# Chapter 60
# Alveolar Epithelial Fluid Transport in Lung Injury

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**Abstract** This chapter discusses the regulation of lung fluid transport by lung epithelial active ion transport mechanisms. Ion transport occurs across both the alveolar and the distal airway epithelium. Both experimental models of pulmonary edema and clinical studies are considered to demonstrate how active $Na^+$ and active $Cl^-$ transport participate and regulate alveolar edema resolution. Some of the material in this chapter has been discussed in recent reviews. For decades, it was believed that Starling forces, i.e. differences in hydrostatic and protein osmotic pressures, accounted for removal of excess air space fluid. This misconception remained partly because experiments measuring solute fluxes across the lung epithelial and endothelial barriers were done at room temperature and the animal species used was the dog, a species that later was demonstrated to have a very low rate of active ion and fluid transport. Also, until the late 1970s and early 1980s, there were few good animal models to study the resolution of pulmonary edema. Furthermore, isolation and culture of alveolar epithelial type II cells was just evolving to become a useful experimental technique. Although removal of interstitial pulmonary edema by lung lymphatics and lung microcirculation had been discussed by Staub in his review of pulmonary edema, there was still no information on how pulmonary edema was removed from the distal air spaces of the mature lung.

**Keywords** Lung fluid transport • Ion transportation • Airway epithelium • Pulmonary edema • Vascular permeability

## 1 Introduction

This chapter discusses the regulation of lung fluid transport by lung epithelial active ion transport mechanisms. Ion transport occurs across both the alveolar and the distal airway epithelium. Both experimental models of pulmonary edema and clinical studies are considered to demonstrate how active $Na^+$ and active $Cl^-$ transport participate and regulate alveolar edema resolution. Some of the material in this chapter has been discussed in recent reviews [1, 2].

For decades, it was believed that Starling forces, i.e. differences in hydrostatic and protein osmotic pressures, accounted for removal of excess air space fluid. This misconception remained partly because experiments measuring solute fluxes across the lung epithelial and endothelial barriers were done at room temperature [3] and the animal species used was the dog, a species that later was demonstrated to have a very low rate of active ion and fluid transport [4]. Also, until the late 1970s and early 1980s, there were few good animal models to study the resolution of pulmonary edema. Furthermore, isolation and culture of alveolar epithelial type II cells was just evolving to become a useful experimental technique. Although removal of interstitial pulmonary edema by lung lymphatics and lung microcirculation had been discussed by Staub in his review of pulmonary edema [5], there was still no information on how pulmonary edema was removed from the distal air spaces of the mature lung.

## 2 Lung Fluid Absorption

The human lung consists of a series of highly branched hollow tubes that end blindly in alveoli, with conducting airways (cartilaginous trachea, bronchi, and membranous bronchioles) occupying the first 16 airway generations [6]. Gas exchange occurs in the branches making up the last seven generations, which include respiratory bronchioles, alveolar ducts, alveolar sacs, and alveoli [7]. The airways, approximately 1.4 $m^2$, and alveoli, approximately 143 $m^2$, constitute the interface between the lung internal environment and the external environment and are lined by a continuous epithelium [8]. Distally, the airway epithelium is composed of polarized epithelial cells having the ability to transport $Na^+$ and $Cl^-$, including ciliated Clara cells and nonciliated cuboidal cells. The alveoli themselves are composed of a thin alveolar epithelium (0.1-0.2 μm)

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that covers 99% of the air space surface area in the lung and contains thin, squamous type I cells and cuboidal type II cells [8]. The alveolar type I cell covers 95% of the alveolar surface [8]. The close apposition between the alveolar epithelium and the vascular endothelium facilitates the efficient exchange of gases, but forms a tight barrier to movement of liquid and proteins from the interstitial and vascular spaces, thus assisting in maintaining relatively dry alveoli [9]. The large alveolar surface area favors the idea that most fluid absorption occurs at the alveolar level, although active fluid absorption may occur across all segments of the pulmonary epithelia. The contribution of each anatomic segment of the lung to fluid absorption remains to be established.

The tight junction is another critical structure for the barrier function of the alveolar epithelium. This junction connects adjacent epithelial cells near their apical surfaces, thereby maintaining apical and basolateral cell polarity [10]. Ion transporters are asymmetrically distributed on opposing cell surfaces, conferring vectorial transport properties to the epithelium. It was originally thought that tight junctions were rigid structures, physically restricting passage of larger molecules. However, the permeability of tight junctions is dynamic and regulated by cytoskeletal proteins and intracellular $Ca^{2+}$ concentration [10]. Studies of tight junctions in the alveolar epithelium indicate that diffusion of water-soluble solutes between alveolar epithelial cells is much slower than through the intercellular junctions of the adjacent lung capillary endothelium [5, 11, 12]. In addition, studies of protein flux across the endothelial–epithelial barrier of the sheep lung suggested that 92% of the resistance to albumin flux resides in the epithelium [13]. Removal of large quantities of soluble protein from the air spaces appears to occur primarily by restricted diffusion [14, 15], although there is evidence for endocytosis and transcytosis of albumin across alveolar epithelium [14–18].

The general model for transepithelial fluid movement is that active salt transport drives osmotic water transport. This paradigm has proven to be true for fluid absorption from the distal airs paces on the basis of several animal and human studies [19]. The results of in vivo studies demonstrated that changes in hydrostatic or protein osmotic pressures cannot account for removal of excess fluid from the distal air spaces [20–23]. Pharmacologic inhibitors of $Na^+$ transport also reduce the rate of fluid absorption in the lungs [4, 24–26]. Additionally, there is good evidence that isolated alveolar epithelial cells actively transport $Na^+$ and other ions [27–29].

Multiple preparations have been used to study fluid and protein transport from the distal air spaces, including isolated perfused lungs, in situ lung perfusions, surface fluorescence, and in vivo lung preparations (30 min to 144 h). The advantages and disadvantages of these preparations have been reviewed in some detail [1, 19].

The initial in vivo evidence of active ion transport that may account for pulmonary edema fluid removal in mature lungs came from anesthetized, ventilated sheep [21, 22]. In those studies, the key discovery was that isosmolar fluid absorption occurred in spite of a rising alveolar protein concentration and protein oncotic pressure over 4-24 h. After 4 and 24 h, air space protein concentration had risen from approximately 6.5–8.4 g/dL, respectively, to 12.9 g/dL. Plasma protein concentration never changed. This rise in protein concentration was equivalent to an increase in air space protein osmotic pressure (oncotic pressure) from 25 to 65 $cmH_2O$. As the epithelial barrier was intact and prevented protein leak and no rapid protein absorption or secretion occured in the lung, the only way that the protein concentration could have increased was if water had been absorbed. This process has since been confirmed in multiple animal species and humans [24–26].

Elimination of ventilation to one lung did not change the rate of alveolar fluid clearance, thus ruling out changes in transpulmonary airway pressure as a major determinant of alveolar fluid clearance [20]. Furthermore, if active ion transport was responsible for alveolar fluid clearance, then alveolar fluid clearance should be temperature-dependent. In fact, in an in situ rat lung preparation, alveolar fluid clearance was inhibited by low temperature [30]. This was also true in an in situ perfused goat lung preparation [31] and in ex vivo human lung studies [26]. Additional evidence for active ion transport was obtained with the use of pharmacologic blockade of $Na^+$ uptake by amiloride, an inhibitor of $Na^+$ uptake by the apical membrane of alveolar and distal airway epithelia. Amiloride inhibits between 40 and 70% of basal alveolar fluid clearance in sheep, rabbit, rat, guinea pig, mouse, and human lungs [1]. To further explore the role of active $Na^+$ transport, experiments were designed to inhibit the $Na^+/K^+$-ATPase. This turned out to be more challenging in vivo since ouabain, the commonly used $Na^+/K^+$-ATPase blocker, is cardiotoxic. However, in isolated rat lungs, ouabain inhibits more than 90% of alveolar fluid clearance [32]. Following the development of an in situ sheep preparation for measuring alveolar fluid clearance, it was reported that ouabain inhibited more than 90% of alveolar fluid clearance [20].

## 3 Ion Transport in Alveolar and Distal Airway Epithelia

The success in isolating and culturing alveolar epithelial type II cells was pivotal to the researchers' success in studying the transport properties of these cells. Initial studies showed that when alveolar epithelial type II cells were grown on a nonporous surface such as plastic, they formed a continuous confluent layer of polarized cells [33, 34]. Then, after 3–5 days, small domes of fluid were observed where the substratum was detached. These domes were later shown to form from active ion transport from the apical to the basal surface, because they were inhibited by both the replacement of $Na^+$ by another cation and pharmacologic inhibitors of $Na^+$ transport, such as amiloride

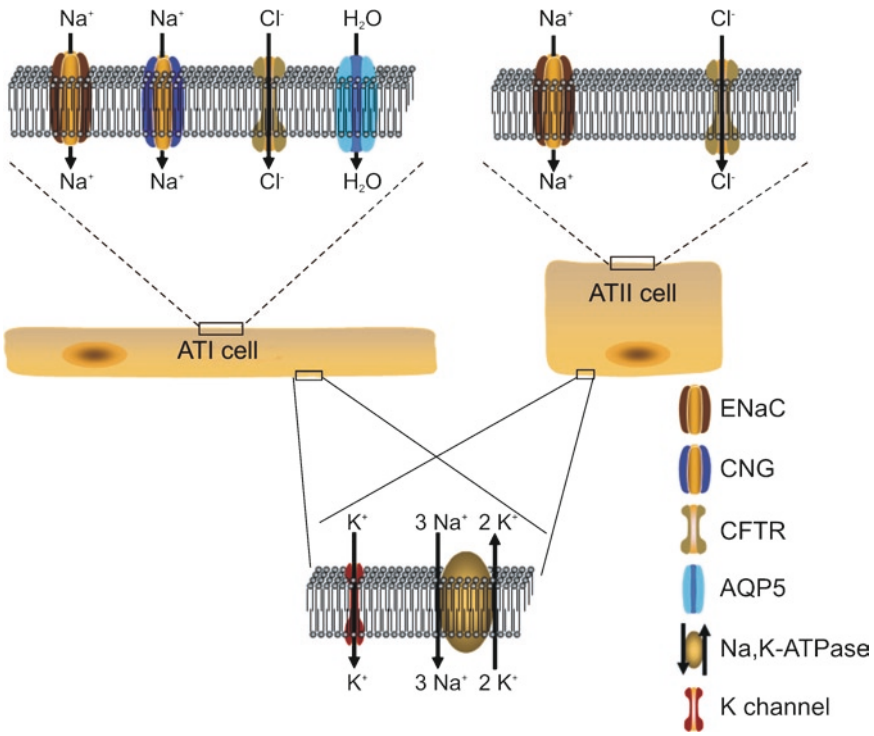


**Fig. 1** $Na^+$ absorption occurs from the apical surface of both alveolar epithelial type I and alveolar epithelial type II cells via the epithelial $Na^+$ channel (ENaC) and via cyclic-nucleotide-gated (*CNG*) channels (seen only in alveolar epithelial type I cells). $Cl^-$ transport occurs through the cystic fibrosis transmembrane conductance regulator in alveolar epithelial type I and type II cells. $K^+$ may be transported from the alveolar epithelial cells via basolateral $K^+$ channels in alveolar epithelial type I and type II cells. Aquaporin 5 is expressed at the apical cell membrane of the alveolar epithelial type I cell. The basolaterally expressed $Na^+/K^+$-ATPase provides the driving force for $Na^+$ absorption through the ENaC and CNG channels in the apical membrane

and ouabain. More information on the nature of ion transport across alveolar epithelial type II cells was gained by culturing these cells on porous supports and mounting them in Using chambers and measuring short-circuit current ($I_{sc}$) and ion flux under voltage-clamp conditions [1, 33]. $Na^+$ entering the epithelial cells passively through specialized pathways apically is pumped out of the cells basolaterally by the $Na^+/K^+$-ATPase (Fig. 1). Because of its continuous pumping, the $Na^+$ electrochemical potential is lower inside the cell, $Na^+$ flows down its electrochemical gradient, and is extruded basolaterally. Owing to the pump activity, $K^+$ electrochemical potential is larger inside the cell, and $K^+$ leaks through the basolateral cell membrane and is then recycled by the $Na^+/K^+$-ATPase. $Na^+$ entry pathways into alveolar epithelial type II cells are numerous (Fig. 1). Amiloride inhibited dome formation [33, 34] and decreased $I_{sc}$ in in vitro studies [35], an observation that supported the critical importance of $Na^+$ uptake through an amiloride-sensitive pathway present in the apical membrane of the alveolar epithelial cells, e.g. the epithelial $Na^+$ channel (ENaC). A detailed discussion of pharmacologic, biophysical, and molecular bases for fluid clearance across the alveolar and distal airway epithelium is available in other reviews [1, 36].

The role of alveolar epithelial type I cells in vectorial fluid transport in the lung is becoming clearer as these cells have now been successfully isolated. On the basis of studies in freshly isolated alveolar epithelial type I cells, it has been shown that these cells have a high osmotic permeability to water with apical surface expression of aquaporin 5 [37] (Fig. 1). Also, studies have reported that the $Na^+/K^+$-ATPase is expressed in the alveolar epithelial type I cells [28, 29, 38] (Fig. 1). The presence of the $Na^+/K^+$-ATPase could be consistent with a role for the alveolar epithelial type I cell in vectorial fluid transport, although this is not conclusive, because the $Na^+/K^+$-ATPase can also be used to maintain cell volume. Recent data demonstrate that alveolar epithelial type I cells express a multitude of $Na^+$ transporting proteins, such as the amiloride-sensitive ENaC and amiloride-insensitive cyclic-nucleotide-gated $Na^+$ channels [29] (Fig. 1). There are also new data confirming the importance of ENaC for alveolar fluid clearance using RNA interference (RNAi) against the αENaC subunit [39]. In those studies, inhibition of αENaC expression by specific small interfering RNA against αENaC attenuated the amiloride sensitivity of both normal and stimulated alveolar fluid clearance. In addition, the alveolar epithelial type I cells express $Cl^-$ transporters, i.e. the cystic fibrosis transmembrane regulator (CFTR), and thus have the ion transporters needed to participate in vectorial ion transport and clearance of alveolar fluid.

The large surface area that the alveolar epithelium covers in the lung suggests that removal of edema fluid from the lung may primarily occur across the alveolar epithelium. However, it has been demonstrated that the distal airway epithelium also actively transports $Na^+$, a process that depends on amiloride-inhibitable uptake of $Na^+$, likely by ENaC, on the apical surface and extrusion of $Na^+$ through a basolateral $Na^+/K^+$-ATPase [1]. Clara cells actively absorb and transport $Na^+$ from the apical to the basal surface [40]. This information provides support for a possible role of distal airway epithelia in fluid clearance. Thus, even though their surface area is limited, a contribution from distal airway epithelia to the overall fluid transport is likely, especially as cells from distal airway epithelia primarily transport salt from the apical to the basolateral surface.

## 4 Regulation of Lung Fluid Absorption

This section considers how the rate of vectorial fluid transport across the distal pulmonary epithelium is regulated by catecholamine-dependent [cyclic AMP (cAMP)-dependent] or catecholamine-independent mechanisms. Studies in newborn animals indicated that endogenous catecholamine release, particularly epinephrine, is critical for stimulation of reabsorption of fetal lung fluid from the newborn lung air spaces [41, 42]. In most adult mammalian species, $\beta_2$-adrenoceptor stimulation increases alveolar fluid clearance [43, 44]. The stimulatory effect occurs rapidly after administration of epinephrine or terbutaline, and is completely prevented by a nonspecific β-receptor antagonist (propranolol).

### 4.1 Catecholamine Regulation

The increased alveolar fluid clearance by $\beta_2$-adrenoceptor agonists can be prevented by amiloride or RNAi against αENaC, indicating that stimulation is related to increased transepithelial $Na^+$ transport [39]. In anesthetized ventilated sheep, terbutaline-induced stimulation of alveolar fluid clearance was also associated with an increase in lung lymph flow, a finding that reflected increased removal of the alveolar fluid volume to the lung interstitium [43]. Although terbutaline increased pulmonary blood flow, this is not important, because control studies with nitroprusside, an agent that increased pulmonary blood flow, did not increase alveolar fluid clearance. Other studies have demonstrated that β-adrenoceptor agonists increased alveolar fluid clearance in rats, dogs, guinea pigs, mice, and humans [1] (Table 1). The existence of $\beta_1$- and $\beta_2$-adrenoceptors on alveolar epithelial type II cells has been shown in vivo by autoradiographic and immunochemistry techniques [1].

It has been difficult to quantify the effect of catecholamines on the rate of alveolar fluid clearance and edema resolution in humans [45]. However, studies of alveolar fluid clearance in isolated human lungs have shown that β-adrenoceptor agonist therapy increases alveolar fluid clearance, and this increased alveolar fluid clearance can be inhibited by propranolol or amiloride [26]. The magnitude of the stimulatory effect is similar to that observed in other species, with a β-adrenoceptor-agonist-dependent doubling of alveolar fluid clearance over baseline levels. These data are particularly important because aerosolized β-adrenoceptor agonist treatment in some patients with pulmonary edema might be used to accelerate the resolution of alveolar edema.

On the basis of in vitro studies, it was proposed that increased intracellular cAMP levels, such as those observed after β-adrenoceptor stimulation, resulted in increased $Na^+$ transport across alveolar epithelial type II cells by an independent upregulation of apical $Na^+$ conductive pathways and basolateral $Na^+/K^+$-ATPases. Proposed mechanisms for upregulation of $Na^+$ transport proteins by cAMP include augmented $Na^+$ channel open probability, increased $Na^+/K^+$-ATPase α-subunit phosphorylation, and increased delivery of ENaCs to the apical membrane and of $Na^+/K^+$-ATPases to the basolateral cell membrane [1, 46] (Table 1).

Most experimental studies have attributed a role for active $Na^+$ transport in the apical-to-basolateral transport of salt and water across the alveolar epithelium of the lung. The potential role of $Cl^-$, especially in mediating the cAMP-mediated upregulation of fluid clearance across distal lung epithelium, has also been the subject of a few recent studies. A study in cultured alveolar epithelial cells suggested that vectorial $Cl^-$ transport across the alveolar epithelium occurs paracellularly under basal conditions and perhaps transcellularly in the presence of cAMP stimulation [47]. A second study in cultured alveolar epithelial type II cells suggested that cAMP-mediated apical $Na^+$ uptake may depend on an initial $Cl^-$ uptake [48]. A third study of cultured alveolar epithelial type II cells under apical surface–air interface conditions reported that β-adrenoceptor agonists produced acute activation of apical $Cl^-$ channels with enhanced $Na^+$ absorption [49] (Table 1). However, the results of these studies are considered inconclusive [50] because the data depend on cultured cells of an uncertain phenotype. Furthermore, studies of isolated alveolar epithelial type II cells do not address the possibility that vectorial fluid transport may be mediated by other epithelial cells, including alveolar epithelial type I cells as well as distal airway epithelial cells.

To better define the role of $Cl^-$ transport for active salt and water transport across the distal pulmonary epithelia, in vivo lung studies were designed to study mechanisms regulating $Cl^-$ transport [51]. This approach is important because studies in several species have indicated that both distal airway and air-space epithelia are capable of ion transport and both ENaC and CFTR are expressed in alveolar and distal airway epithelia. Inhibition and ion substitution studies demonstrated that $Cl^-$ transport was necessary for basal alveolar fluid clearance. The role of CFTR under basal and cAMP-stimulated conditions was tested using conditions in which CFTR was not functional because of failure in CFTR trafficking to the cell membrane, the most common human mutation in cystic fibrosis (Δ1F508). The results suggested that CFTR was essential for cAMP-mediated upregulation of fluid clearance from the distal air spaces, because alveolar fluid clearance could not be increased in Δ1F508 mice either with β-adrenoceptor agonists or with forskolin [51] (Table 1). Studies using pharmacologic CFTR inhibition in both mouse and human lungs with glibenclamide or $CFTR_{Inh\text{-}172}$ supported the results [51, 52]. Although CFTR absence in upper airways results in

**Table 1** Overview of factors that regulate transepithelial ion transport across alveolar and airway epithelia and modulate lung fluid balance

| Factor | Effect on transepithelial ion transport or lung fluid transport |
|---|---|
| β-Adrenergic agonists | Increase $Na^+$ transport and fluid clearance [24, 33, 35, 43, 56, 86, 92, 93] |
| | Down-regulate fluid clearance with prolonged delivery [94, 95] |
| Dopamine | Increase fluid clearance [67, 68] |
| | No effect [96] |
| Cyclic AMP | Increase fluid clearance [97, 98] |
| $Cl^-$ transport (CFTR) | Increase $Na^+$ transport [48, 49] |
| | Increase $Na^+$ transport and fluid clearance [48, 49, 51, 52] |
| Glucocorticoids | Increase fluid clearance [56, 99], ENaC, and $Na^+/K^+$-ATPase expression [100, 101] |
| Mineralocorticoids | No effect in alveolar cells [102] or in upper airways [103] |
| | Increase fluid clearance [57, 58] |
| Thyroid hormones | Increase fluid clearance [56] and $Na^+/K^+$-ATPase expression [59] |
| Growth factors (KGF, EGF, TGFα) | Increase fluid clearance [60–64] and $Na^+/K^+$-ATPase activity in alveolar epithelial type II cells [63] |
| Cytokines (TNFα, IL-1, TGF-$\beta_1$) | Increase fluid clearance [66, 104] |
| | Decrease fluid clearance [83, 84, 105] |
| Serine proteases | Increase $Na^+$ transport and fluid clearance [70, 71] |
| Hyperoxia | Increase $Na^+$ transport [32, 106, 107] and $Na^+/K^+$-ATPase expression [108] |
| | No effect [109] |
| | Decrease $Na^+$ transport and fluid clearance [110] |
| Hypoxia | Decrease $Na^+$ transport [75, 111] and fluid clearance [72] |
| Nitric oxide and nitrate products | Decrease $Na^+$ absorption [81, 82, 112, 113] |
| Anesthetics | Decrease fluid clearance [77, 79] |
| | No effect [114] |

enhanced $Na^+$ absorption, the data in these studies provide evidence that CFTR absence prevents cAMP-upregulated distal air space fluid clearance, a finding that is similar to the results from studies on the importance of CFTR in mediating cAMP-stimulated $Na^+$ absorption in human sweat ducts [53]. Additional studies suggested that the lack of CFTR results in greater accumulation of pulmonary edema in hydrostatic stress, thus demonstrating the potential physiologic importance of CFTR in upregulating fluid transport from the distal air spaces [51]. There is new evidence that functional CFTR $Cl^-$ channels are present in both adult rat alveolar epithelial type I and adult rat alveolar epithelial type II cells on the basis of the findings of whole-cell patch-clamp experiments [29].

In conjunction with progress in experimental studies of lung fluid balance under clinically relevant pathologic conditions, further studies should be done to test the potential role of catecholamine-dependent therapies that may enhance the resolution of clinical pulmonary edema. Recent experimental data in a rat model of acute lung injury suggest that $\beta_2$-adrenoceptor agonist therapy can decrease lung endothelial permeability, increase alveolar fluid clearance, and decrease pulmonary edema even when given after lung injury has developed [54]. The feasibility of delivering therapeutic concentrations of aerosolized β-adrenoceptor agonist therapy to the distal air spaces of ventilated patients has also been demonstrated [55]. Therefore, clinical trials could be carried out to test the potential value of this therapy for enhancing the resolution of pulmonary edema and improving clinical outcomes.

## 4.2 Non-catecholamine Regulation

Several interesting catecholamine-independent mechanisms have been identified that can upregulate fluid transport in the distal air spaces. Hormonal factors such as glucocorticoids and mineralocorticoids upregulate $Na^+$ transport by transcriptional mechanisms [56–58] (Table 1), whereas thyroid hormones work by a posttranslational mechanism [59] (Table 1). Growth factors such as epidermal growth factor, transforming growth factor-α, and keratinocyte growth factor (KGF) work by either transcriptional or direct membrane effects, or by enhancing the number of alveolar epithelial type II cells [60–65] (Table 1). For example, KGF is a potent mitogen for alveolar epithelial type II cells and distal air space administration of KGF increased alveolar fluid clearance by 66% [62]. KGF can also enhance $Na^+$ and fluid transport in injured rat lungs [63, 64]. KGF also increases $Na^+$ transport protein expression [65]. There is also evidence that a proinflammatory cytokine, tumor necrosis factor-α (TNFα), can rapidly upregulate $Na^+$ uptake and fluid transport [66] (Table 1). The effect of TNFα is amiloride-inhibitable in both rats and isolated A549 human cells [66]. Another catecholamine-independent mechanism is represented by dopamine. Dopamine is a vasoactive agonist that has been described to impair $Na^+$ reabsorption in renal tubules. In rat lungs, the effect of dopamine is opposite to that in the renal tubular epithelium, as dopamine increased fluid clearance in isolated perfused rat lungs [67, 68] (Table 1). This increase depended on $Na^+$ transport and was mediated by $D_1$ receptors in

alveolar epithelial type II cells and not via the β-adrenoceptor. In alveolar type II cells, dopamine increased expression of basolateral $Na^+/K^+$-ATPase $\alpha_1$-subunits [69]. Finally, serine proteases can regulate ENaC activity and potentially increase alveolar fluid clearance [70, 71] (Table 1). These catecholamine-independent mechanisms are also explored in more detail in a recent review [1].

## 5 Mechanisms Impairing Alveolar Edema Resolution

Several mechanisms have also been identified that can impair fluid transport from the distal air spaces. This section considers three conditions that have relevance to human disease: hypoxia, the use of anesthetics, and the presence of reactive oxygen and nitrogen species. In addition, this section reviews mechanisms that impair fluid transport under pathologic conditions.

### 5.1 Hypoxia

Hypoxia is a condition that may occur during residence or recreation at high altitudes and under a variety of pathologic conditions associated with acute and chronic respiratory disease. Therefore, it is important to understand the effect of hypoxia on ion and fluid transport across the lung epithelia. In anesthetized rats, hypoxia decreased alveolar fluid clearance by inhibition of the amiloride-sensitive ENaC [72] (Table 1). The effect of hypoxia could not be explained by transcriptional effects on ENaC or $Na^+/K^+$-ATPase. Instead, the results suggested a posttranslational mechanism, i.e. a change of ENaC activity or ENaC transport to the plasma membrane. This hypothesis was supported by the normalization of alveolar fluid clearance by a β-adrenoceptor agonist (terbutaline), which increased trafficking of $Na^+$ transporter proteins from the cytoplasm to the membrane [73, 74]. Direct evidence for this mechanism in hypoxic alveolar epithelial type II cells was demonstrated for ENaC [75] as well as for an inhibitory effect of hypoxia on $Na^+/K^+$-ATPase activity [76].

### 5.2 Anesthesia

Anesthetics represent another group of agents that have the potential to inhibit $Na^+$ transport across the air space epithelia and thereby reduce alveolar fluid clearance (Table 1). In alveolar epithelial cells, halogenated anesthetics affect $Na^+$ transport. In the rat, halothane and isoflurane decreased alveolar fluid clearance by inhibition of the amiloride-sensitive ENaC. This effect was rapidly reversible after cessation of halothane exposure [77]. In vitro, exposure to a low halothane concentration (1%) for a short time (30 min) induced a reversible decrease in $Na^+/K^+$-ATPase activity and amiloride-sensitive [22] Na influx via ENaC in rat alveolar epithelial type II cells [78]. The mechanisms whereby halothane induced this decrease in $Na^+$ transport protein activity have not been elucidated, but they are not related to a decrease in intracellular adenosine triphosphate content or to a change in cytosolic free calcium concentration. Taken together, these observations suggest that halogenated anesthetics may interfere with the clearance of alveolar edema.

Lidocaine is a local anesthetic that is widely used in patients with acute cardiac disorders and has been recently implicated as a possible cause of pulmonary edema following liposuction. In experimental rat studies, both intravenous and intra-alveolar administration of lidocaine reduced alveolar fluid clearance by 50% [79]. Because lidocaine did not inhibit ENaC when expressed in oocytes, the inhibitory effect on vectorial ion and fluid transport was likely through an effect on the $Na^+/K^+$-ATPase activity or through an indirect effect via blockade of $K^+$ channels, a well-known property of lidocaine. The effect of lidocaine was also completely reversible with $\beta_2$-adrenoceptor agonist therapy, further excluding direct effects on the $Na^+$ transport machinery [79].

### 5.3 Reactive Oxygen Species

During many pathologic conditions, in response to proinflammatory cytokines activated neutrophils and macrophages may localize in the lungs and migrate into the air spaces. There they release reactive oxygen species by the membrane-bound enzyme complex nicotinamide adenine dinucleotide phosphate oxidase and nitric oxide (NO). NO has been shown to decrease $I_{sc}$ across cultured rat alveolar epithelial type II cells without affecting transepithelial resistance and to inhibit 60% of the amiloride-sensitive $I_{sc}$ across alveolar epithelial type II cell monolayers [80] (Table 1). NO reacts with superoxide ($\cdot O_2$) to form peroxynitrite ($ONOO^-$), which is a potent oxidant and nitrating species that directly oxidizes a wide spectrum of biologic molecules, such as DNA constituents, lipids, and proteins [36]. Boluses of peroxynitrite delivered into suspensions of freshly isolated rabbit alveolar epithelial type II cells decreased the amiloride-inhibitable $Na^+$ uptake by approximately 65% without affecting cell viability (Table 1). Some investigators also have reported that macrophage products, including NO, downregulate $Na^+$ transport in endotoxin-stimulated fetal distal lung epithelium [81]. These data suggest that oxidation of critical amino acid residues in ENaC might be responsible. This matches well with other studies that have shown that protein nitration and oxidation

by reactive oxygen and nitrogen species are associated with diminished function of important proteins in the alveolar spaces, including surfactant protein A [82].

### 5.4 Cytokines

Specific factors may also decrease ENaC function and thereby reduce alveolar fluid clearance. As an example of this, there is new evidence that transforming smooth factor-$\beta_1$ decreases expression of ENaC and alveolar epithelial $Na^+$ and fluid transport by an extracellular signal-regulated kinase 1 and 2 (ERK1/2)-dependent mechanism in both primary rat and human alveolar epithelial type II cells [83] (Table 1). Interleukin-1β is a proinflammatory cytokine that has been associated with membrane dysfunction and a reduced fluid absorptive capacity in the lungs [84] (Table 1).

### 5.5 Pathologic Conditions

In the clinical setting, fluid clearance from the distal air spaces has been measured in mechanically ventilated patients having acute respiratory failure from pulmonary edema. In addition, there have been numerous studies with animal models of relevant pathologic conditions [85–88]. Studies of alveolar fluid clearance in humans have been done in intubated, ventilated patients by measuring the concentration of total protein in sequential samples of undiluted pulmonary edema fluid aspirated from the distal air spaces with a standard suction catheter passed through the endotracheal tube into a wedged position in the distal airways [23, 45, 89]. This method for measuring alveolar fluid clearance in patients was adapted from the method for aspirating fluid from the distal air spaces in experimental studies in small and large animals [19, 43]. The clinical procedure has been validated in patients by demonstrating that there is a relationship between alveolar fluid clearance and improvement in oxygenation and chest radiographs [45, 89].

In patients with severe hydrostatic pulmonary edema, net alveolar fluid clearance existed in most of them during the first 4 h after endotracheal intubation and onset of positive-pressure ventilation [23]. Upon a more detailed investigation, it was found that the rate of alveolar fluid clearance in these patients varied between maximal (more than 14%/h) in 38% of the patients and submaximal (3–14%/h) in 37% of the patients. Overall, 75% of the patients had intact alveolar fluid clearance. There was no significant correlation between the levels of alveolar fluid clearance and endogenous plasma epinephrine levels, although twice as many of the patients with intact alveolar fluid clearance received aerosolized β-adrenoceptor therapy as did those with impaired alveolar fluid clearance; this difference did not reach statistical significance, perhaps because the total number of patients studied was modest.

Most patients with increased permeability edema and acute lung injury have impaired alveolar epithelial fluid transport, a finding that is associated with more prolonged respiratory failure and a higher mortality. In contrast, a few patients can remove alveolar edema fluid rapidly, and these patients have a higher survival rate [45, 89]. These results indicate that a functional, intact distal lung epithelium is associated with a better outcome in patients with acute lung injury, thus supporting the hypothesis that the degree of injury to the distal lung epithelium is an important determinant of the outcome in patients with increased permeability pulmonary edema from acute lung injury. What are the mechanisms that impair alveolar fluid clearance in the patients with acute lung injury? As already explained, alveolar hypoxia can depress alveolar epithelial fluid transport. In addition to hypoxia, viral or bacterial lung infection can depress alveolar fluid transport, by interfering with normal ion transport, by inducing apoptosis or necrosis of the distal lung epithelium, and/or by affecting the sensitivity of the alveolar epithelium to β-adrenoceptor stimulation [90, 91]. There are also some clinical data indicating that a decrease in alveolar fluid clearance may be associated with higher levels of nitrate and nitrite in pulmonary edema fluid, a finding that supports the hypothesis that nitration and oxidation of proteins essential to epithelial fluid transport may occur in some patients with lung injury, further depressing their ability to remove alveolar edema fluid [82].

## 6 Summary

Remarkable progress has been made in understanding the basic mechanisms that regulate salt and water transport across the distal airway and alveolar epithelia. Removal of excess air space fluid, particularly alveolar edema resolution, is driven by active ion transport. The rate of alveolar fluid clearance can be upregulated by cAMP-dependent stimulation, including endogenous catecholamines or exogenous, aerosolized $\beta_2$-adrenoceptor agonists. Impaired alveolar ion and fluid transport may contribute to the severity of lung edema in multiple pathologic conditions, including clinical acute lung injury. Therapies hastening the repair of the injured alveolar epithelium or upregulating alveolar epithelial ion and fluid transport may have clinical value in reducing morbidity and mortality in patients with acute pulmonary edema.

## References

1. Matthay MA, Folkesson HG, Clerici C (2002) Lung epithelial fluid transport and the resolution of pulmonary edema. Physiol Rev 82:569–600
2. Folkesson HG, Matthay MA (2006) Alveolar epithelial ion and fluid transport – recent progress. Am J Respir Cell Mol Biol 35:10–19

3. Taylor AE, Guyton AC, Bishop VS (1965) Permeability of the alveolar epithelium to solutes. Circ Res 16:353–362
4. Berthiaume Y, Broaddus VC, Gropper MA, Tanita T, Matthay MA (1988) Alveolar liquid and protein clearance from normal dog lungs. J Appl Physiol 65:585–593
5. Staub NC (1974) Pulmonary edema. Physiol Rev 54:678–811
6. Phalen RF, Oldham MJ (1983) Airway structures. Tracheobronchial airway structure as revealed by casting techniques. Am Rev Respir Dis 128:S1–S4
7. Staub NC, Albertine KH (1988) The structure of the lungs relative to their principal function. In: Murray JF, Nadel JA (eds) Textbook of respiratory medicine, vol 1. Saunders, Philadelphia, pp 12–36
8. Weibel ER (1989) Lung morphometry and models in respiratory physiology. In: Chang HK, Paiva M (eds) Respiratory physiology. An analytical approach, vol. 40. Dekker, New York, pp 1–56
9. West JB, Mathieu-Costello O (1992) Strength of the pulmonary blood-gas barrier. Respir Physiol 88:141–148
10. Schneeberger EE, Lynch RD (1992) Structure, function, and regulation of cellular tight junctions. Am J Physiol Lung Cell Mol Physiol 262:L647–L661
11. Hakim TS, Minnear FC, van der Zee H, Barie PS, Malik AB (1981) Adrenoreceptor control of lung fluid and protein exchange. J Appl Physiol 51:68–72
12. Parker JC, Crain M, Grimbert F, Rotili G, Taylor AE (1981) Total lung lymph flow and fluid compartmentation in edematous dog lungs. J Appl Physiol 51:1268–1277
13. Gorin AB, Stewart PA (1979) Differential permeability of endothelial and epithelial barriers to albumin flux. J Appl Physiol 47:1315–1324
14. Folkesson HG, Weström BR, Karlsson BW (1990) Permeability of the respiratory tract to different-sized macromolecules after intratracheal instillation in young and adult rats. Acta Physiol Scand 139:347–354
15. Hastings RH, Grady M, Sakuma T, Matthay MA (1992) Clearance of different-sized proteins from the alveolar space in humans and rabbits. J Appl Physiol 73:1310–1316
16. Kim KJ, LeBon TR, Shinbane JS, Crandall ED (1985) Asymmetric ($^{14}$C)albumin transport across bullfrog alveolar epithelium. J Appl Physiol 59:1290–1297
17. Folkesson HG, Matthay MA, Weström BR, Kim KJ, Karlsson BW, Hastings RH (1996) Alveolar epithelial clearance of protein. J Appl Physiol 80:1431–1445
18. Hastings RH, Folkesson HG, Matthay MA (2004) Mechanisms of alveolar protein clearance in the intact lung. Am J Physiol Lung Cell Mol Physiol 286:L679–L689
19. Matthay MA, Folkesson HG, Verkman AS (1996) Salt and water transport across alveolar and distal airway epithelium in the adult lung. Am J Physiol Lung Cell Mol Physiol 270:L487–L503
20. Sakuma T, Pittet JF, Jayr C, Matthay MA (1993) Alveolar liquid and protein clearance in the absence of blood flow or ventilation in sheep. J Appl Physiol 74:176–185
21. Matthay MA, Landolt CC, Staub NC (1982) Differential liquid and protein clearance from the alveoli of anesthetized sheep. J Appl Physiol 53:96–104
22. Matthay MA, Berthiaume Y, Staub NC (1985) Long-term clearance of liquid and protein from the lungs of unanesthetized sheep. J Appl Physiol 59:928–934
23. Verghese GM, Ware LB, Matthay BA, Matthay MA (1999) Alveolar epithelial fluid transport and the resolution of clinically severe hydrostatic pulmonary edema. J Appl Physiol 87:1301–1312
24. Jayr C, Garat C, Meignan M, Pittet J-F, Zelter M, Matthay MA (1994) Alveolar liquid and protein clearance in anesthetized ventilated rats. J Appl Physiol 76:2636–2642
25. Norlin A, Finley N, Abedinpour P, Folkesson HG (1998) Alveolar liquid clearance in the anesthetized ventilated guinea pig. Am J Physiol Lung Cell Mol Physiol 274:L235–L243
26. Sakuma T, Okaniwa G, Nakada T, Nishimura T, Fujimura S, Matthay MA (1994) Alveolar fluid clearance in the resected human lung. Am J Respir Crit Care Med 150:305–310
27. Goodman BE, Fleischer RD, Crandall ED (1983) Evidence for active $Na^+$ transport by cultured monolayers of pulmonary alveolar epithelial cells. Am J Physiol Cell Physiol 245: C78–C83
28. Borok Z, Liebler JM, Lubman RL et al (2002) Alveolar epithelial ion and fluid transport: Na transport proteins are expressed by rat alveolar epithelial type I cells. Am J Physiol Lung Cell Mol Physiol 282:L599–L608
29. Johnson MD, Bao HF, Helms MN et al (2006) Functional ion channels in pulmonary alveolar type I cells support a role for type I cells in lung ion transport. Proc Natl Acad Sci USA 103: 4964–4969
30. Rutschman DH, Olivera W, Sznajder JI (1993) Active transport and passive liquid movement in isolated perfused rat lungs. J Appl Physiol 75:1574–1580
31. Serikov VB, Grady M, Matthay MA (1993) Effect of temperature on alveolar liquid and protein clearance in an in situ perfused goat lung. J Appl Physiol 75:940–947
32. Olivera W, Ridge K, Wood LD, Sznajder JI (1994) Active sodium transport and alveolar epithelial Na-K-ATPase increase during subacute hyperoxia in rats. Am J Physiol Lung Cell Mol Physiol 266:L577–L584
33. Mason RJ, Williams MC, Widdicombe JH, Sanders MJ, Misfeldt DS, Berry LCJ (1982) Transepithelial transport by pulmonary alveolar type II cells in primary culture. Proc Natl Acad Sci USA 79:6033–6037
34. Goodman BE, Crandall ED (1982) Dome formation in primary cultured monolayers of alveolar epithelial cells. Am J Physiol Cell Physiol 243:C96–C100
35. Cheek JM, Kim KJ, Crandall ED (1989) Tight monolayers of rat alveolar epithelial cells: bioelectric properties and active sodium transport. Am J Physiol Cell Physiol 256:C688–C693
36. Matalon S, O'Brodovich H (1999) Sodium channels in alveolar epithelial cells: Molecular characterization, biophysical properties, and physiological significance. Annu Rev Physiol 61:627–661
37. Dobbs LG, Gonzalez R, Matthay MA, Carter EP, Allen L, Verkman AS (1998) Highly water permeable type I alveolar epithelial cells confer high water permeability between the airspace and vasculature in rat lung. Proc Natl Acad Sci U S A 95: 2991–2996
38. Ridge KM, Rutschman DH, Factor P, Katz AI, Bertorello AM, Sznajder JL (1997) Differential expression of Na-K-ATPase isoforms in rat alveolar epithelial cells. Am J Physiol Lung Cell Mol Physiol 273:L246–L255
39. Li T, Folkesson HG (2006) RNA interference for a-ENaC inhibits rat lung fluid absorption in vivo. Am J Physiol Lung Cell Mol Physiol 290:L649–L660
40. van Scott MR, Chinet TC, Burnette AD, Paradiso AM (1995) Purinergic regulation of ion transport across nonciliated bronchiolar epithelial (Clara) cells. Am J Physiol Lung Cell Mol Physiol 269:L30–L37
41. Walters DV, Olver RE (1978) The role of catecholamines in lung liquid absorption at birth. Pediatr Res 12:239–242
42. Finley N, Norlin A, Baines DL, Folkesson HG (1998) Alveolar epithelial fluid clearance is mediated by endogenous catecholamines at birth in guinea pigs. J Clin Invest 101:972–981
43. Berthiaume Y, Staub NC, Matthay MA (1987) Beta-adrenergic agonists increase lung liquid clearance in anesthetized sheep. J Clin Invest 79:335–343
44. Sakuma T, Folkesson HG, Suzuki S, Okaniwa G, Fujimura S, Matthay MA (1997) Beta-adrenergic agonist stimulated alveolar fluid clearance in ex vivo human and rat lungs. Am J Respir Crit Care Med 155:506–512

45. Matthay MA, Wiener-Kronish JP (1990) Intact epithelial barrier function is critical for the resolution of alveolar edema in humans. Am Rev Respir Dis 142:1250–1257
46. Matalon S (1991) Mechanisms and regulation of ion transport in adult mammalian alveolar type II pneumocytes. Am J Physiol Cell Physiol 261:C727–C738
47. Kim KJ, Cheek JM, Crandall ED (1991) Contribution of active $Na^+$ and $Cl^-$ fluxes to net ion transport by alveolar epithelium. Respir Physiol 85:245–256
48. Jiang X, Ingbar DH, O'Grady SM (1998) Adrenergic stimulation of $Na^+$ transport across alveolar epithelial cells involves activation of apical $Cl^-$ channels. Am J Physiol Cell Physiol 275: C1610–C1620
49. Jiang X, Ingbar DH, O'Grady SM (2001) Adrenergic regulation of ion transport across adult alveolar epithelial cells: effects on $Cl^-$ channel activation and transport function in cultures with an apical air interface. J Membr Biol 181:195–204
50. Lazrak A, Nielsen VG, Matalon S (2000) Mechanisms of increased $Na^+$ transport in ATII cells by cAMP: we agree to disagree and do more experiments. Am J Physiol Lung Cell Mol Physiol 278:L233–L238
51. Fang X, Fukuda N, Barbry P, Sartori C, Verkman AS, Matthay MA (2002) Novel role for CFTR in fluid absorption from the distal airspaces of the lung. J Gen Physiol 119:199–208
52. Fang X, Song Y, Hirsch J et al (2006) Contribution of CFTR to apical-basolateral fluid transport in cultured human alveolar epithelial type II cells. Am J Physiol Lung Cell Mol Physiol 290:L242–L249
53. Reddy MM, Light MJ, Quinton PM (1999) Activation of the epithelial $Na^+$ channel (ENaC) requires CFTR $Cl^-$ channel function. Nature 402:301–304
54. McAuley DF, Frank JA, Fang X, Matthay MA (2004) Clinically relevant concentrations of $\beta_2$-adrenergic agonists stimulate maximal cyclic adenosine monophosphate-dependent airspace fluid clearance and decrease pulmonary edema in experimental acid-induced lung injury. Crit Care Med 32:1470–1476
55. Atabai K, Ware LB, Snider ME et al (2002) Aerosolized $\beta_2$-adrenergic agonists achieve therapeutic levels in the pulmonary edema fluid of ventilated patients with acute respiratory failure. Intensive Care Med 28:705–711
56. Folkesson HG, Norlin A, Wang Y, Abedinpour P, Matthay MA (2000) Dexamethasone and thyroid hormone pretreatment upregulate alveolar epithelial fluid clearance in adult rats. J Appl Physiol 88:416–424
57. Olivera WG, Ciccolella DE, Barquin N et al (2000) Aldosterone regulates Na,K-ATPase and increases lung edema clearance in rats. Am J Respir Crit Care Med 161:567–573
58. Suzuki S, Tsubochi H, Suzuki T et al (2001) Modulation of transalveolar fluid absorption by endogenous aldosterone in adult rats. Exp Lung Res 27:143–155
59. Lei J, Mariash CN, Bhargava M, Wattenberg EV, Ingbar DH (2008) T3 increases Na-K-ATPase activity via a MAPK/ERK1/2-dependent pathway in rat adult alveolar epithelial cells. Am J Physiol Lung Cell Mol Physiol 294:L749–L754
60. Sznajder JI, Ridge KM, Yeates DB, Ilekis J, Olivera W (1998) Epidermal growth factor increases lung liquid clearance in rat lungs. J Appl Physiol 85:1004–1010
61. Folkesson HG, Pittet J-F, Nitenberg G, Matthay MA (1996) Transforming growth factor-$\alpha$ increases alveolar liquid clearance in anesthetized ventilated rats. Am J Physiol Lung Cell Mol Physiol 271:L236–L244
62. Wang Y, Folkesson HG, Jayr C, Ware LB, Matthay MA (1999) Alveolar epithelial fluid transport can be simultaneously upregulated by both KGF and $\beta$-agonist therapy. J Appl Physiol 87:1852–1860
63. Guery BPH, Mason CM, Dobard EP, Beaucaire G, Summer WR, Nelson S (1997) Keratinocyte growth factor increases transalveolar sodium reabsorption in normal and injured rat lungs. Am J Respir Crit Care Med 155:1777–1784
64. Guo J, Yi ES, Havill AM et al (1998) Intravenous keratinocyte growth factor protects against experimental pulmonary injury. Am J Physiol Lung Cell Mol Physiol 275:L800–L805
65. Borok Z, Lubman RL, Danto SI et al (1998) Keratinocyte growth factor modulates alveolar epithelial cell phenotype in vitro: expression of aquaporin 5. Am J Respir Cell Mol Biol 18:554–561
66. Börjesson A, Norlin A, Wang X, Andersson R, Folkesson HG (2000) TNF$\alpha$ stimulates alveolar liquid clearance during intestinal ischemia-reperfusion in rats. Am J Physiol Lung Cell Mol Physiol 278:L3–L12
67. Barnard ML, Olivera WG, Rutschman DM, Bertorello AM, Katz AI, Sznajder JI (1997) Dopamine stimulates sodium transport and liquid clearance in rat lung epithelium. Am J Respir Crit Care Med 156:709–714
68. Saldías FJ, Lecuona E, Comellas AP, Ridge KM, Sznajder JI (1999) Dopamine restores lung ability to clear edema in rats exposed to hyperoxia. Am J Respir Crit Care Med 159: 626–633
69. Barnard ML, Ridge KM, Saldias F et al (1999) Stimulation of the dopamine 1 receptor increases lung edema clearance. Am J Respir Crit Care Med 160:982–986
70. Planès C, Leyvraz C, Uchida T et al (2005) In vitro and in vivo regulation of transepithelial lung alveolar sodium transport by serine proteases. Am J Physiol Lung Cell Mol Physiol 288:L1099–L1109
71. Rossier BC (2004) The epithelial sodium channel. Activation by membrane-bound serine proteases. Proc Am Thorac Soc 1:4–9
72. Vivona ML, Matthay MA, Chabaud MB, Friedlander G, Clerici C (2001) Hypoxia reduces alveolar epithelial sodium and fluid transport in rats: reversal by $\beta$-adrenergic agonist treatment. Am J Respir Cell Mol Biol 25:554–561
73. Kleyman TR, Ernst SA, Coupaye-Gerard B (1994) Arginine vasopressin and forskolin regulate apical cell surface expression of epithelial $Na^+$ channels in A6 cells. Am J Physiol Renal Physiol 266:F506–F511
74. Snyder PM (2000) Liddle's syndrome mutations disrupt cAMP-mediated translocation of the epithelial $Na^+$ channel to the cell surface. J Clin Invest 105:45–53
75. Planès C, Blot-Chabaud M, Matthay MA, Couette S, Uchida T, Clerici C (2002) Hypoxia and $\beta_2$-agonists regulate cell surface expression of the epithelial sodium channel in native alveolar epithelial cells. J Biol Chem 277:47318–47324
76. Dada LA, Chandel NS, Ridge KM, Pedemonte C, Bertorello AM, Sznajder JI (2003) Hypoxia-induced endocytosis of Na,K-ATPase in alveolar epithelial cells is mediated by mitochondrial reactive oxygen species and PKC-$\zeta$. J Clin Invest 111:1057–1064
77. Rezaiguia-Delclaux S, Jayr C, Luo DF, Saidi N-E, Meignan M, Duvaldestin P (1998) Halothane and isoflurane decrease alveolar epithelial fluid clearance in rats. Anesthesiology 88:751–760
78. Molliex S, Dureuil B, Aubier M, Friedlander G, Desmonts JM, Clerici C (1998) Halothane decreases Na,K-ATPase, and Na channel activity in alveolar type II cells. Anesthesiology 88:1606–1613
79. Laffon M, Jayr C, Barbry P et al (2002) Lidocaine induces a reversible decrease in alveolar epithelial fluid clearance in rats. Anesthesiology 96:392–399
80. Guo Y, DuVall MD, Crow JP, Matalon S (1998) Nitric oxide inhibits its $Na^+$ absorption across cultured alveolar type II monolayers. Am J Physiol Lung Cell Mol Physiol 274:L369–L377
81. Compeau CG, Rotstein OD, Tohda H et al (1994) Endotoxin-stimulated alveolar macrophages impair lung epithelial $Na^+$ transport by an L-Arg-dependent mechanism. Am J Physiol Cell Physiol 266:C1330–C1341
82. Zhu S, Ware LB, Geiser T, Matthay MA, Matalon S (2001) Increased levels of nitrate and surfactant protein a nitration in the

pulmonary edema fluid of patients with acute lung injury. Am J Respir Crit Care Med 163:166–172
83. Frank J, Roux J, Kawakatsu H et al (2003) Transforming growth factor-$\beta_1$ decreases expression of the epithelial sodium channel αENaC and alveolar epithelial vectorial sodium and fluid transport via an ERK1/2-dependent mechanism. J Biol Chem 278:43939–43950
84. Roux J, Kawakatsu H, Gartland B et al (2005) Interleukin-1β decreases expression of the epithelial sodium channel α-subunit in alveolar epithelial cells via a p38 MAPK-dependent signaling pathway. J Biol Chem 280:18579–18589
85. Folkesson HG, Matthay MA, Hébert CA, Broaddus VC (1995) Acid aspiration induced lung injury in rabbits is mediated by interleukin-8 dependent mechanisms. J Clin Invest 96:107–116
86. Pittet J-F, Wiener-Kronish JP, McElroy MC, Folkesson HG, Matthay MA (1994) Stimulation of lung epithelial liquid clearance by endogenous release of catecholamines in septic shock in anesthetized rats. J Clin Invest 94:663–671
87. Berthiaume Y, Matthay MA (2007) Alveolar edema fluid clearance and acute lung injury. Respir Physiol Neurobiol 159:350–359
88. Guidot DM, Folkesson HG, Jain L, Sznajder JI, Pittet JF, Matthay MA (2006) EB2006 symposium report: Integrating acute lung injury and regulation of alveolar fluid clearance. Am J Physiol Lung Cell Mol Physiol 291:L301–L306
89. Ware LB, Matthay MA (2001) Alveolar fluid clearance is impaired in the majority of patients with acute lung injury and the acute respiratory distress syndrome. Am J Respir Crit Care Med 163:1376–1383
90. van Schaik SM, Vargas I, Welliver RC, Enhorning G (1997) Surfactant dysfunction develops in BALB/c mice infected with respiratory syncytial virus. Pediatr Res 42:169–173
91. Davis IC, Xu A, Gao Z et al (2007) Respiratory syncytial virus induces insensitivity to β-adrenergic agonists in mouse lung epithelium in vivo. Am J Physiol Lung Cell Mol Physiol 293:L281–L289
92. Goodman BE, Brown SE, Crandall ED (1984) Regulation of transport across pulmonary alveolar epithelial cell monolayers. J Appl Physiol 57:703–710
93. Saldías F, Lecuona E, Friedman E, Barnard ML, Ridge KM, Sznajder JI (1998) Modulation of lung liquid clearance by isoproterenol in rat lungs. Am J Physiol Lung Cell Mol Physiol 274:L694–L701
94. Morgan EE, Hodnichak CM, Stader SM et al (2002) Alveolar epithelial ion and fluid transport: prolonged isoproterenol infusion impairs the ability of $\beta_2$-agonists to increase alveolar liquid clearance. Am J Physiol Lung Cell Mol Physiol 282:L666–L674
95. Sartori C, Fang X, McGraw DW et al (2002) Selected contribution: long-term effects of $\beta_2$-adrenergic receptor stimulation on alveolar fluid clearance in mice. J Appl Physiol 93:1875–1880
96. Tibayan FA, Chesnutt AN, Folkesson HG, Eandi J, Matthay MA (1997) Dobutamine increases alveolar liquid clearance in ventilated rats by β-2 receptor stimulation. Am J Respir Crit Care Med 156:438–444
97. Goodman BE, Anderson JL, Clemens JW (1989) Evidence for regulation of sodium transport from airspace to vascular space by cAMP. Am J Physiol Lung Cell Mol Physiol 257:L86–L93
98. Berthiaume Y (1991) Effect of exogenous cAMP and aminophylline on alveolar and lung liquid clearance in anesthetized sheep. J Appl Physiol 70:2490–2497
99. Norlin A, Baines DL, Folkesson HG (1999) Role of endogenous cortisol in basal liquid clearance from distal air spaces in adult guinea-pigs. J Physiol 519:261–272
100. Barquin N, Ciccolella DE, Ridge KM, Sznajder JI (1997) Dexamethasone upregulates the Na-K-ATPase in rat alveolar epithelial cells. Am J Physiol Lung Cell Mol Physiol 273:L825–L830
101. Dagenais A, Denis C, Vives M-F et al (2001) Modulation of a-ENaC and $a_1$-$Na^+$-$K^+$-ATPase by cAMP and dexamethasone in alveolar epithelial cells. Am J Physiol Lung Cell Mol Physiol 281:L217–L230
102. O'Brodovich H, Rafii B, Perlon P (1992) Arginine vasopressin and atrial natriuretic peptide do not alter ion transport by cultured fetal distal lung epithelium. Pediatr Res 31:318–322
103. Grubb BR, Boucher RC (1998) Effect of in vivo corticosteroids on $Na^+$ transport across airway epithelia. Am J Physiol Cell Physiol 275:C303–C308
104. Rezaiguia S, Garat C, Delclaux C et al (1997) Acute bacterial pneumonia in rats increases alveolar epithelial fluid clearance by a tumor necrosis factor-α-dependent mechanism. J Clin Invest 99:325–335
105. Laffon M, Lu LN, Modelska K, Matthay MA, Pittet JF (1999) α-Adrenergic blockade restores normal fluid transport capacity of alveolar epithelium after hemorrhagic shock. Am J Physiol Lung Cell Mol Physiol 277:L760–L768
106. Nici L, Dowin R, Gilmore-Hebert M, Jamieson JD, Ingbar DH (1991) Upregulation of rat lung Na-K-ATPase during hyperoxic injury. Am J Physiol Lung Cell Mol Physiol 261:L307–L314
107. Yue G, Russell WJ, Benos DJ, Jackson RM, Olman MA, Matalon S (1995) Increased expression and activity of sodium channels in alveolar type II cells of hyperoxic rats. Proc Natl Acad Sci USA 92:8418–8422
108. Johnson CR, Guo Y, Helton ES, Matalon S, Jackson RM (1998) Modulation of rat lung $Na^+$,$K^+$-ATPase gene expression by hyperoxia. Exp Lung Res 24:173–188
109. Carter EP, Wangensteen OD, O'Grady SM, Ingbar DH (1997) Effects of hyperoxia on type II cell Na-K-ATPase function and expression. Am J Physiol Lung Cell Mol Physiol 272:L542–L551
110. Olivera WG, Ridge KM, Sznajder JI (1995) Lung liquid clearance and Na,K-ATPase during acute hyperoxia and recovery in rats. Am J Respir Crit Care Med 152:1229–1234
111. Tomlinson LA, Carpenter TC, Baker EH, Bridges JB, Weil JV (1999) Hypoxia reduces airway epithelial sodium transport in rats. Am J Physiol Lung Cell Mol Physiol 277:L881–L886
112. Ding JW, Dickie J, O'Brodovich H et al (1998) Inhibition of amiloride-sensitive sodium-channel activity in distal lung epithelial cells by nitric oxide. Am J Physiol Lung Cell Mol Physiol 274:L378–L387
113. DuVall MD, Zhu S, Fuller CM, Matalon S (1998) Peroxynitrite inhibits amiloride-sensitive $Na^+$ currents in *Xenopus* oocytes expressing αβγ-rENaC. Am J Physiol Cell Physiol 274: C1417–C1423
114. Nielsen VG, Baird MS, Geary BT, Matalon S (2000) Halothane does not decrease amiloride-sensitive alveolar fluid clearance in rabbits. Anesth Analg 90:1445–1449