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Genetic variation of the alpha subunit of the epithelial Na^+ channel influences exhaled Na^+ in healthy humans

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ABSTRACT

Epithelial Na^+ channels (ENaC) are located in alveolar cells and are important in β_2-adrenergic receptor-mediated lung fluid clearance through the removal of Na^+ from the alveolar airspace. Previous work has demonstrated that genetic variation of the alpha subunit of ENaC at amino acid 663 is important in channel function: cells with the genotype resulting in alanine at amino acid 663 (A663) demonstrate attenuated function when compared to genotypes with at least one allele encoding threonine (T663, AT/TT). We sought to determine the influence of genetic variation at position 663 of ENaC on exhaled Na^+ in healthy humans. Exhaled Na^+ was measured in 18 AA and 13 AT/TT subjects (age = 27 ± 8 years vs. 30 ± 10 years; ht. = 174 ± 12 cm vs. 171 ± 10 cm; wt. = 68 ± 12 kg vs. 73 ± 14 kg; BMI = 22 ± 3 kg/m^2 vs. 25 ± 4 kg/m^2, mean ± SD, for AA and AT/TT, respectively). Measurements were made at baseline and at 30, 60 and 90 min following the administration of a nebulized β_2-agonist (albuterol sulfate, 2.5 mg diluted in 3 ml normal saline). The AA group had a higher baseline level of exhaled Na^+ and a greater response to β_2-agonist stimulation (baseline = 3.1 ± 1.8 mmol/l vs. 2.3 ± 1.5 mmol/l; 30 min-post = 2.1 ± 0.7 mmol/l vs. 2.2 ± 0.8 mmol/l; 60 min-post = 2.0 ± 0.5 mmol/l vs. 2.3 ± 1.0 mmol/l; 90 min-post = 1.8 ± 0.8 mmol/l vs. 2.6 ± 1.5 mmol/l, mean ± SD, for AA and AT/TT, respectively, $p < 0.05$). The results are consistent with the notion that genetic variation of ENaC influences β_2-adrenergic receptor stimulated Na^+ clearance in the lungs, as there was a significant reduction in exhaled Na^+ over time in the AA group.

1. Introduction

Fluid levels in the alveolar space are normally tightly regulated to optimize gas transfer of oxygen and carbon dioxide between the blood and the airspace within the alveoli (Aarseth et al., 1975; Snyder et al., 2006; Wallin and Leksell, 1994). Conditions such as heart failure and cystic fibrosis disrupt fluid regulation causing either flooding of the alveolar space, or drying of the airways resulting in mucous accumulation. Both conditions result in compromised gas transfer (Boucher, 2004; Chua and Coats, 1995).

Lung fluid balance is normally tightly regulated by the interplay between different ion channels transporting salts and the water, which ultimately follows the flux of these ions. When ions such as sodium (Na^+) or chloride (Cl^-) are transported from the alveolar space to the interstitium, this causes an osmotic gradient to form, resulting in the translocation of water from the alveoli to the interstitial space (Starling, 1896). One important factor in this ion transport is the epithelial Na^+ channel (ENaC, encoded by the gene SCNN1A), a protein found on the luminal surface of many cells including the type I and II epithelia of the alveolar wall (Garty and Palmer, 1997). The primary function of ENaC is to allow cations, in particular Na^+, to pass from the apical side (alveolar space) to the basolateral side (interstitial space) of these airway epithelial cells. This results in an overall flux of water out of the alveoli of the lung, a critical element in the regulation of overall fluid balance (Eaton et al., 2004). Over-activity or over-expression of Na^+ channels can cause the lungs to be dry, while lower than normal activity can lead to flooding within the alveolar space. Two additional channel proteins critically involved in fluid balance are the cystic fibrosis transmembrane regulator (CFTR) and the calcium activated chloride channels (Fang et al., 2006; Hartzell et al., 2005). These apical membrane mechanisms allow for the outward flux of Cl^- into the alveolar space, serving as a balance for the effects of channels such as ENaC. Various stimuli have effects directly and/or indirectly on these channels and transporters, modifying their expression or function. Epithelial Na^+ channel function can be modulated by endogenous (such as epinephrine) and exogenous (albuterol) stimuli (Berdiev et al., 2009; Li and Folkesson, 2006; Morris and Schafer, 2002). The β_2-adrenergic receptor (ADRB2) stimulates ENaC, and previous research has shown increased ENaC expression with

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long-term β_2-agonist exposure (Berthiaume et al., 1999; Minakata et al., 1998). Studies in rats have shown a strong increase in fluid clearance from the alveolar space after over-expression of ADRB2. Lung fluid clearance has also been found to be attenuated by agents that block Na^+ channel function pointing to a role for ENaC in the clearance process (Dumasius et al., 2001; Ma et al., 2000).

Each of the three ENaC subunits (α, β, γ) has been found to exhibit genetic variants in the form of single nucleotide polymorphisms (SNPs). The effects of these alterations in the protein have most commonly been studied in the kidney, where the renin–angiotensin–aldosterone system plays a critical role in Na^+ and fluid regulation through ENaC and other channel proteins. Polymorphisms increasing the function of ENaC, primarily those in β and γ ENaC subunits, have been associated with inheritable forms of hypertension, such as Liddle's syndrome, a form of salt sensitive hypertension (Azad et al., 2009; Snyder, 2000). The alpha subunit of ENaC shows an especially significant effect on channel function when altered, particularly with respect to the pulmonary epithelium (Tong et al., 2006). Within the alpha subunit, the T663 variant has been associated with an increase in overall ENaC function when compared with the A663 homozygous variant (Samaha et al., 2004; Tong et al., 2006) although others have shown little to no functional effect from the mutation (Ambrosius et al., 1999). One limitation of much of the previous work is that it has been primarily performed in cultured cells. There are very few functional human studies exploring the phenotypic consequences of genetic variation of the alpha subunit of ENaC, beyond those that demonstrate susceptibility to hypertension. Previous investigators have theorized that the increase in function in the T663 variants observed in previous studies are likely due to a higher number of active Na^+ channels on the apical membrane because of increased insertion or retention of the channels in the membrane, or by increasing the activity of closed channels (Samaha et al., 2004). The overactive T663 group may have an increased response to exogenous stimuli due to overall individual channel activity, or according to these previous theories a diminished response to stimuli will occur as levels of ENaC expression and activity may be maximally reached by the SNP variant alone.

No published studies, to date, have investigated the relationship between exhaled Na^+ or lung diffusion (DLCO, D_M, Vc) and genetic variation of ENaC in humans. β_2-Adrenergic stimulation is known to cause an increase in expression of the ENaC protein leading to a greater number of available channel units, a more active channel (possibly due to an increase in the time the channel spends in its open position), or both (Berthiaume et al., 1999; Minakata et al., 1998). Here, we sought to determine the influence of genetic variation in the alpha subunit of ENaC at amino acid position 663, on exhaled Na^+ in healthy individuals prior to, and following, administration of nebulized albuterol, a common inhaled β_2-agonist. It is important to note that the direct measurement of ENaC activity was not part of this study and is outside of the scope of the study. We used a surrogate marker, exhaled sodium in exhaled breath condensate (EBC) fluid, to demonstrate the ultimate effect of increased ENaC activity, which is the clearance of sodium and water from the fluid lining the lower airway. Based on cell work, we predicted that those with at least one allele encoding the T663 variant (more functional in cell work) would show decreased exhaled Na^+ in response to a β-agonist as compared to those homozygous for the wild type (A663) variant.

2. Methods

2.1. Subjects

Thirty-one healthy subjects between the ages of 18 and 45 were recruited to participate in the study. The protocol was reviewed and approved by the University of Arizona Institutional Review Board, with all subjects providing written informed consent prior to participation, and all aspects of the study conformed to the Declaration of Helsinki. Following genotyping, subjects were stratified into two groups based on SCNN1A genotype where individuals homozygous for SCNN1A resulting in alanine at amino acid 663 were classified as AA and individuals with at least allele resulting in threonine at amino acid 663 were classified as AT/TT.

2.2. Protocol

The subjects were asked to come to the laboratory in a fasted state, were instructed not to use caffeine, and were not taking any medications that could influence the response to albuterol (β-agonist, β-blocker). Subjects had a 20-gauge venous catheter inserted into a prominent antecubital vein for multiple blood sampling to assess hemoglobin. Prior to the administration of albuterol and 30, 60 and 90 min post-nebulization, exhaled breath condensate (EBC) was collected for 25 min on the Jaeger EcoScreen (Cardinal Health, Yorba Linda, CA). During each collection, subjects were seated wearing a nose clip while performing calm tidal breathing. A blood sample was taken at the midway point of the EBC collection. Following each EBC collection, measurement of diffusion capacity of the lungs for carbon monoxide and nitric oxide (DLCO/DLNO) was assessed in triplicate. Subjects received albuterol (2.5 mg diluted in 3 ml normal saline) via nebulization using a Power Neb2 nebulizer (Drive Medical, Port Washington, NY). Albuterol administration was conducted with subjects seated and quietly breathing while wearing a nose piece until all liquid had been nebulized, typically 10–12 min. During administration subjects were also instructed to take a deep breath every 2 min to allow for dispersal of the nebulized particles into the lower airways. Once this was completed subjects rested for 10 min to allow the drug to take effect.

2.3. Determination of ENaC genetic polymorphism

Buccal swabs were collected from the inside of each cheek and placed into a stabilizing solution for storage. Samples were sent to the University of Arizona Genetics Core for genotyping of the ENaC amino acid at position 663 of both alleles using a Taqman SNP assay for rs#2228576 (Applied Biosystems, Carlsbad, CA). Initial DNA quantitation and QC was performed using PicoGreen (Life Technologies). Pre-validated primers and probe sets for TaqMan Allelic Discrimination Assay were obtained from Life Technologies. Reactions were run at 10 μl, containing TaqMan Universal PCR Master Mix, No AmpEraseR UNG (Life Technologies), 10 ng total DNA, and 1× Assay Mix. All samples processed and analyzed on 7900 Real-Time PCR System (Life Technologies) with cycling conditions (95 °C for 10 min, 50 cycles of 92 °C for 15 s and 60 °C for 1 min) and Genotyper software (SDS system, version 2.3).

2.4. Quantification of serum hemoglobin and ion concentrations

From the 12 ml venous blood sample at the midway point of the exhaled breath collection (10–12 min), hemoglobin was measured by a cyanide-free hemoglobin method on an ADVIA 2120 Hematology system. Serum Na^+ concentrations were determined from the same venous blood sample using ion-selective electrodes at the University of Arizona Medical Center Pathology Laboratory.

2.5. Collection of exhaled breath condensate

The condensing system used in the present study was the Jaeger Ecoscreen cooling unit described previously (Wheatley et al., 2010). Briefly, sample collection cups were screwed onto the bottom of the