Nasal potential difference to detect Na$^+$ channel dysfunction in acute lung injury

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Mac Sweeney R, Fischer H, McAuley DF. Nasal potential difference to detect Na$^+$ channel dysfunction in acute lung injury. Am J Physiol Lung Cell Mol Physiol 300: L305–L318, 2011. First published November 26, 2010; doi:10.1152/ajplung.00223.2010.— Pulmonary fluid clearance is regulated by the active transport of Na$^+$ and Cl$^-$ through respiratory epithelial ion channels. Ion channel dysfunction contributes to the pathogenesis of various pulmonary fluid disorders including high-altitude pulmonary edema (HAPE) and neonatal respiratory distress syndrome (RDS). Nasal potential difference (NPD) measurement allows an in vivo investigation of the functionality of these channels. This technique has been used for the diagnosis of cystic fibrosis, the archetypal respiratory ion channel disorder, for over a quarter of a century. NPD measurements in HAPE and RDS suggest constitutive and acquired dysfunction of respiratory epithelial Na$^+$ channels. Acute lung injury (ALI) is characterized by pulmonary edema due to alveolar epithelial-interstitial-endothelial injury. NPD measurement may enable identification of critically ill ALI patients with a susceptible phenotype of dysfunctional respiratory Na$^+$ channels and allow targeted therapy toward Na$^+$ channel function.

acute respiratory distress syndrome; ENaC; sodium channel

PROPER REGULATION AND MAINTENANCE of pulmonary fluid balance is crucial to health. In utero, the lungs are net fluid secretors with the volume of fluid being vital for optimal lung growth (100). Pulmonary hypoplasia occurs when too little fluid is produced, such as in oligohydramnios (143), whereas excess fluid produces pulmonary hyperplasia (144, 234). Importantly, fetal fluid secretion is under the control of Cl$^-$ excess fluid produces pulmonary hyperplasia (144, 234). Importantly, fetal fluid secretion is under the control of Cl$^-$ secretion by the respiratory epithelium (133–134). At birth the sudden dependency on pulmonary gas exchange requires a dramatic change in lung fluid dynamics. As gaseous exchange requires a relatively dry alveolus the respiratory epithelium transforms to a net fluid absorber in a remarkably short space of time. In the few days before birth the lungs begin to produce less fluid, and during labor the physical passage of the fetus through the birth canal forces fluid from the lungs (20). Simultaneous increases in fetal sympathetic output and catecholamine levels help activate alveolar Na$^+$ channels necessary for fluid absorption (29, 169). Additionally, the manifold increase in fetal oxygenation ex utero further stimulates Na$^+$ channels (9, 172).

After birth, a small volume of airway surface liquid (ASL) is required for normal airway function (i.e., microbial killing and clearance). The ASL consists of a periciliary layer, in which the cilia beat, of ~7 μm height (215) and an overlying mucus blanket of variable volume (dependent on the amount of mucus produced). Excessive ASL volume interferes with airway function, which most notably is observed in cystic fibrosis (CF), resulting in airway narrowing and blockage (185).

Optimal alveolar gas exchange is dependent on an even thinner alveolar lining fluid (ALF) of 0.1–0.2 μm height (15). The ALF consists of an analogous dual layer, with surfactant covering an aqueous subphase. ALF even the air-liquid interface, enables surfactant precursors to reach the surfactant layer, and allows movement of surfactant within this layer. Pulmonary edema results in fluid deposition in the alveolar region. The resolution of pulmonary edema is vitally dependent on the active absorption of Na$^+$ and Cl$^-$ from the alveolar air space into the interstitium creating an osmotic gradient for the movement of water out of the alveolar air space (150). From the interstitium, water is cleared by the lymphatic system (107, 229).

Acute lung injury (ALI) is a form of increased permeability pulmonary edema seen in critically ill patients. It is a significant problem, with a yearly incidence of 86 per 100,000 person-years, translating to 190,600 cases per year in the USA (186). Mortality rates range from 20–60% (30, 130) and an estimated 74,500 Americans die from this condition annually (186). Survivors suffer from muscle wasting, weakness, fatigue, pulmonary dysfunction, cognitive disability, and affecting disorders, and just 50% return to work (5, 86).

ALI results from an inflammatory injury to the alveolar epithelial-interstitial-endothelial complex (177) caused by either a pulmonary or extrapulmonary insult (1, 16). The neu-
trophil-mediated disruption of this physical barrier causes increased permeability pulmonary edema (230). Alveolar flooding is dependent on the balance of pulmonary edema formation and clearance (194).

Dysfunction of any of the components needed for alveolar fluid clearance (AFC) can predispose to the development of pulmonary edema. This review will evaluate the evidence for a spectrum of Na⁺ channel function, with decreased Na⁺ channel function predisposing to both the development of, and a worse outcome from, ALI. Additionally, we review the possible role nasal potential difference (NPD) measurement could play in the identification of this susceptible phenotype and suggest implications if this hypothesis is correct.

Alveolar Epithelial Cell Types

The alveolar epithelium constitutes ~99% of the internal surface area of the lung (45) and is one of the tightest epithelia in the body forming transepithelial resistances of >2,000 Ω·cm² (71, 117). It is ~0.1–0.2 μm thick and is composed of two cell types: large, squamous type 1 alveolar cells (AT1) with a diameter of 50–100 μm (44) and smaller cuboidal type 2 alveolar cells (AT2) with a diameter of 10 μm (150). Alveoli are ~250 μm in diameter (233) and at its thinnest the alveolar interstitium consists of only a fused basement membrane between the epithelial and endothelial layers (207). Although AT1 cells compose 66% of the cell population of the alveolus, because of their large size they constitute >95% of the alveolar surface (207). Alveolar epithelial cells form tight junctions, which represent a barrier between the functionally different apical and basolateral cell membranes with specifically localized ion channels and pumps (195). This functional division of apical and basolateral membranes is a prerequisite for vectorial transport of ions and water across the alveolar epithelial layer.

Major Alveolar Channels, Transporters, and Receptors

Na⁺ channels. Apical membrane Na⁺ channels are expressed in the alveolar epithelium and contribute to Na⁺ absorption (13, 72) (see Fig. 1). Two main classes of Na⁺ channels have been found: the epithelial Na⁺ channel (ENaC) and the cyclic nucleotide-gated channel (CNG).

Three subtypes of ENaC have been described on the basis of the subunit composition: a highly selective cation channel (HSC) that is usually referred to as the ENaC channel; two types of poorly selective cation channel (PSC), types 1 and 2, differentiated by their unit conductance; and a nonselective cation channel (NSC) (56, 125). ENaC channels are present in the apical membranes of both AT1 and AT2 cells (91). HSC is a heterotrimeric channel composed of α, β, and γ-ENaC.
Table 1. Activators and inhibitors of ion channels and pumps

<table>
<thead>
<tr>
<th>Activator</th>
<th>Inhibitor</th>
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<tr>
<td>ENaC</td>
<td>Amiloride</td>
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<tr>
<td>CNG</td>
<td>Pimozide</td>
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<tr>
<td>CFTR</td>
<td>Isoprenaline</td>
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<tr>
<td>Na⁺⁺K⁺-ATPase</td>
<td>Ouabain</td>
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ENaC, epithelial Na⁺ channel; CNG, cyclic nucleotide gated channel; CFTR, cystic fibrosis transmembrane conductance regulator.

subunits (33). It has a unit conductance of 4–5 picoSiemens (pS; a measure of the ease of movement of ions through the ion channel) and a Na⁺/K⁺ selectivity of >40 (91, 125). PSC is composed of a combination of α- with either β- or γ–ENaC subunits and has a Na⁺/K⁺ selectivity of 5–8, with type 1 PSCs having unit conductance of 8–9 pS and type 2 PSCs a unit conductance of 56 pS (56, 125). NSC is composed solely of α–ENaC subunits (33), has a Na⁺/K⁺ selectivity of 1.5 and a unit conductance of 19–24 pS (91). Efficient Na⁺ transport requires all three subunits (33, 138). HSC, PSC, and NSC channels are inhibitable by amiloride (Table 1) (125).

One isoform of the CNG channel, CNG1, is present in the distal airway and AT1 cells (54) and is likely to be involved in distal airway and alveolar fluid balance (93, 97, 196). This channel is nonselective, amiloride insensitive, but pimozide sensitive and has a unit conductance of 2–8 pS (91). Other Na⁺⁻ dependent cotransporters, such as glucose or amino acid cotransporters, may contribute to amiloride-insensitive Na⁺ absorption (14, 52, 212). In addition, atypical ENaC channels, composed of different subunit stoichiometries, rendering them potentially insensitive to amiloride, could also contribute to amiloride-insensitive Na⁺ transport (165, 178).

$Cl^-$ channels. The cystic fibrosis transmembrane conductance regulator (CFTR) is a CAMP-regulated amiloride-sensitive $Cl^-$ channel expressed in both AT1 and AT2 cells (28, 91, 153). Alveolar CFTR has a unit conductance of ~4 pS (AT1, similar to airway CFTR) to 8 pS (AT2) and can be stimulated by forskolin (91). CFTR consists of two membrane-spanning domains, two nucleotide-binding domains, and a regulatory domain (197). Both airway (213) and alveolar (66) CFTR-mediated apical $Cl^-$ movement may be bidirectional and under the control of adenosine. Although the absence of CFTR does not hinder basal alveolar fluid balance, it is necessary for maximal AFC during β-agonist therapy (167). CFTR activation increases amiloride-sensitive Na⁺ absorption (68, 181), suggesting that both Na⁺ and $Cl^-$ transport determine alveolar fluid absorption (69, 147). The exact interaction between CFTR and ENaC remains to be determined.

K⁺ channels. Respiratory epithelial K⁺ channels mediate a diverse range of physiological functions including oxygen sensing, inflammation, and epithelial repair (8). The primary role of these channels is to regulate the cell membrane potential to allow maintenance of the transepithelial driving force for ion movement and subsequent regulation of the airway and alveolar liquid layers (8). K⁺ channels have been localized to the apical and the basolateral membrane where they serve differing functions (167). Apical K⁺ channels support K⁺ secretion into the ALF resulting in the relatively high K⁺ concentration of the ALF (59). In contrast, basolateral K⁺ channels support recycling of K⁺ across the basolateral membrane in support of the Na⁺⁻K⁺-ATPase (see Na⁺⁺K⁺-ATPase) and to hyperpolarize the cell. Classes of K⁺ channels found in alveolar cells include inwardly rectifying K⁺ channels (K⁺ ir), Ca²⁺-activated K⁺ channels, and voltage-gated K⁺ channels (K⁺ v) (167). K⁺ ir channels have been localized to the basolateral membrane, and the α-subunit of the K⁺ v channel has been localized to the apical membrane. A barium-sensitive K⁺ channel with a unit conductance of ~5–6 pS has been identified in the apical membrane of AT1 (91).

ATP-sensitive K⁺ channels (K⁺ATP) have also been described in the alveolus. Inhibition of K⁺ATP reduced amiloride-sensitive Na⁺ currents and forskolin-stimulated Cl⁻ currents in AT2, whereas K⁺ATP activation increased both ion fluxes (110–111). Similarly, ENaC and CFTR expression responded to K⁺ATP modification, with increased expression occurring with K⁺ATP activation and decreased expression seen with K⁺ATP inhibition (111). The interplay between K⁺, ENaC, and CFTR provides further evidence for the complexity of the relationship between epithelial ion channels.

$Na^+-K^+\text{-ATPase.}$ The Na⁺⁻K⁺-ATPase is ubiquitously expressed and is the primary active transport process that generates the gradients necessary for epithelial Na⁺ absorption. It is a heterodimeric protein composed of an α- and a β-subunit and is located in the basolateral membrane of AT1 (91–92) and AT2 (91, 168) cells. The α-subunit allows exchange of three intracellular Na⁺ for two extracellular K⁺ during ATP hydrolysis (198). The β-subunit is required for protein assembly and insertion into the cell membrane (136). Four α- and three β-subunits exist and different associations of an α- and a β-subunit, plus different posttranscriptional processing, creates a range of Na⁺⁻K⁺-ATPase isoenzymes with different functional characteristics (19). AT1 cells contain α₁-, α₂-, and β₁-subunits, whereas AT2 cells express α₁- and β₁-subunits (92, 182).

The regulation of Na⁺⁻K⁺-ATPase and ENaC occurs in parallel, with increased apical Na⁺ transport being matched by increased Na⁺⁻K⁺-ATPase activity (43). Upregulating stimuli, such as glucocorticoids (47) and catecholamines (141), increase expression and function of both proteins. Likewise, downregulating stimuli, such as hypoxia, reduce levels (237) and function (225) of these membrane transporters.

Water channels. The aquaporins (AQP) are a family of small (30-kDa monomer), integral membrane proteins that function as mercury-sensitive water channels. AQP5 has been located in the apical membrane of both AT1 cells (154) and AT2 cells (127), AQP3 is located in the basolateral membrane of AT2 cells (105), and AQP1 is located in microvascular endothelia. AQP4 is also probably present in AT1 cells (105). Water permeability is required in both apical and basolateral membranes for efficient transcellular water transport, although paracellular water transport also occurs. AQP5s increase the water permeability of epithelial membranes by 5- to 10-fold (23) with AT1 cells having the highest water permeability of any mammalian cell (55). Murine alveolar osmotic water permeability is reduced ~10-fold by AQP1 or AQP5 deletion (6), and >30-fold reduced by combined AQP1/AQP5 deletion (119). Active, near-isosmolar
alveolar fluid clearance (AFC) was unaffected, however. Similarly, AQP deletion did not affect the rate of AFC in newborn mice or mice with experimentally induced pulmonary edema including ALI (203), suggesting that other pathways for water exist. AQPzs do not seem to play a major role in the maintenance of the ASL (204). The lack of effect of AQP deletion has been suggested to be due to the slower rate of active fluid absorption in the lung compared with organs such as the renal proximal tubule or the salivary gland (131). It appears that AQPs are not required for physiologically relevant functions in pulmonary fluid balance (224).

**β-Adrenergic receptors.** Four β-adrenergic receptor subtypes exist (149), with over 90% of all pulmonary β-receptors being located in the alveoli, predominantly in the form of the β2-receptor (34). Both β1- and β2-receptors are expressed on the cell membranes of both AT1 (114) and AT2 (64) cells. β-Stimulation may increase the activity of ENaC or Na+/K+-ATPase, improve pulmonary lymphatic flow (201), or enhance K+ channel activity appears to play a role in AFC. The functional significance of K+ATP was suggested by pharmacological stimulation in human lungs (190). In that study, activation of KATP channels resulted in increased K+ secretion into the alveolar space, suggesting a role in ALF regulation.

The contribution of Na+/K+-ATPase to AFC was confirmed with its specific inhibitor ouabain in animal perfused lung preparations (81). In the ex vivo human lung ouabain reduced AFC by almost 50% (187–188). Similarly, overexpression of either the β1- or α2-subunit of Na+/K+-ATPase increases AFC (65, 182, 217). The exact role of Na+/K+-ATPase inhibition required to impair AFC is unknown. Neither the α1- nor α2-subunits function near their maximal capacity under normal conditions (118, 232). Mice that are 50% protein deficient in both α1- and α2-subunits have a submaximal response to stimulated AFC, although a normal basal rate. This suggests a partial impairment of the Na+/K+-ATPase may not be a rate-limiting factor for Na+ transport and correspondingly AFC.

**Mechanism of alveolar Na+ transport and fluid absorption.** See Fig. 1; numbered paragraphs below refer to circled numbers in the figure.

1) The Na+/K+-ATPase pump exchanges intracellular Na+ for extracellular K+. The activity of the Na+/K+-ATPase hyperpolarizes the basolateral membrane potential and establishes an outward K+ and an inward Na+ gradient.

2) Intracellular K+ follows its chemical gradient across basolateral K+ channels, which hyperpolarizes the basolateral membrane. In an epithelial setting, basolateral hyperpolarization results in apical hyperpolarization by electrical coupling of the two membranes across the tight junctions.

3) This creates a large electrochemical gradient for entry of luminal Na+ through the apical membrane Na+ channels, including HSC, PSC, NSC, and CNG channels.

4) The resulting Na+ absorption depolarizes the apical membrane potential to allow for Cl− entry across CFTR.

5) The osmotic gradient created by the movement of Na+ and Cl− causes water to move from the air space to the interstitium both transeellularly and paracellularly.
6) $\text{Cl}^-$ leaves the basolateral membrane through an as yet undetermined pathway thought to be a $\text{Cl}^-/K^+$ cotransporter to maintain electroneutrality (91).

7) $K^+$ secretion through the apical membrane enables maintenance of the transepithelial electrochemical gradient needed for ion and water movement.

8) AFC may be upregulated in a catecholamine-dependent or independent fashion (239). $\text{Na}^+$ movement occurs by using the same $\text{Na}^+-\text{K}^+-\text{ATPase}$ driven mechanism, but in an upregulated manner. The exact means of upregulation remains unclear but several theories have been suggested (65). The upregulation may be primarily due to an increase in $\text{Na}^+$ absorption (80) caused by an increase in any of the following: ENaC delivery to the apical membrane (32), ENaC open probability (124, 212), $\text{Na}^+-\text{K}^+-\text{ATPase}$ delivery to the basolateral membrane (17), $\text{Na}^+-\text{K}^+-\text{ATPase}$ $\alpha$-phosphorylation (109) and $\text{Na}^+-\text{K}^+-\text{ATPase}$ activity (212). In addition, the increase, in AFC may be mediated through CFTR and $\text{Cl}^-$ conductance. Increased $\text{Cl}^-$ movement may be required electrically to initiate or maintain increased $\text{Na}^+$ movement (90, 99, 166). Studies utilizing CFTR knockout mice have demonstrated an inhibition of upregulated AFC (69–70). The exact role of CFTR and ENaC in AFC remains uncertain, but there are indications that both are required for efficient water clearance.

**ALI as an Ion Channelopathy**

It is intuitive that a disorder of any of the components necessary for the regulation of the respiratory tract surface liquid layer could be implicated in disorders of pulmonary fluid balance. Decreased expression of ENaC has been reported in experimental models of ALI including bleomycin-induced injury (73), viral pneumonia (218), and canine ischemia-reperfusion injury (210). The ability to maintain a maximal or submaximal rate of AFC (129, 231), and similarly a smaller magnitude of extravascular lung water (42), has been associated with improved survival in ALI. Although this varying ability to resorb edema fluid could represent differing degrees of alveolar injury, it is also possible that it could represent underlying $\text{Na}^+$ channel function, with those with the most functional $\text{Na}^+$ channels having the best outcome. Cardiogenic pulmonary edema serves as a useful comparative model as it is a condition with much less physical injury to the alveolus (41, 53). Patients with this condition who maintain an intact rate of AFC also have superior outcomes to those who do not (222). This suggests that rates of AFC in the critically ill may depend on more than just the degree of alveolar epithelial injury.

Consistent with this theory of a spectrum of $\text{Na}^+$ channel activity being associated with abnormal pulmonary fluid handling, genetic variation in the $\beta_2$-adrenergic receptor has been associated with a susceptibility to the development of pulmonary edema (201). A large body of evidence exists linking $\text{Na}^+$ channel activity with pulmonary edema (see *Nasal Potential Difference as a Surrogate Measure of Alveolar Ion Channel Function*).

**Potential Difference as a Measure of Ion Channel Function**

The movement of ions across epithelia results in a transepithelial voltage, or potential difference (PD). The transepithelial PD is determined by the sum of apical and basolateral membrane PDs. As transepithelial conductances or ion gradients change, the PD changes accordingly. Placing electrodes on both sides of an epithelial membrane allows this PD to be measured via a high-impedance voltmeter. Topically perfusing the apical membrane with compounds to activate or inhibit ion channels allows the functionality of these channels to be investigated.

Respiratory epithelial PD has been measured throughout the respiratory tract, including nose (104), trachea (103), bronchi (50, 103), and alveolus (157–159). Although the alveolus is the site of interest, the measurement of alveolar PD is highly invasive, requiring alveolar micro puncture with microelectrode insertion, and to our knowledge has not been performed in humans. Lower airway PD measurement is also invasive requiring bronchoscopy.

NPD measurement is easily performed in humans, is well tolerated, and has been shown to correlate closely with the PD found in the distal respiratory epithelium (101, 103). NPD has been used as a diagnostic technique in cystic fibrosis for over 25 years. The basal NPD is largely determined by the potential generated by epithelial $\text{Na}^+$ absorption (104). Initial studies established the $\text{Na}^+$ dependence and amiloride sensitivity of NPD (104). Later, Knowles and colleagues (102) employed a simple modification of the perfusate to a $\text{Cl}^-$ free solution to establish a $\text{Cl}^-$ gradient across the epithelium. This allowed a measure of $\text{Cl}^-$-selective NPD, which was a key approach to study the defective $\text{Cl}^-$ conductance in CF.

Over the years, and across the globe, the technique has been refined by local investigators to suit their needs and preferences; however, no common standard approach for NPD has been established (2, 202). Large variation in the multiple components of the NPD circuit (NPD catheter, solutions, electrodes, bridges, voltmeters, etc.), the technique of measuring NPD (location in the nose, method of passage and fixation of catheter, solution flow rate and temperature, etc.) and methods of scoring readings have made comparisons between studies less clear. At present NPD has been optimized for the measurement of $\text{Cl}^-$ transport in a relatively healthy outpatient population for the purpose of diagnosing cystic fibrosis. The optimal method of measuring baseline NPD, and thus $\text{Na}^+$ channel function, has been a secondary consideration in the CF literature. However, a few studies have examined the effects of components of the measurement technique on baseline/basal NPD. Baseline/basal NPD as measured on the floor of the nose is similar to that measured under the inferior turbinate (4). Room temperature solutions are equivalent to body temperature solutions (27), and ECG cream is similar to agar for completing the electrical circuit (202).

Here we describe a generic technique to measure $\text{Na}^+$ transport (Fig. 2). Measurement of NPD involves the placement of a double-lumen catheter, which acts as the measuring (luminal) electrode, in the nose. A reference (electrically central luminal) electrode is placed either subcutaneously (using an electrolyte/agar-filled needle) or over an area of abraded skin, typically on the forearm (3). Abrading the skin breaks the epithelial skin barrier and short-circuits the skin potential, allowing electrical contact to the subepithelial space. The measuring electrode catheter is placed either under the inferior turbinate (104) or along the floor of the nose (4), and the site of greatest PD is sought. The catheter is secured at this site. Different solutions are infused through the double lumen catheter to activate or inhibit specific ion channels and enable in
vivo ion channel function to be investigated. For Na$^+$ transport, the following measurements can be made (see Fig. 3).

Baseline NPD. This is a composite value of all ion transport but is predominantly reflective of Na$^+$ transport.

Amiloride infusion. Amiloride (0.1 mM) blocks the ENaC class (HSC, PSC, NSC) of Na$^+$ channels, leading to luminal Na$^+$ retention and a fall in NPD (due to the accumulation of the positively charged Na$^+$ cation making a negative PD less negative).

Nasal Potential Difference as a Surrogate Measure of Alveolar Ion Channel Function

There are several reasons why NPD potentially could serve as a noninvasive, surrogate measure of alveolar ion channel function.

Firstly, the epithelium of the respiratory tract has a continuous function: to regulate the depth of the overlying fluid layer (8). At baseline each nostril contains 800 µl fluid (94). Human lungs hold 3–7 ml/kg body wt of extravascular water (98) including 20–30 ml of ALF (31). The mechanism of nasal (200), airway (205), and alveolar (43, 116) fluid regulation is largely the same: the modification of transepithelial Na$^+$ retention and a fall in NPD (due to the accumulation of the positively charged Na$^+$ cation making a negative PD less negative).

Secondly, indirect evidence for the utility of NPD as a surrogate measure of more distal ion channel function comes from human studies of disorders of the airway liquid layer. CF is the archetypal respiratory ion channel disorder. It is a genetic disease of CFTR with autosomal recessive inheritance. To date over 1,600 mutations have been described (49), leading to varying amounts of the CFTR gene being expressed and CFTR protein being produced, assembled, trafficked to the cell membrane, inserted, and functioning correctly (185). The exact pathophysiology of CF remains debated; however, the low ASL volume theory proposes that CFTR dysfunction causes excessive airway Na$^+$ resorption via ENaC (126) and an inability to secrete Cl$^-$ to increase ASL height when necessary (213). This result in decreased ASL causing a dry, inspissated mucus layer, which hinders airway clearance and promotes a cycle of repeated airway infection, bronchial damage, and the development of bronchiectasis. NPD readings from patients with CF typically demonstrate a large baseline NPD [approximately −45 mV (236) to −70 mV (101)], a large amiloride-sensitive fraction, and a lack of response to low Cl$^-$ and isoprenaline perfusion. This identifies Na$^+$ hyperabsorption, an inability of CFTR to allow Cl$^-$ movement, and the interdependency of ENaC and CFTR function. CF patients with

![Fig. 2. Measurement of nasal potential difference (NPD).](image)

![Fig. 3. Typical NPD tracing from a healthy volunteer (see text for details). 1 = Perfusion with Ringers solution; 2 = perfusion with amiloride solution; vertical line indicates onset of amiloride perfusion.](image)

![Fig. 4. Schematic representation of respiratory potential differences with associated height of respiratory liquid layers. Data adapted from Refs. 33, 81, 84, 127, 129.](image)
lower, more normal NPDs have a milder phenotype than those with higher NPDs (67).

Systemic pseudohypoaldosteronism is a rare autosomal recessive disorder of ENaC caused by loss-of-function mutations (35, 209). Excessive ASL occurs due to an inability to absorb Na+ and thus water from the air space (97). This is partly compensated for by increased removal via mucociliary clearance. The excess air space fluid causes luminal narrowing, wheezing and repeated pulmonary infection (40, 238). Baseline NPD is approximately one-third that of healthy controls and not affected by amiloride, reflecting abnormal ENaC-mediated Na+ absorption. In addition, nasal surface liquid Na+ concentration is increased and liquid volume more than doubled compared with healthy volunteers (97).

Thirdly, and most importantly, further indirect evidence comes from human studies associating abnormal NPD with alveolar dysfunction in the form of pulmonary edema. Neonatal RDS is a form of pulmonary edema suffered by premature babies. Both surfactant and ENaC subunit (216, 226) production are dependent on gestational age, and if birth occurs before sufficient production has occurred respiratory dysfunction is likely. RDS occurs with a deficiency of both surfactant and ENaC, whereas transient tachypnea of the newborn (TTN) occurs with ENaC deficiency alone (63). Premature babies with RDS have lower mRNA levels of all three ENaC subunits than premature babies without RDS (85). Similarly, premature babies with RDS have lower NPDs than otherwise healthy premature babies (10). As these babies grow older their NPD increases in keeping with enhanced ENaC production. Dexamethasone upregulates ENaC expression (216) and pretreatment for the premature fetus decreases the incidence of RDS (115), albeit via numerous mechanisms (7). Babies with TTN have a lower amiloride-sensitive fraction of NPD than healthy babies, with this value recovering to normal after 3 days (83). Interestingly, newborn babies’ NPD varies with mode of delivery (83).

High-altitude pulmonary edema (HAPE) is a form of pulmonary edema typically suffered at altitudes above 2,500 m. The pathophysiology is complex, consisting of excessive hypoxic pulmonary vasoconstriction, endothelial stress failure, excessive inflammation, nitric oxide dysfunction, and, importantly, impaired amiloride-sensitive Na+ transport (12). When measured at low altitude, mountaineers prone to the development of HAPE have significantly reduced baseline NPD compared with those resistant to the condition (192). This reduction was due to the amiloride-sensitive fraction of Na+ transport, localizing the defective Na+ movement to the ENaC class of Na+ channels. Pretreating this group of HAPE prone mountaineers with salmeterol, a β2-agonist known to upregulate respiratory Na+ transport, more than halved the incidence of HAPE upon reexposure to high altitude. When HAPE prone and resistant mountaineers underwent NPD measurement at high altitude, both groups had reduced baseline NPDs compared with their low-altitude values. This decrease was due to impaired amiloride-insensitive Na+ transport and localized the defect to CNG channels (193).

These findings suggest that a constitutive defect in the ENaC class of Na+ channels, by interfering with the mechanism of AFC, may predispose certain individuals to the development of pulmonary edema and that an acquired transient defect in CNG channels, perhaps due to an environmental factor such as hypoxia, may compound this constitutive defect and further impair AFC.

Fourthly, there is direct evidence from animal studies that nasal epithelial function may be a surrogate for alveolar epithelium. Transgenic mice, with endogenous murine α-ENaC replaced by partly functional rat α-ENaC, have been used to investigate the possible role of a constitutively impaired ENaC in the generation of pulmonary edema (60). These mice grow normally and appear otherwise healthy until physiologically challenged. The baseline NPD of these mice is reduced by ~50%, with the defect, as expected, being linked to the amiloride-sensitive fraction. Similarly, the rate of AFC is reduced by ~50% compared with wild-type mice. When subjected to experimentally induced lung injury, the transgenic mice are more prone to the development of pulmonary edema, develop a more severe pulmonary edema, and exhibit slower rates of AFC. NPD was also significantly correlated with AFC. Further comparison of NPD and AFC comes from another mouse study investigating the effects of inducible nitric oxide synthase (iNOS), a possible regulator of amiloride-sensitive Na channels, on AFC (84). iNOS(+/-) mice had higher NPD and similar AFC values to iNOS(−/−) mice. In the presence of amiloride both NPD and AFC decreased in the controls but there was little change in the knockout mice, again suggesting that nasal Na+ transport reflects alveolar Na+ transport.

Several factors could limit the relationship between measured NPD and alveolar ion transport, especially in the intensive care unit (ICU). The nasal epithelium is subjected to a different environment than the alveolus. It has been suggested that altered NPDs seen at high altitude may be reflective of a lack of stimulated Cl− secretion at this cold dry climate (121). This is less likely to be of significance in an ICU. Abrasion of the nasal epithelium abolishes NPD (101) and therefore placement of nasogastric and nasotracheal tubes may affect NPD measurements. However, NPD does not vary between nostrils (104), allowing either nostril to be used.

Even relatively mild ischemic tissue injury may lead to loss of the polarized cellular structure affecting Na+ transport, as has been shown for renal tubular cells (221). During severe ALI denudement of the alveolar epithelium may lead to an electrical discordance between a functioning nasal epithelium and a physically damaged, poorly functioning alveolar epithelium. This could be a strength of the procedure, by permitting measurement of the underlying constitutive epithelial function and not losing this signal due to alveolar epithelial injury. Exogenous infusions of catecholamines could potentially upregulate either nasal or alveolar ion transport, or both. To date, no association has been shown between catecholamine therapy for cardiovascular support and rates of AFC in patients with ALI (231). Similarly, anesthesia and sedation could have effects on Na+, Cl−, and K+ channels affecting the applicability of readings in the ICU. Lower airway PD values are the same when measured during general anesthesia or sedation (103). Sedation is not required for the measurement of NPD and is not expected to alter its measurement. Volatile general anesthetics have inconsistent effects on AFC (155, 179). Volatile anesthetics are rarely used as a means of sedation in ICU. In addition, importantly mechanical ventilation does not appear to modify NPD in a mouse model (60).
Modifiers of NPD and AFC in ALI

Although NPD can identify a defect in ion transport, it cannot identify the cause of the defect. A detailed review of the many potential causes of defects in Na\(^+\) transport are outside the scope of this article. However, it is interesting to briefly touch on three factors present in ICU that could affect both NPD and AFC: hypoxia, glucocorticoids, and inflammatory mediators.

The effect of hypoxia on NPD has been variable, perhaps dependent on the setting in which the measurement is made (120). Hypoxia has inhibitory effects on Na\(^+\) transport via the impairment of ENaC and Na\(^+\)-K\(^+\)-ATPase at multiple levels. In hypoxic conditions, alveolar ENaC suffers from reduced mRNA expression (174–175), apical membrane abundance (174–175), and reduced activity (225). Hemoglobin acts as an O\(_2\) sensor and regulates ENaC function, inhibiting the channel during hypoxia (227). Na\(^+\)-K\(^+\)-ATPase is similarly affected at transcription (175, 237), translation (237), and posttranslational modification (46, 211).

The specific effects of hypoxia on Cl\(^-\) channel function are poorly understood. Inhibition of alveolar apical membrane Na\(^+\) transport, via amiloride and phloridzin, a blocker of the Na\(^+\)-glucose transporter, causes Cl\(^-\)-driven fluid secretion into the alveolus in the setting of hypoxia (151). This suggests that unopposed Cl\(^-\) secretion generates a gradual accumulation of pulmonary fluid and could reflect the basic blueprint for respiratory ion channel function (Cl\(^-\) secretion in hypoxia, i.e., in utero; Na\(^+\) absorption in normoxia, i.e., ex utero). Although ALI patients are relatively hypoxemic (usually PaO\(_2\) <11 kPa), they are rarely absolutely hypoxemic (PaO\(_2\) <8 kPa) with few (<10%) dying from refractory hypoxemia (145, 206).

Glucocorticoids are often proposed for their anti-inflammatory and antifibrotic effects in ALI/acute RDS (ARDS) (139). The presence of a glucocorticoid response element in the α-ENaC subunit is suggestive of a role in the regulation of alveolar Na\(^+\) transport (38). Inhibition of cortisol synthesis decreases ENaC mRNA and AFC, whereas administration of dexamethasone reverses this effect (161). Dexamethasone modulates expression and activity of both ENaC and Na-K-ATPase (11, 47, 108), with resulting increases in transepithelial current (47), and AFC (74, 160).

Inflammatory mediators are present in virtually all critically ill patients and have been suggested as a possible cause of ALI/ARDS (18, 61, 82). Several mediators have been shown to inhibit AFC, via negative effects on the expression and function of Na\(^+\) transport proteins. Conversely, proinflammatory cytokines may have positive effects on Na\(^+\) transport and edema resolution, making the overall picture difficult to interpret at present.

Reduction of pulmonary Na\(^+\) transport occurs in association with a range of cytokines, including interleukin-1β (184), TGF-β\(_1\) (76), and TNF-α (48). TNF-α can, however, also increase AFC (22, 78, 180). Reactive oxygen and nitrogen species (RONS) similarly diminish Na\(^+\) transport and AFC in the setting of mycoplasma infection (87) and ventilator-induced lung injury (75). Interleukin-4 (IL-4) (79) can lower β\(_1\)- and γ-ENaC subunit expression and amiloride-sensitive current in vitro, while increasing CFTR mRNA, CFTR function, and Cl\(^-\) current, suggesting a net effect of increased fluid secretion (79). Against this, TGF-β1 decreases CFTR gene and protein expression with an associated reduction in nasal Cl\(^-\) transport (176) and cAMP-mediated AFC (183). Non-CFTR Cl\(^-\) channels are also affected (39, 240).

K\(^+\) channel function could also be regulated by inflammation, as has been briefly reviewed by Bardou et al. (8). There is little direct evidence, but bronchial cells change from a Na\(^+\)-absorbing to a Cl\(^-\)-secreting state, along with an increase in SK4 K\(^+\) transport, when subjected to IL-3 (57).

Na\(^+\)-K\(^+\)-ATPase gene expression and function is similarly affected by cytokines, increasing with IL-1β (152), TGF-β\(_1\) (235), and leukotriene D\(_4\) (199) with concomitant elevated rates of ion transport and fluid clearance. In contrast, mitochondrial reactive oxygen species downregulate alveolar Na\(^+\)-K\(^+\)-ATPase function during hypoxia (46). AQPs may equally be affected, with TNF-α, via activation of NF-κB, decreasing aquaporin 5 mRNA and protein expression in murine lung epithelial cells (219). Likewise, β-receptors could be susceptible to effects of both infection and inflammatory mediators. Respiratory syncytial virus directly (146), and indirectly via inflammatory mediators such as CXCR8 (51, 220), induces β\(_2\)-receptor insensitivity. Nitric oxide (173) and neutrophil-mediated oxidant injury (142), generated by severe hemorrhagic shock, also decrease alveolar β-receptor function and β-agonist-mediated upregulation of AFC.

The differences in Na\(^+\) transport shown between diverse models may be due to multiple effectors, not just cytokines, including severity and duration of injury, degree of hypoxia, generation of RONS, and cell or animal model used (149). The dominant influence of inflammatory mediators on Na\(^+\) transport and AFC remains to be conclusively determined (228).

Future Directions

ALI patients capable of maintaining maximal or submaximal AFC, as measured by increasing protein concentration from sequentially aspirated tracheal fluid samples, have increased survival (231). β\(_2\)-Agonists can increase alveolar Na\(^+\) transport (170). Salbutamol, a β\(_2\)-agonist, has been shown to decrease extravascular lung water in ALI when administered intravenously (171) and in a retrospective study was associated with improved outcomes when inhaled (122). Inhaled salbutamol may increase the resolution of pulmonary edema after lung resection (113). The recently completed ALTA study investigating the effect of inhaled albuterol on outcome in ALI reported negative results (130). Similarly, the recently terminated BALTI-2 study, which investigated the effects of intravenous salbutamol in ALI, was stopped for futility (Gao F et al., unpublished at present). With the increasing understanding of the importance of matching treatment to an individual’s phenotype, it is possible that this trial was negative because of an inability to identify those with constitutively dysfunctional Na\(^+\) channels who would benefit from pharmacological upregulation. To date, Na\(^+\) channel function, as assessed by NPD, has been studied in pediatric meningococcal-associated pulmonary edema and shown not to be affected (62).

If NPD proves to be a useful technique in the ICU, there are possible avenues for its development. Bedside parameters for the purposes of managing pulmonary edema are currently limited. Many ICUs perform daily chest X-rays, partly with the aim of quantifying the degree of pulmonary edema (123). This quantification is known to be poor (106). Cardiac filling pres-
ures, via measurements of central venous pressure (CVP) and pulmonary artery occlusion pressure (PAOP), are commonly used to avoid hypervolemia and the risk of pulmonary edema. Both CVP and PAOP have been shown to be poor indicators of fluid status in ICU (140). Extravascular lung water can be more accurately measured by the single indicator transpulmonary thermodilution technique, via a PiCCO device (98); however, this remains a largely research tool. NPD could potentially improve on these monitoring techniques, by identifying an individual’s Na\textsuperscript{+} transport phenotype and thus the potential to develop, and recover from, pulmonary edema. Sequential NPD measures could also, in theory, map an individual’s Na\textsuperscript{+} transport ability during critical illness, enabling optimization of both respiratory and hemodynamic therapy. Clearly these potential applications require future prospective investigation before implementation.

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Conclusion

A spectrum of respiratory epithelial Na\textsuperscript{+} channel function may exist and contribute to both the development of, and outcome from, ALI. NPD is an in vivo measure of respiratory epithelial ion channel function. Although it is likely to have limitations, substantial evidence supports the possibility this technique could act as a surrogate measure of alveolar Na\textsuperscript{+} channel function.

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DISCLOSURES

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