratory cycle. The inspired air contains miniscule amount of

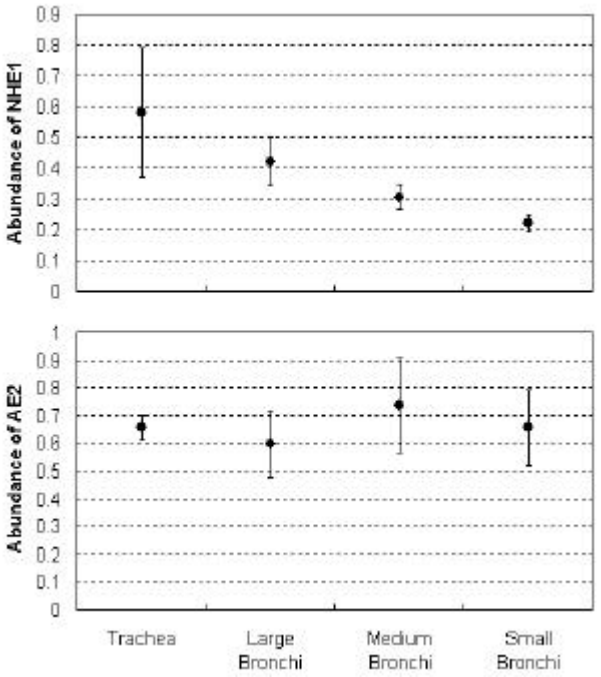

Figure 1. Relative abundance of NHE1 and AE2 at various regions in the mucosa of the human conducting airways. Relative abundance of NHE1 (top) and AE2 (bottom) was determined by calculating the ratio of their density to the density of GAPDH (the internal standard glyceraldehyde-3-phosphate dehydrogenase). Values represent the mean±SEM of 3-5 independent samples from 3-5 organ donors normalized to GAPDH.

CO_2, whereas the expired air has high pCO_2. It is feasible that distal airways by virtue of their proximity to the CO_2-rich alveoli are subjected to minor swings in luminal pCO_2, whereas the tracheal epithelium is exposed to much larger variations in luminal pCO_2, resulting in rapid and large changes in intracellular pCO_2 and hence pH with each respiratory cycle.

We did not detect NHE2 and NHE3 isoforms which are usually localized to the apical membrane of polarized epithelia [15, 29, 35]. The absence of both of these putative apical membrane isoforms, NHE2 and NHE3, in the human proximal and distal airways suggests that the neutral NaCl absorptive process (involving dual ion exchange of Na^+-H^+ and Cl^--HCO_3^-) may be absent in the luminal membranes of the human airway epithelial cells. Further studies are required to confirm this conclusion because other NHE isoforms that we did not investigate such as NHE4 and NHE5 may be localized to luminal membranes of airways.

Recent studies have suggested a role for anion exchangers in the transport of sulfate and Cl^- in sheep trachea and human bronchial cells [2]. There is also evidence for the presence of a Cl^--HCO_3^- exchange activity in equine trachea [3] and a Cl^--HCO_3^- exchange process (Na^+-independent) in the rat type II alveolar epithelial cells [11]. This activity was shown to be localized to the basolateral side of the alveolar epithelial cells [9]. Basolateral localization AE2 polypeptide has also been shown in choroid plexus epithelium and gastric parietal cells and in the human and rat kidney [26], as well as the basolateral membrane of the epithelial cells of the human small intestine and colon [31].

Differential expression of the NHE1 isoform and the uniform distribution of AE2 and brain-AE3 isoforms in the human airways may have functional significance related to The pH or ionic composition of airway surface liquid [1, 5, 32]. An acidic mucus pH increases mucus viscosity [33] and may thus interfere with the efficiency of the mucociliary apparatus, whereas an alkaline pH may enhance bacterial adherence to epithelial cells [34]. At the alveolar level, regulation of lamellar bodies pH by Na^+-H^+ exchange and H^+-ATPase may affect surfactant secretion or metabolism [35]. Further studies of the functional roles of these ion exchangers would be important to better understand the physiology of the alveolar and airway absorption and secretion.

Key words Bicarbonates; Chlorides; Cystic Fibrosis; Respiratory Mucosa; Sodium-Hydrogen Antiporter

Abbreviations AE: anion exchanger; NHE: Na^+-H^+ exchanger; RT-PCR: reverse-transcription polymerase chain reaction

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