Lung Epithelial Fluid Transport and the Resolution of Pulmonary Edema

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I. Introduction

In the last 20 years, the mechanisms that regulate active salt and water transport by the alveolar and distal airway epithelium of the lung have emerged as a new area of research with important implications for understanding lung fluid balance under both normal and pathological conditions. Studies of epithelial fluid transport by the distal pulmonary epithelium have provided important new concepts regarding the resolution of pulmonary edema, a common clinical problem. Before 1982, there was no information on how lung fluid balance was regulated across the distal airway and alveolar epithelial barriers. However, in 1982, new work provided evidence that fluid balance in the lung was regulated by active ion transport mechanisms (131, 214, 229). For many years, it was generally believed that differences in hydrostatic and protein osmotic pressures (Starling forces) accounted for
the removal of excess fluid from the airspaces of the lung. This misconception persisted in part because some experiments that measured solute flux across the epithelial and endothelial barriers of the lung were done at room temperature (347), and the studies were done in dogs, a species that turned out to have a very low rate of active sodium and fluid transport (28). Also, until the early 1980s, there were no satisfactory animal models to study the resolution of alveolar edema, and the isolation and culture of alveolar epithelial type II cells was just becoming a useful experimental method. Although the removal of interstitial pulmonary edema by lung lymphatics and the lung microcirculation was discussed by Staub in 1974 in his review of pulmonary edema (333), there was no information on how pulmonary edema was removed from the distal airspaces of the lung.

This review provides a perspective on how in vivo and in vitro experiments in the last 20 years have provided a new understanding of the regulation of lung fluid balance by active transport mechanisms across both the alveolar and distal airway epithelium. On balance, there is convincing evidence that the vectorial transport of salt and water across the alveolar and distal airway epithelium is the primary determinant of fluid clearance, thus accounting for the ability of the lung to remove alveolar fluid at the time of birth as well as in the mature lung when pathological conditions lead to the development of pulmonary edema. Most of this review is focused on the function of the adult, not the fetal or the perinatal, lung.

II. SALT AND WATER TRANSPORT ACROSS THE DISTAL PULMONARY EPITHELIUM

With few exceptions, the general model for transepithelial fluid movement is that active salt transport drives osmotic water transport. This paradigm is probably correct for fluid clearance from the distal airspaces of the lung (22, 30, 91, 92, 94, 228, 229). The results of several in vivo studies have demonstrated that changes in hydrostatic or protein osmotic pressures cannot account for the removal of excess fluid from the distal airspaces (22, 28, 30, 94, 215, 219, 226–229, 343). Furthermore, pharmacological inhibitors of sodium transport can reduce the rate of fluid clearance in the lungs of several different species, including the human lung (30, 110, 164, 217, 228, 258, 266, 308, 327). In addition, there is good evidence that isolated epithelial cells from the distal airspaces of the lung actively transport sodium and other ions. Less than 10 years ago, the pathways for water clearance across alveolar and distal airway epithelium were unknown. Since then, several studies have demonstrated the presence of several functional water channels, aquaporins, in the lung.

This section reviews relevant morphological features of the distal lung epithelium with particular reference to their potential contribution to solute and water transport capacity. Next, based on in vivo and in vitro studies, there will be a discussion of some of the evidence supporting the hypothesis that active ion transport is the primary mechanism for driving vectorial fluid transport from the airspaces to the interstitium of the lung. The last section describes the evidence for the role of epithelial sodium channel (ENaC), \( \text{Na}^+ - \text{K}^+ - \text{ATPase} \), and aquaporins in mediating salt and water transport in distal lung epithelia.

A. Structural Features of the Distal Pulmonary Epithelia

Although the large surface area of the alveoli seems to favor the hypothesis that most fluid reabsorption occurs at the alveolar level, active fluid reabsorption could occur across all of the different segments of the pulmonary epithelium of the distal airspaces of the lung. The precise contribution of each of the anatomic segments of the distal airspaces to fluid reabsorption is not firmly established. Therefore, it is important to review briefly the morphological features of the distal lung epithelia and some of the information regarding their transport characteristics.

The human lung consists of a series of highly branched hollow tubes that end blindly in the alveoli, with the conducting airways (the cartilaginous trachea, bronchi, and the membranous bronchioles) occupying the first 16 generations of airways (281). Gas exchange primarily occurs in the branches that make up the last seven generations including respiratory bronchioles, alveolar ducts, alveolar sacs, and alveoli (334). The airways, \( \sim 1.4 \, \text{m}^2 \) in the adult human lung (376), and alveoli, \( \sim 143 \, \text{m}^2 \) in the adult human lung (376), constitute the interface between lung parenchyma and the external environment and are lined by a continuous epithelium. The distal airway epithelium is composed of terminal respiratory and bronchiolar units with polarized epithelial cells that have the capacity to transport sodium and chloride including ciliated Clara cells and nonciliated cuboidal cells. The alveoli themselves are composed of a thin alveolar epithelium (0.1–0.2 \( \mu \text{m} \)) that covers 99% of the airspace surface area in the lung and contains thin, squamous type I cells and cuboidal type II cells (334, 376) (Fig. 1). The alveolar type I cell covers 95% of the alveolar surface (376). The close apposition between the alveolar epithelium and the vascular endothelium facilitates efficient exchange of gases, but also forms a tight barrier to movement of liquid and proteins from the interstitial and vascular spaces, thus assisting in maintaining relatively dry alveoli (377).

The tight junctions are the critical structures for the barrier function of the alveolar epithelium. Tight junctions connect adjacent epithelial cells near their apical
surfaces, thereby maintaining apical and basolateral cell polarity (318). Ion transporters and other membrane proteins are asymmetrically distributed on opposing cell surfaces, conferring vectorial transport properties to the epithelium. In addition, the tight junction itself might contain discrete ion-selective pores (139). In the past, it was thought that tight junctions were rigid structures, which physically restrict passage of larger molecules; however, the permeability of tight junctions is dynamic and regulated, in part, by cytoskeletal proteins and intracellular calcium concentration (318). Physiological studies of the barrier properties 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 capillaries (145, 279, 317, 320, 333). Based on tracer fluxes of small water-soluble solutes across the air-blood barrier of the distal airspaces, the effective pore radii were 0.5–0.9 nm in the distal respiratory epithelium and 6.5–7.5 nm in the capillary endothelium (346). In addition, studies of protein flux across both the endothelial and epithelial barriers of the sheep lung indicate that 92% of the resistance to albumin flux across the alveolar barrier resides in the epithelium (134). Removal of large quantities of soluble protein from the airspaces appears to occur primarily by restricted diffusion (119, 150, 151), although there is evidence for some endocytosis and transcytosis of albumin across alveolar epithelium (27, 114, 119, 150, 167, 177, 178, 221).

The most extensively studied cell in the distal pulmonary epithelium is the alveolar type II cell, partly because type II cells can be readily isolated from the lung and studied in vitro. The alveolar type II cell is responsible for the secretion of surfactant (65, 66) as well as vectorial transport of sodium from the apical to the basolateral surface (67, 132, 214, 216, 217, 228, 238). The active transport of sodium by type II cells appears to provide a major driving force for removal of fluid from the alveolar space. Sodium uptake occurs on the apical surface, partly through amiloride-sensitive and amiloride-insensitive channels. Subsequently, sodium is pumped actively from the basolateral surface into the lung interstitium by Na+/K+-ATPase (Fig. 1). An ENaC that participates in sodium movement across the cell apical membrane was cloned and characterized in 1994 (52), and work by other investigators has provided new insight into the molecular and biochemical basis for sodium uptake in alveolar epithelial cells (264, 370, 387) (see details below).

The role of the alveolar type I cell in vectorial fluid transport in the lung is unknown, although several investigators are currently trying to assess the potential contribution of the alveolar type I cell to vectorial fluid transport. On the basis of studies in freshly isolated type I cells, it is known that these cells have a high osmotic permeability to water with expression of aquaporin-5 on the apical surface (84). Immunocytochemical studies in the intact lung by some investigators failed to demonstrate the presence of Na+/K+-ATPase in these cells in vivo (319). However, more recent studies have reported the presence of the α1- and α2-subunits of Na+/K+-ATPase in both type I like cells in vitro (303). The presence of the Na+/K+-ATPase could be consistent with a role for this cell in vectorial fluid transport, although it is not conclusive since the Na+/K+-ATPase may be needed to maintain cell volume. Also, recent studies of freshly isolated alveolar type I cells from rats demonstrated that these cells express the Na+/K+-ATPase α1- and β1-subunit isoforms, but not the α2-subunit (38). In the same study, there was
evidence for $\alpha_1$ Na\(^+\)-K\(^+\)-ATPase expression on the basolateral surface of the alveolar epithelial type I cells in situ in the rat lung. In addition, there is evidence for expression of all the subunits of ENaC in freshly isolated alveolar type I cells from two laboratories (38, 168) as well as in situ in the rat lung (168). Finally, there is some evidence that \( ^{22} \)Na uptake can be partially inhibited by amiloride in the freshly isolated rat alveolar type I cells (168), although definitive studies of cultured, polarized type I cells have not yet been achieved. There is also recent preliminary evidence that \( \beta \)-receptors are expressed on type I cells (201), although this issue is not clear since evidence also has been brought forward for the lack of such expression in type I cells (249). Also, type I cells might be involved in the transport of macromolecules because of the presence of vesicles and caveolin in these cells (50, 171, 247). Although the inability to study alveolar type I cells in culture has hindered progress in assessing their capacity for ion transport and the role they may play in vectorial fluid transport across the alveolar epithelium, the new evidence provides suggestive though not conclusive evidence that alveolar type I cells may participate in vectorial salt transport in the lung.

The alveolar epithelium comprises 99% of the surface area of the lung, a finding that suggests that removal of edema fluid from the lung might primarily occur across the alveolar epithelium. However, this conclusion may be misleading. It has been demonstrated that the distal airway epithelium actively transports sodium, a process that depends on amiloride-inhibitable uptake of sodium on the apical surface and extrusion of sodium through a basolateral Na\(^+\)-K\(^+\)-ATPase (5, 13, 14, 41, 42, 159, 382). It appears that in Airways <200 μm in diameter, sodium absorption predominates (5, 13, 14) (Fig. 1). Secretion of chloride may occur in the trachea and larger airways, and perhaps in distal airspaces of some animal species in the presence of cAMP stimulation (254). In support of the potential role of distal airway cells is evidence that Clara cells actively absorb sodium and transport from an apical to basal direction (358, 359). Also, there are new data on the possible role of cystic fibrosis transmembrane conductance regulator (CFTR) in upregulating cAMP fluid clearance (see sect. III). This information provides support for a possible role of distal airway epithelia in fluid clearance, since CFTR is expressed abundantly in distal airway epithelial cells. Also, there is new evidence that some alveolar fluid may be removed by convective surface active forces that might propel alveolar edema fluid into the distal airways where some absorption may occur across the respiratory bronchiolar epithelium (263, 372). Thus, even though their surface area is limited, a contribution from distal airway epithelia to the overall fluid transport is probable, especially since cells from the distal airway epithelium primarily transport salt from the apical to the basolateral surface (5, 13, 14, 41, 42, 159).

**B. Vectorial Fluid Transport in the Intact Lung**

A substantial number of innovative experimental methods have been used to study fluid and protein transport from the distal airspaces of the intact lung including isolated perfused lung preparations, in situ lung preparations, surface fluorescence methods, and intact lung preparations in living animals for short time periods (30–240 min) or for extended time periods (24–144 h). The advantages and disadvantages of these preparations have been reviewed in some detail (228, 313).

The first in vivo evidence that active ion transport could account for the removal of alveolar edema fluid across the distal pulmonary epithelium was obtained in studies of anesthetized, ventilated sheep (229). In those studies, the critical discovery was that isosmolar fluid clearance of salt and water occurred in the face of a rising concentration of protein in the airspaces of the lung, whether the instilled solution was autologous serum or an isosmolar protein solution. The initial protein concentration of the instilled protein solution was the same as the circulating plasma. After 4 h, the concentration of the protein had risen from ~6.5 to 8.4 g/100 ml, while the plasma protein concentration was unchanged. In longer term studies in unanesthetized, spontaneously breathing sheep, alveolar protein concentrations increased to very high levels. After 12 and 24 h, the alveolar protein concentration increased to 10.2 and 12.9 g/100 ml, respectively (226). The overall rise in protein concentration was equivalent to an increase in distal airspace protein osmotic pressure from 25 to 65 cmH\(_2\)O (Fig. 2). The concentration of protein in the lung lymph draining the lung interstitium declined, providing further evidence that pro-

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**FIG. 2.** In spontaneously breathing unanesthetized sheep, alveolar fluid protein concentration increased from 6.0 g/dl to a maximum of 13.8 g/dl (x-axis) after 24 h. This increase in protein concentration was paralleled by a marked increase in protein oncotic pressure (y-axis). [Data adapted from Matthay et al. (226).]
tein-free fluid was being reabsorbed from the distal airspaces into the lung interstitium (30, 229). Also, morphological studies showed that the interstitial fluid did not contain the Evans blue dye-labeled alveolar protein (224). These data provided convincing evidence that active ion transport must be responsible for the fluid clearance, especially in the face of a rising alveolar protein osmotic pressure that was ~40 cmH2O greater than the protein osmotic pressure in the vascular or interstitial spaces of the lung (226, 229).

Other studies in the intact lung have supported the hypothesis that removal of alveolar fluid requires active transport processes. For example, elimination of ventilation to one lung did not change the rate of fluid clearance in sheep, thus ruling out changes in transpulmonary airway pressure as a major determinant of fluid clearance, at least in the uninjured lung (309). Furthermore, if active ion transport were responsible for fluid clearance, then fluid clearance should be temperature dependent. In an in situ perfused goat lung preparation, the rate of fluid clearance progressively declined as temperature was lowered from 37 to 18°C (323). Similar results were obtained in perfused rat lungs (306) and ex vivo human lung studies (308) in which hypothermia inhibited sodium and fluid transport.

Additional evidence for active ion transport was obtained in intact animals with the use of amiloride, an inhibitor of sodium uptake by the apical membrane of alveolar epithelium and distal airway epithelium. Amiloride inhibited 40–70% of basal fluid clearance in sheep, rabbits, rats, guinea pigs, mice, and in the human lung (21, 22, 30, 74, 94, 121, 164, 258, 266, 308, 327, 386). Amiloride also inhibited sodium uptake in distal airway epithelium from sheep and pigs (5, 13). To further explore the role of active sodium transport, experiments were designed to inhibit Na+/K+-ATPase. It has been difficult to study the effect of ouabain in intact animals because of cardiac toxicity. However, in the isolated rat lung, ouabain inhibited >90% of fluid clearance (21, 270). Subsequently, after the development of an in situ sheep preparation for measuring fluid clearance in the absence of blood flow, it was reported that ouabain inhibited 90% of fluid clearance over a 4-h period (309).

Important species differences in the basal and stimulated rates of fluid clearance have been identified; not all species respond to cAMP agonists (Table 1). To normalize for differences in lung size or the available surface area, different instilled volumes were used ranging from 1.5 to 13.0 ml/kg. The slowest fluid clearance was measured in dogs (28, 135), intermediate rates of fluid clearance in sheep and goats (30, 226, 229, 323), and the highest basal fluid clearance rates in rabbits, guinea pigs, rats, and mice (121, 124, 164, 258, 327). The basal rate of fluid clearance in the human lung has been difficult to estimate, but based on the isolated, nonperfused human lung model, basal fluid clearance rates appear to be intermediate to fast (308). In fact, the fluid clearance in the ex vivo human lung is approximately one-half of the rate in the ex vivo rat lung (307). However, as discussed in section V, studies in patients suggest that the rate of maximal fluid clearance from the human lung may be fast. The explanation for the species differences is not apparent, although it may be related to the number or activity of sodium or chloride channels or the density of Na+/K+-ATPases in alveolar epithelium in different species. Morphometric studies (76, 282) have shown no significant difference in the number of alveolar type II cells in different species. It is also possible that the contribution of the distal airway epithelium may not be uniform in all species.

C. Sodium Transport in Cultured Alveolar Type II Cells

The success in obtaining nearly pure cultures of alveolar epithelial type II cells from rats made it possible to study the transport properties of these cells and relate the results to the findings in the intact lung studies. The initial

**TABLE 1. Fluid clearance in different animal species under control conditions and after stimulation with cAMP agonists or inhibition with amiloride or ouabain**

<table>
<thead>
<tr>
<th>Species</th>
<th>Baseline, Fluid Clearance Over 1 h, %</th>
<th>% Increase Over Baseline</th>
<th>Amiloride Sensitivity, % Decrease Below Baseline</th>
<th>Ouabain Sensitivity, % Decrease Below Baseline</th>
<th>Reference Nos.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mouse</td>
<td>48–60</td>
<td>58–71</td>
<td>63–91</td>
<td>80</td>
<td>103, 121, 122, 146, 155, 236</td>
</tr>
<tr>
<td>Guinea pig</td>
<td>38</td>
<td>32</td>
<td>26</td>
<td>NA</td>
<td>258</td>
</tr>
<tr>
<td>Rabbit</td>
<td>36</td>
<td>0</td>
<td>81</td>
<td>NA</td>
<td>327</td>
</tr>
<tr>
<td>Sheep†</td>
<td>8</td>
<td>50–60</td>
<td>42</td>
<td>90</td>
<td>30, 226, 229</td>
</tr>
<tr>
<td>Dog†‡</td>
<td>2–4</td>
<td>73–150</td>
<td>31</td>
<td>84</td>
<td>28, 135, 191, 210</td>
</tr>
<tr>
<td>Human‡</td>
<td>3</td>
<td>72–100</td>
<td>42</td>
<td>50</td>
<td>307, 308</td>
</tr>
</tbody>
</table>

All values are adapted from other studies. † Values are corrected for 25-min instillation time. † Studies were done originally over 4 h, and the values are extrapolated to 1 h in the table. ‡ Resected lung lobe model. § Ouabain was only applied to the apical surface in the human lung; thus basolateral penetration may have been incomplete. NA, not available.
studies showed that when type II cells were cultured on a nonporous surface such as plastic, they would form a continuous confluent layer of polarized cells after 2–3 days (131, 214). Interestingly, after 3–5 days, small domes of fluid could be appreciated from where the substratum was detached (Fig. 3). The domes were thought to result from active ion transport from the apical to the basal surface with water following passively since they were inhibited by the replacement of sodium by another cation or by pharmacological inhibitors of sodium transport, such as amiloride and ouabain (132). More detailed information on the nature of ion transport across alveolar type II cells was obtained by culturing these cells on porous supports and mounting them in Ussing chambers and measuring short-circuit current and ion flux under voltage-clamp conditions. Confluent monolayers with tight junctions were formed by 4–5 days later, and with considerable care to the culture conditions, it has been possible in some, but not all studies, to obtain monolayers with a high transepithelial resistance (2,000 \( \Omega \) \( \text{cm} \) \(^2 \)) (60). Alveolar type II cell monolayers are capable of generating spontaneous transmembrane potential difference (PD) related to electrogenic transport, and it is interesting to note that the spontaneous PD values (0.2–10 mV with apical membrane negative) (60, 73, 337) are similar to those obtained in vivo between the alveolar lumen and the pleural surface (255). When the apical and basolateral compartments are bathed with solutions with identical ionic compositions and the transepithelial potential is maintained (clamped) to zero, the measured current is termed short-circuit current \( (I_{sc}) \). The \( I_{sc} \) in alveolar type II cells monolayers corresponded closely to the net sodium absorption; substitution of sodium by equimolar concentration of choline in the apical side of monolayers or addition of ouabain that have the ability to block Na\(^+\)-K\(^+\)-ATPase at the basolateral side, quickly decreased \( I_{sc} \) to zero (60). With the use of labeled sodium (\(^{22}\)Na), unidirectional flux from the apical to the basolateral surface exceeded the flux in the opposite direction (176), a finding that was similar to results in isolated perfused lung preparations (21, 22, 74, 93, 94, 133).

The coordinated role of apical and basolateral sodium transport has been studied in several in vitro studies. Sodium ions that enter the epithelial cells at the apical membrane are pumped out of the cells at the basolateral membrane by the Na\(^+\)-K\(^+\)-ATPase enzyme. Because of the continuous pumping, sodium chemical potential is lower inside the cell. The entry step is passive and then sodium flows down a chemical potential gradient through specialized pathways, where basolateral transport requires energy to move ions against the gradient. Because of the pump activity, potassium electrochemical potential is larger inside the cell, and potassium leaks through the basolateral membrane and is then recycled by the Na\(^+\)-K\(^+\)-ATPase. The pathways for sodium entry into alveolar type II cells are numerous. Amiloride blocked dome formation (131) and decreased \( I_{sc} \) in the in vitro studies (60), a finding that supported the critical importance of sodium uptake through an amiloride-sensitive pathway in the apical membrane of alveolar epithelial cells. As already discussed, the efficacy of amiloride as an inhibitor of fluid clearance in the intact lung was demonstrated in several in vivo studies, although the fraction of amiloride-sensitive transport was as low as 40–50% in some lung preparations, particularly the rat and the human lung. The amiloride-insensitive sodium influx may be represented in vivo in part by the Na\(^+\)-glucose cotransport (21, 22). In vitro, the amiloride-insensitive sodium pathways are not clearly defined, but several sodium cotransporters such as Na-amino acid, Na-phosphate, Na-proton, as well as other cation channels may be involved (46, 68, 69, 256).

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**FIG. 3. A:** light micrograph of domes from primary culture of alveolar epithelial type II cells. The domes formed spontaneously after 3 days in culture. Dome formation was inhibited by amiloride. **B:** schematic representation of a dome of fluid accumulation between cultured alveolar epithelial type II cell monolayers. The type II cells actively transport sodium from the culture medium to beneath the monolayer (dashed arrows), which results in fluid accumulation between the cell monolayer and the culture dish. The apical cell surface (microvilli) is shown in contact with the medium; the basolateral surface is shown facing the culture dish. [From Mason et al. (214), with permission from the National Academy of Sciences USA.]
D. Phenotype of Cultured Alveolar Type II Cells

One important unresolved issue is to what extent cultured alveolar type II cells retain their in vivo phenotype. Within the first 24 h of culture, some of the morphological and functional characteristics of the alveolar type II cells are lost (81, 86). Most surfactant protein expression is downregulated (202), and some transport proteins such as Na-phosphate and Na$^+\cdot$K$^+\cdot$Cl$^-$ cotransport vanish (68, 69). However, active ion transport can be measured for up to 7 days. Also, some data indicate that culture conditions may help the cells to retain features of type II cells. For example, alveolar type II cells cultured on collagen gels with culture medium containing fetal calf serum and with an apical surface exposed to air retain morphological characteristics of alveolar type II cells for a longer duration (85). These cells express surfactant proteins and their mRNAs and express a plasma membrane marker specific for alveolar type II cells. Recent work (163) indicates that in vitro culture conditions are an important determinant for the biophysical characteristics of sodium channels. Alveolar type II cells grown on a permeable surface with an air interface and glucocorticoids in the medium maintain alveolar type II cell phenotype and express highly selective sodium channel while cells cultured on glass and in air-liquid interface express a low selective sodium channel.

E. Lung Epithelial Fluid Transport

This section considers pharmacological, biophysical, and the molecular basis for fluid clearance across the alveolar and distal airway epithelium.

1. Amiloride-sensitive sodium channels and ENaC

A number of studies using patch-clamp techniques, measurements of $^{22}$Na uptake, and binding of amiloride analogs have demonstrated the presence of amiloride-sensitive sodium channels in alveolar type II cells. On the basis of the biophysical and pharmacological characterization, at least three types of amiloride-sensitive sodium channels have been described in the apical membrane of cultured adult alveolar type II cells (220). The most frequently observed in both inside-out and cell-attached configurations is a nonselective cation (NSC) channel with a conductance of ~21 pS, which was equally permeable to sodium and potassium ($P_N/P_K \sim 1$, voltage-independent calcium-activated and completely blocked by 1 $\mu$M amiloride (105). A similar channel was described in fetal distal lung epithelial cells (213, 274). Also, others have recorded a channel with a conductance of 27 pS with a high relative permeability of sodium to potassium (7:1), completely blocked by 1 $\mu$M amiloride or its analog N-ethyl-N-isopropropylamiloride (EIPA) (388). Finally, the presence of highly selective cation (HSC) channels has been identified in adult alveolar type II cells cultured on permeable support, in air-liquid interface with aldosterone. These channels recorded on cell-attached configuration have a low conductance (5–7 pS) and were inhibited by a low concentration of amiloride (<0.1 $\mu$M) (163). These properties are similar to those described in fetal alveolar epithelial cells (370). Treatment of A549 cells with dexamethasone also shifted the conductance of the channel from 8 to 4 pS (194). A variety of other channels have been recorded in alveolar type II cells including ones regulated by G proteins (220).

Molecular identification of the proteins involved in amiloride-sensitive sodium influx has been achieved in the last few years. Three homologous subunits, entitled $\alpha$, $\beta$, and $\gamma$-ENaC, correspond to the pore-forming subunits. The three subunits share in common a structure predicting two hydrophobic membrane-spanning regions, intracellular amino and carboxy termini, two transmembrane spanning domains, and a large extracellular loop with highly conserved cysteine residues (51, 298, 330). Not all investigators agree on the numbers of subunits that form functional channels; some propose that ENaC is a tetramer made of two $\alpha$-, one $\beta$-, and one $\gamma$-subunit that assemble pseudosymmetrically around the channel pore to form a high amiloride affinity sodium-selective pore (111, 184), whereas others contend that ENaC is a much larger complex of nine (3 $\alpha$, 3 $\beta$, 3 $\gamma$) subunits (97, 329). An important role has been established for ENaC in the distal renal tubule and the collecting ducts and in the colon (15, 96, 223, 299). Some of the data suggest that individual ENaC subunits may have independent functions. For example, there are differences in the onset of expression of the three genes in the fetal lung (78, 348), and the relative levels of mRNA for the subunits can vary significantly in different regions of the lung, kidney, and colon (15, 87, 96, 104, 126, 223, 232). Xenopus oocyte studies predict a greater dependence of amiloride-sensitive sodium transport on $\alpha$-ENaC than on $\beta$-ENaC or $\gamma$-ENaC (52, 237). Low levels of sodium transport (1% of maximal) could be detected in oocytes injected with $\alpha$-ENaC alone but not in oocytes that expressed the $\beta$- or $\gamma$-subunits. Also, coinjection of $\beta$- or $\gamma$-subunits with $\alpha$-ENaC increased sodium transport approximately fivefold, and maximal current required expression of all the three subunits. When coexpressed, the three subunits reproduce the expected properties of the HSC channel. In A549 lung epithelial cells, dexamethasone increases the expression of $\beta$- and $\gamma$-ENaC and markedly shifts the IC$^{50}$ for amiloride (194).

The critical role of ENaC in the absorption of salt and fluid by lung epithelium has been confirmed by the generation of knockout mice with inactivated subunits of ENaC. After inactivation of murine $\alpha$-ENaC, deficient neonates develop respiratory distress syndrome and die within 40 h
of birth, primarily from failure to clear their lungs of fluid (154). In contrast, β- or γ-ENaC knockout mice were able to clear fluid from lungs at birth, although at a slower rate than in wild-type control, but these mice died from abnormal electrolyte reabsorption in the kidney resulting in fatal hyperkalemia (16, 233). These results suggest that small levels of activity provided by the remaining intact subunits (αβ-subunit or αγ-subunits) are enough to maintain respiratory function but are not sufficient to absorb sodium in the kidney. More definitive answers regarding the role of ENaC should be forthcoming from inducible knockout ENaC mice, i.e., CRE-lox mice in which tetracycline can transiently suppress ENaC.

In humans, loss-of-function mutations in the α-, β-, and γ-ENaC subunits have been described in systemic pseudohypoaldosteronism (173, 316). Patients had no respiratory symptoms at birth but developed, within weeks or months after birth, respiratory illnesses characterized by chest congestion and cough caused by an excessive volume of liquid that resulted from sodium channel dysfunction in the airways.

In situ hybridization studies, as well as Northern blot analyses done in mice, rats, and humans, have identified the presence of mRNA for all three subunits of ENaC in alveolar epithelial type II cells both in vivo and in vitro with usually a greater expression of α- than β- and γ-subunits (64, 75, 78, 162, 220, 223, 232, 238, 264, 315, 345, 348). The relative amounts of ENaC mRNA could explain the difference in ENaC activity and amiloride sensitivity between tissues and species, although a direct relationship between mRNA and functional protein cannot be predicted. As previously mentioned, patch-clamp studies using freshly isolated or cultured rat alveolar type II cells have reported the presence of a nonselective cation (NSC) channel and have often failed to identify the classical HSC channel that would be expected from ENaC expression. Recent studies have provided evidence that HSC channels are expressed in the apical membrane of adult alveolar type II cells under specific culture conditions (163, 194). All studies showing the presence of a 4-pS channel were done after exposure of alveolar type II cells or A549 cells to aldosterone or dexamethasone. In these cells, the number of HSC channels decreased after treatment with antisense oligonucleotides to any of the three subunits of ENaC: α-antisense reduced all channel types while treatment with β- or γ-antisense increased NSC channels (163). Therefore, in alveolar type II cells, ENaC may be expressed in different subunit combinations that determine sodium channel characteristics and render them less sensitive to amiloride (35, 268). This observation raises the possibility that ENaC-like channels could be present in vivo. As described in section II, new evidence has localized the three subunits of ENaC protein to alveolar type I cells also (38, 168).

In intact rat, sheep, mouse, or human lung, a high concentration of amiloride (~1 mM) was required to inhibit 50–60% of fluid clearance. The discrepancy between in vivo and in vitro studies in which 1 μM is required to completely block epithelial sodium channels (105, 388) is not fully understood but could be due in part to the binding of amiloride to protein, a rapid degradation of amiloride after instillation (386), and/or escape from the airspaces (146, 266). Nevertheless, in vivo studies suggest that amiloride has poor affinity for sodium channels that are present in alveolar epithelium. In one rat study, EIPA, a pharmacological inhibitor primarily of low-affinity amiloride channels, inhibited an equal fraction of fluid clearance as amiloride (386). These results suggest that active sodium transport in vivo involves in part low-affinity amiloride-sensitive channels, i.e., NSC channels.

Several investigators have investigated how ENaC is regulated by transcriptional, translational, and posttranslational mechanisms (275, 276, 304). It is clear that understanding the regulation of ENaC processing, trafficking to, and stability at the cell surface is of fundamental importance (304).

2. Amiloride-insensitive sodium transport

Studies in several species including sheep, rabbit, guinea pig, rat, and human lungs demonstrated that there was a substantial fraction of fluid clearance that could not be inhibited by amiloride (30, 164, 258, 266, 308, 327, 386). In the rat and human lung, for example, 40–50% of the basal fluid clearance is amiloride insensitive while in mice ~20% of fluid clearance is insensitive to amiloride, although the latter result has been mainly identified in CD-1 mice (121). In CD57BL/6 mice, ~40% of the fluid clearance is amiloride insensitive (146).

There is some limited evidence that the amiloride-insensitive fraction of 8-BrcGMP stimulated INa and sodium uptake in rat tracheal epithelia was inhibited by dichlorobenzamil or l-cis-diltiazem, both inhibitors of cyclic nucleotide-gated cation channels (CNG) (322). In subsequent studies, dichlorobenzamil inhibited a significant fraction of lung fluid absorption in sheep (170), a finding that supported the hypothesis that CNG channels could play a role in fluid absorption from the distal airspaces. The CNG channels were originally identified and cloned from vertebrate rod photoreceptors (109) and the olfactory neuroepithelium (246). Three different isoforms have been cloned (33). The CNG1 channel has a wide tissue distribution including expression in the lung; mRNA for CNG1 has been localized to distal lung epithelium and alveolar epithelial cells (82). In a recent rat study, l-cis-diltiazem inhibited a fraction of terbutaline or cGMP-stimulated fluid clearance that was not inhibited by amiloride, suggesting that part of the amiloride-insensitive fraction fluid clearance in the distal lung could be mediated through CNG channels, activated by cGMP (82, 261).
Also, there is recent data that inducible nitric oxide synthase (iNOS) (-/-) mice have normal rates of basal fluid clearance, but none of the basal fluid clearance is inhibited by amiloride (146).

In the intact rat, the amiloride-insensitive part of fluid clearance may be mediated in part through the Na\(^{+}\)-glucose cotransporter (23). The amiloride-insensitive part of fluid clearance is abolished when sodium is replaced by choline in the instillate or when phlorizin, a specific inhibitor of the Na\(^{+}\)-glucose cotransport, is added in the instillate. However, in one study, only sodium channel inhibition slowed lung water clearance in neonatal animals (265). On balance, the importance of Na\(^{+}\)instillate. However, in one study, only sodium channel inhibition slowed lung water clearance in neonatal animals (265). On balance, the importance of Na\(^{+}\)-glucose cotransport for fluid clearance from the airspaces remains unproven. Surprisingly, although SGLT1 mRNA is detected in mouse lungs, suggesting the presence of Na\(^{+}\)-glucose transporter, luminal glucose has no influence on fluid clearance (155). In vitro there is no evidence that the Na\(^{+}\)-glucose cotransporter plays a major role in transepithelial sodium transport (174). Also, the role of other cotransporters such as Na-amino acid and Na-phosphate that are specifically expressed in the apical membranes of human, mouse, and rat type II alveolar epithelial cells, remains to be defined (46, 69, 354). Some recent work indicates that SGLT1 can function as a cotransporter of water (89). Finally, regulation of vectorial fluid transport could be indirectly influenced by Na\(^{+}/\)H\(^{+}\) exchange, especially in the setting of chronic metabolic acidosis (160).

3. Na\(^{+}\)-K\(^{+}\)-ATPase and lung epithelial fluid transport

The Na\(^{+}\)-K\(^{+}\)-ATPase enzyme is a ubiquitous plasma membrane ion-transporting ATPase that maintains electrochemical sodium and potassium gradients across the plasma membrane by pumping sodium out of the cell and potassium into the cell against their respective concentration gradients, fueled by hydrolysis of ATP. The electrochemical gradient generated by extrusion of sodium from and uptake of potassium into the cell is essential for the secondary active movement of sodium ions. In most of the epithelia, including the alveolar epithelium, the Na\(^{+}\)-K\(^{+}\)-ATPase is confined to the basolateral domain of the cells; this polarized distribution is essential for the vectorial transport of sodium followed isosmotically by that of water while also controlling in its housekeeping mode cell volume and composition. In the alveolar epithelium, the Na\(^{+}\)-K\(^{+}\)-ATPase protein is detected primarily in type II cells with a weak expression in type I cells consistent perhaps with a predominant role of type II cells in resorbing alveolar fluid. The Na\(^{+}\)-K\(^{+}\)-ATPase is a heterodimeric transmembrane protein composed of one \(\alpha\)-and one \(\beta\)-subunit in a 1:1 ratio, although there are multiple isoforms of both subunits usually expressed in a tissue-specific pattern. The \(\alpha\)-isoform catalyzes the movement of sodium and potassium, is phosphorylated by ATP, and binds the specific inhibitor ouabain (326). The \(\alpha\)-isoform is the predominant form in alveolar type II cells, but cultured type I cells may express \(\alpha\) (303). The \(\beta\)-subunit has three isoforms and targets the assembled molecule to the plasma membrane. It is believed that a heterodimeric form composed of the \(\alpha\)- and \(\beta\)-subunits is the predominant Na\(^{+}\)-K\(^{+}\)-ATPase isoform in alveolar epithelial cells. It has been convincingly demonstrated that both \(\alpha\)- and \(\beta\)-isoforms are required for functional insertion of Na\(^{+}\)-K\(^{+}\)-ATPase in the membrane (235). In the lung, adenoviral gene transfer of the \(\beta\)-, but not \(\alpha\)-, subunit in the alveolar epithelium increases Na\(^{+}\)-K\(^{+}\)-ATPase expression and fluid clearance in the adult rat (102) and in confluent monolayers of fetal alveolar type II cells (351).

Removing edema fluid from the airspaces is accomplished by the polar distribution of Na\(^{+}\)-K\(^{+}\)-ATPases and channels on opposite poles of epithelial cells (129). Na\(^{+}\)-K\(^{+}\)-ATPase activity in the plasma membrane is regulated acutely by covalent or allosteric modification and/or acute trafficking of Na\(^{+}\)-K\(^{+}\)-ATPases between the plasma membrane and intracellular endosomal pools, and chronically by regulation of the abundance of Na\(^{+}\)-K\(^{+}\)-ATPases in the membrane by changes in Na\(^{+}\)-K\(^{+}\)-ATPase synthesis and/or degradation rates (31).

4. Aquaporins and lung epithelial fluid transport

In 1993, the first transcellular water channel, aquaporin-1 (AQP1), was cloned (3). Since that time, the list of known aquaporins has grown rapidly to approximately 10 mammalian water channels (182, 366). Four aquaporins have been localized to the lung with nonoverlapping distribution (365). In rat and mouse lung, AQP1 is present in both apical and basolateral membrane of microvascular endothelial cells and in fibroblasts, while AQP3, -4, and -5 polarize to the apical or basolateral membrane at different locations in the respiratory epithelium (179). Recent work in the human lung demonstrates that there are many similarities between the distribution of aquaporins in the rodent and human lung, but there are some additional areas of expression in the human respiratory tract (185). In addition to its expression on the apical surface of alveolar type I cells, AQP5 is located in the apical membrane of airway epithelium as well as in the superficial epithelium of the nasopharynx (185). AQP3 was expressed in the apical membrane of columnar cells as well as in basal cells. There seems to be some AQP3 in type II cells and AQP4 in alveolar epithelial cells (185).

The identification of aquaporins in the lung naturally suggested the hypothesis that aquaporins might contribute to the developmental regulation of lung fluid balance in the formation or reabsorption of fetal lung fluid, the pathogenesis or resolution of pulmonary edema in the mature lung, or in the humidification of the airways.
These possibilities have been studied in several lung preparations over the last few years.

The initial studies measured the water permeabilities of the endothelial and epithelial barriers in the lung in conjunction with studies that determined the cellular expression of aquaporins in the mature lung. Water permeability across a simple barrier separating two compartments is characterized by an osmotic water permeability coefficient ($P_f$) defined as the volume flux ($J_v$) induced by an osmotic gradient: $P_f = J_v/(V_w C)$, where $S$ is the barrier surface area, $V_w$ is the partial molar volume of water, and $C$ is the osmotic gradient. High $P_f$ (generally >0.0005–0.01 cm/s) suggests a facilitated water pathway involving molecular water channels (53). More detailed description of the biophysics of measuring water permeability is available in several sources (2, 3, 54, 362, 364).

In the lung, osmotic water permeability was found to be high in the intact sheep lung across the alveolar-capillary barrier, 0.02 cm/s (113). Additional studies were done in mice with a novel fluorescence method that provided similar data, namely, a high water permeability across the alveolar-capillary barriers, 0.017 cm/s. Aquaporin localization studies had already demonstrated that AQP1 was distributed primarily throughout the lung capillary endothelium, whereas AQP5 was localized primarily to the apical surface of alveolar epithelial type I cells (113, 181, 252, 321). Interestingly, measurements of osmotic water permeability of isolated alveolar epithelial I cells indicated that these cells have a high water permeability, 0.07 cm/s (84). A relatively high water permeability was also measured across isolated microperfused distal airways from guinea pigs, 0.005 cm/s (112). All of these studies provided indirect evidence for a possible functional role of aquaporins in lung fluid balance in the lung as potential rate-limiting channels for isosmolar fluid clearance from the distal airspaces of the lung. However, because there are no reliable, nontoxic inhibitors of water transport, it was necessary to generate specific aquaporin knockout mice to test the importance of specific aquaporins in isosmolar vectorial fluid transport under physiological conditions across the distal pulmonary epithelium.

AQP1 knockout mice had a modest decrease in lung interstitial fluid accumulation in the presence of a hydrostatic stress, but there was no effect on isosmolar fluid clearance across the distal pulmonary epithelium (9). The potential contribution of AQP5 to isosmolar fluid clearance in knockout mice was tested under both basal and stimulated conditions. Even when isosmolar fluid transport in the mouse was increased to a maximum level of 30% in 15 min, the absence of this apical type I cell membrane water channel did not slow the rate of fluid clearance (9, 331, 364, 365, 367). It is unlikely that the discovery of other water channels would alter the results of these studies because deletion of AQP1 or AQP5 each produced a 10-fold decrease in osmotically driven water transport between the airspace and capillary compartments.

Although aquaporin expression is strongly upregulated near the time of birth (180, 355, 385), AQP1, AQP4, and AQP5 knockout mice have the same ability to clear lung fluid in the perinatal period as wild-type controls (331). One study suggested that aquaporins might play some role in lung fluid balance after pneumonia because there was decreased expression of AQP4 and AQP5 in the mouse lung after acute viral pneumonia (353). However, the decreased expression may simply have reflected loss of functional epithelial cells from the pneumonia. Furthermore, knockout of AQP1, -4, and -5 had no effect on the formation or resolution of experimental pulmonary edema from hyperoxia, thiourea, or acid instillation (331). Finally, recent data indicate that the elimination of AQP3 does not delay the normal humidification process in the airways of mice (332).

The insensitivity of fluid clearance to aquaporin deletion is probably the consequence of the substantially lower rate of active fluid absorption per unit surface area in the lung compared, for example, with the proximal tubule of the kidney or the salivary gland, organs in which aquaporin deletion does alter fluid absorption or secretion (362, 363). Slower rates of active fluid transport probably do not require very high cell membrane water permeabilities.

Although a major role of AQP5 in physiological transalveolar epithelial water movement appears unlikely, aquaporins may have effects on other cell functions, especially volume regulation, particularly in the highly specialized type I cell (152, 199), in alveolar fluid homeostasis (206), or in the regulation of fluid secretion from mucus secreting cells (207).

III. REGULATION OF SALT AND WATER TRANSPORT

This section considers how vectorial fluid transport across the distal pulmonary epithelium can be upregulated by either catecholamine-dependent or catecholamine-independent mechanisms. The potential relevance of these mechanisms under pathological conditions is evaluated in section V.

A. Catecholamine-Dependent Regulation

Until recently, most studies focused primarily on the active transport of sodium as the primary determinant for regulating catecholamine-dependent transport across distal pulmonary epithelium. New evidence indicates that cAMP-stimulated uptake of chloride may be an important mechanism for regulating fluid clearance across distal lung epithelium (103, 165, 166, 267). Therefore, this sec-
tion is divided into the effects of catecholamines on fluid transport based primarily on studies that examined the effects of sodium transport inhibitors. Section 1A2 considers the new evidence regarding a potential role for chloride transport in cAMP-mediated transport.

1. Upregulation of fluid transport by sodium-dependent mechanisms

Studies in newborn animals indicate that endogenous release of catecholamines, particularly epinephrine, may stimulate reabsorption of fetal lung fluid from the airspaces of the lung (45, 110, 259, 260, 371). In most adult mammal species, stimulation of \( \beta_2 \)-adrenergic receptors either by salmeterol, terbutaline, or epinephrine increases fluid clearance (30, 59, 74, 164, 210, 290, 307). This stimulatory effect occurs rapidly after intravenous administration of epinephrine or instillation of terbutaline in alveolar space and is completely prevented by either a nonspecific \( \beta_2 \)-receptor antagonist, propranolol, or in rats by a specific \( \beta_2 \)-antagonist (210, 290). In some species, such as the guinea pig, there is also evidence that stimulation of the \( \beta_1 \)-receptor will augment fluid clearance (258).

The increased fluid clearance by \( \beta_2 \) agonists can be prevented by amiloride, indicating that the stimulation was related to an increased transepithelial sodium transport (164, 258, 290). In anesthetized ventilated sheep, terbutaline-induced stimulation of fluid clearance was associated with an increase in lung lymph flow, a finding that reflected removal of some of the alveolar fluid volume to the interstitium of the lung (30). Although terbutaline increased pulmonary blood flow, this effect was not important, since control studies with nitroprusside, an agent that increased pulmonary blood flow, did not increase fluid clearance. Other studies have demonstrated that \( \beta \)-adrenergic agonists increased fluid clearance in rat (164), dog (28, 191), guinea pig (258), mouse (121, 124, 155), as well as human lung (307, 308). The presence of \( \beta_1 \) and \( \beta_2 \)-receptors on alveolar type II cells has been demonstrated in vivo by autoradiographic and immunochemistry techniques (19, 98, 201). In vitro, studies in cultured alveolar type II cells indicate that \( \beta \)-adrenergic agonists added at the apical or the basolateral sides increased \( I_{sc} \) that was abolished by amiloride (132, 217). In addition, terbutaline stimulated net apical-to-basolateral flux of \( ^{22} \text{Na} \), and the magnitude of this stimulation was similar to the steady-state increase in \( I_{sc} \) (60). Experiments in isolated lungs (130, 313) and in vitro studies provided further evidence that cAMP is a second messenger for the \( \beta \)-adrenergic effects, whereas activation of protein kinase C does not appear to be involved (26, 29). Interestingly, \( \beta \)-adrenergic agonist therapy does not increase fluid clearance in rabbits and hamsters (129, 327). The explanation for this lack of effect is unclear, particularly since there are \( \beta \)-receptors in rabbit type II cells that stimulate surfactant secretion (234). One study in rabbits suggested that cAMP agonists might stimulate chloride secretion in rabbits, thus perhaps offsetting any increase in vectorial fluid clearance from the distal airspaces (254).

On the basis of studies of the resolution of alveolar edema in humans, it has been difficult to quantify the effect of catecholamines on the rate of fluid clearance (230). However, studies of fluid clearance in the isolated human lung have demonstrated that \( \beta \)-adrenergic agonist therapy increases fluid clearance, and the increased fluid clearance can be inhibited with propranolol or amiloride (Fig. 4) (307, 308). Subsequent studies showed that long-acting lipid-soluble \( \beta \)-agonists are more potent than hydrophilic \( \beta \)-agonists in the ex vivo human lung (307). The magnitude of the effect is similar to that observed in other species, with a \( \beta \)-agonist-dependent doubling of fluid clearance over baseline levels. These data are particularly important because aerosolized \( \beta \)-agonist treatment in some patients with pulmonary edema might accelerate the resolution of alveolar edema (see sect. v).
the absence of short actin filaments (25). Thus the increase in open probability may have been the result of increased phosphorylation of short actin filaments or other cytoskeleton proteins. Finally terbutaline, a β₂-adrenergic agonist, may promote insertion of new channel protein from a cytoplasmic pool to the apical membrane.

In fetal distal lung cells, brefeldin A, an inhibitor of protein trafficking, prevented terbutaline-induced increase in open probability of Ca²⁺-activated nonselective channels (160).

One investigator reported that stimulation of thyroid epithelial cells transfected with α, β₁, and γ-ENaC with cAMP translocated ENaC from the cytoplasm to the cell surface, and that effect was dependent on a sequence (PPPXY) in the COOH terminus of each subunit (328). Existing evidence also suggests that agents that increase cAMP may increase sodium influx through an increase in apical chloride conductance without affecting apical membrane sodium conductance (165, 267).

One recent study using patch clamping of rat alveolar type II cells demonstrated that treatment with a β₂-adrenergic agonist, terbutaline, increased the number of highly selective sodium channels, an effect that was mediated by PKA (61). Also, in the same study, terbutaline increased both intracellular calcium levels and the open probability of NSC channels, an effect that was blocked by a calcium chelator (61). The authors concluded that β-adrenergic stimulation increase intracellular cAMP and the activation of PKA, and the PKA promotes an increase in the number of highly selective sodium channels and an increase in intracellular calcium. The increase in intracellular calcium increases the open probability of NSC channels. Interestingly, other investigators recently found that both extracellular and intracellular calcium may act as second messengers for β-adrenergic stimulation of fluid clearance from the distal airspaces of near-term fetal guinea pigs (260). Other investigators have also demonstrated that calcium channel activation and cell volume changes alter the rate of sodium transport via NSC channels (211–213).

β-Adrenergic stimulation increases extrusion of sodium at the basolateral side by increasing Na⁺-K⁺-ATPase activity. In alveolar type II cells, isoproterenol increased the maximal velocity of the enzyme independently of sodium permeability (340). The stimulation of Na⁺-K⁺-ATPase activity was associated with an increase in ouabain binding and expression of the α₁-subunit at the membrane (32). This effect occurred rapidly (<15 min) and was independent of protein kinase phosphorylation and mediated by subunit recruitment from late endosomes into the plasma membrane. There is some evidence that long-term exposure of alveolar type II cells to β-adrenergic agonists increased Na⁺-K⁺-ATPase and sodium channel activity, at least in part through a transcriptional effect, since cAMP increased transiently the α₁-subunit of Na⁺-K⁺-ATPase (239) and the three subunits of ENaC (77). Increased activity of PKA could lead to phosphorylation of the cAMP-responsive element protein, which could bind to the cAMP responsive element (CRE) site on the gene promoter. A CRE site has been demonstrated on the promoter region of α₁-subunit of Na⁺-K⁺-ATPase (4) and in the 5′-flanking region of γ-ENaC (350). However, other...
investigators have failed to demonstrate change in mRNA levels of Na\(^+\)-K\(^+\)-ATPase after long exposure to isoproterenol, whereas they found a posttranscriptional regulation via mitogen-activated protein kinase/ERK and rapamycin-sensitive pathways (280).

2. Potential role of CFTR in cAMP-mediated upregulated fluid transport

While most experimental studies have attributed a primary role for active sodium transport in the vectorial transport of salt and water from the apical to the basal surface of the alveolar epithelium of the lung, the potential role of chloride, especially in mediating the cAMP-mediated upregulation of fluid clearance across distal lung epithelium, has been the subject of a few recent studies. One older study of cultured alveolar epithelial cells concluded that vectorial transport of chloride across alveolar epithelium occurs by a paracellular route under basal conditions and perhaps by a transcellular route in the presence of cAMP stimulation (176). Another study of cultured alveolar epithelial type II cells suggested that cAMP-mediated apical uptake of sodium might depend on an initial uptake of chloride (165). A more recent study of cultured alveolar type II cells under apical air interface conditions reported that \(\beta\)-adrenergic agonists produced acute activation of apical chloride channels with enhanced sodium absorption (166). However, the results of these studies have been considered to be inconclusive by some investigators (193, 378, 379), partly 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 several different epithelial cells including alveolar epithelial type I cells as well as distal airway epithelial cells.

It should also be appreciated that the pathways for chloride secretion and absorption in the fetal and the neonatal lung are not well understood. Recent and older studies have demonstrated a role for chloride secretion and absorption in the fetal and perinatal lung (57, 117, 127, 273) as well as in distal fetal rat lung epithelial cells (70, 195), but the molecular basis for chloride transport is not well understood (262).

To define the role of chloride transport in the active transport of salt and water across the distal pulmonary epithelium of the lung, one group has used in vivo lung studies to define the mechanisms and pathways that regulate chloride transport during the absorption of fluid from the distal airspaces of the lung. This approach may be important because studies in several species, as already discussed, have indicated that distal airway epithelia are capable of ion transport (5, 13, 14), and both ENaC and CFTR are expressed in alveolar and distal airway epithelia (104, 123, 185, 284, 299).

Both inhibition and ion substitution studies demonstrated that chloride transport was necessary for basal fluid clearance. The potential role of CFTR under basal and cAMP-stimulated conditions was tested using intact lung studies in which CFTR was not functional because of failure in trafficking of CFTR to the cell membrane, the most common human mutation in cystic fibrosis (\(\Delta F508\) mice). The results supported the hypothesis that CFTR was essential for cAMP-mediated upregulation of isosmolar fluid clearance from the distal airspaces of the lung because fluid clearance could not be increased in the \(\Delta F508\) mice with either \(\beta\)-agonists or with forskolin, unlike the wild-type control mice (103). Additional studies using pharmacological inhibition of CFTR in both the mouse and human lung with glibenclamide supported the same conclusion, namely, that chloride uptake and CFTR-like transport seemed to be required for cAMP-stimulated fluid clearance from the distal airspaces of the lung (103). Glibenclamide can also inhibit potassium channels so the inhibitory effects are not specific for CFTR, but the \(\Delta F508\) mouse studies have provided more direct evidence. Although the absence of CFTR in the upper airways results in enhanced sodium absorption (336), the data in these studies provide evidence that the absence of CFTR prevents cAMP-upregulated fluid clearance from the distal airspaces of the lung, a finding that is similar to work on the importance of CFTR in mediating cAMP-stimulated sodium absorption in human sweat ducts (297). Because CFTR is distributed throughout the distal pulmonary epithelium in distal airway epithelium as well as the alveolar level in the human lung (95), the data also suggest that the cAMP-mediated upregulated reabsorption of pulmonary edema fluid may occur across distal airway epithelium as well as at the level of the alveolar epithelium. Finally, additional studies indicated that the lack of CFTR results in a greater accumulation of pulmonary edema in the presence of a hydrostatic stress, thus demonstrating the potential physiological importance of CFTR in upregulating fluid transport from the distal airspaces of the lung (103).

The new data on a role for CFTR in cAMP-upregulated fluid clearance from the distal airspaces raise further interest in determining how CFTR and ENaC interact and contribute to net fluid clearance under isosmolar conditions. The relative conductances for chloride and sodium are difficult to measure in the in vivo lung epithelium but conceivably the debate about the role of either ion in limiting isosmolar fluid clearance is somewhat misleading. Under open-circuit conditions, the net transfer of chloride and sodium across the distal lung epithelium must be equal, i.e., there cannot be a significant net charge accumulation. It is possible that with cAMP stimulation the conductances for both chloride and sodium increase in parallel. This hypothesis would be in accord with recent data on isolated distal lung epithelial cells in which
CFTR protein was immunolocalized to these cells and forskolin-stimulated $I_{sc}$ was inhibited by glibenclamide (195). Other investigators did not find evidence to support the hypothesis that cAMP-stimulated chloride conductance directly controlled sodium uptake in rat fetal distal lung epithelial cells (70), and these investigators concluded that chloride and sodium transport must be linked by some mechanism.

There are several interesting unanswered questions. What is the molecular basis for chloride transport under basal conditions across the distal lung epithelium, since it is clear that CFTR is not required for basal fluid clearance or for clearance of fetal lung fluid at birth (103)? Second, if CFTR is required for cAMP-mediated transport, does this mechanism apply to all levels of the distal lung epithelium including the alveolar epithelial type I and type II cells as well as the distal airway epithelia? Also, does upregulation of fluid clearance by cAMP-independent pathways require functional CFTR (see sect. iv)?

3. Regulation of $\beta_2$-receptor expression

There is new information regarding the effect of upregulating the expression of the $\beta_2$ receptor ($\beta_2$AR) in alveolar type II cells using the SP-C promoter in transgenic mice (236). $\beta_2$AR expression was 4.8-fold greater than that of the nontransgenic mice (939 ± 113 vs. 194 ± 18 fmol/mg protein, $P < 0.001$). Basal fluid clearance in the transgenic mice was 40% greater than that of untreated nontransgenic mice (15 ± 1.4 vs. 10.9 ± 0.6%, $P < 0.005$) and approached that of nontransgenic mice treated with $10^{-5}$ M of the potent $\beta$-agonist formoterol (19.8 ± 2.2%). Adrenalectomy had no effect on nontransgenic mice (11.5 ± 1.0%) but decreased basal fluid clearance in the transgenic mice to 9.7 ± 0.5%. These findings demonstrated that overexpression of the $\beta_2$AR can be an effective means to increase vectorial fluid transport in the absence of exogenous agonists.

Other investigators have used an adenoviral vector to deliver the $\beta_2$AR to the rat lung, which results in a comparable increase in fluid clearance that was also inhibited by adrenalectomy or by $\beta$-antagonists (99). In these studies, the $\beta_2$AR may be expressed in several cells in the distal airspaces of the lung. There was evidence that the $\beta_2$AR overexpression was associated with increased levels of ENaC ($\alpha$-subunit) and Na$^{+}$-K$^{+}$-ATPase in apical and basolateral cell membrane fractions isolated from peripheral lungs (88).

Some recent studies suggest that prolonged exposure to exogenous catecholamines may impair the ability of the alveolar epithelium to remove alveolar edema fluid and that this impairment was associated with a reduction in epithelial $\beta$-receptor number (244). It is important to bear in mind that although a mild impairment was evident at clinically used doses, infused $\beta$-agonist as well as insulin-stimulated $\beta$-agonist stimulated fluid clearance above baseline levels.

B. Catecholamine-Independent Regulation

In the last few years several interesting catecholamine-independent mechanisms have been identified that can upregulate fluid transport across the distal airspaces of the lung as well as in cultured alveolar type II cells. Hormonal factors, such as glucocorticoids, can upregulate transport by transcriptional mechanisms while thyroid hormone may work by a posttranslational mechanism (197). Some growth factors can work by either a transcriptional or direct membrane effect, or by enhancing the number of alveolar type II cells. There is also evidence that a proinflammatory cytokine, tumor necrosis factor-$\alpha$, can rapidly upregulate sodium uptake and fluid transport by a novel mechanism. Finally, serine proteases can regulate the activity of ENaC and potentially increase fluid clearance across the distal airway epithelium.

1. Glucocorticoids

Glucocorticoid hormones regulate sodium uptake and fluid transport in both adult and fetal lungs (20, 158, 299, 348). In adult guinea pig lungs, endogenous plasma levels of cortisol are important for maintaining normal lung fluid balance and distal airspace fluid clearance. Cortisol modulates the amiloride-sensitive sodium transport pathways through regulation of de novo synthesis of sodium channel proteins (257). Dexamethasone, a more potent glucocorticoid agonist than cortisol, regulates transepithelial sodium transport in vivo by increasing lung fluid clearance in anesthetized, ventilated rats (116). Based on in vitro studies, there was a functional increase in $I_{sc}$ across monolayers of alveolar type II cells after dexamethasone treatment (77). In A549 cell studies, dexamethasone increased the whole cell amiloride-sensitive current and decreased the $I_{C_{50}}$ for amiloride and increased the channel conductance (194). Dexamethasone increased Na$^{+}$-K$^{+}$-ATPase and sodium channel activity and expression by acting at both transcriptional and posttranscriptional levels. The molecular studies have reported that glucocorticoids directly stimulated the transcription of sodium transport proteins. In vivo experiments have demonstrated a differential regulation of ENaC subunits by corticosteroids: $\alpha$- but neither $\beta$- nor $\gamma$-ENaC mRNA levels increase within 8 h of treatment. Dexamethasone increases mRNA of the three subunits of ENaC in studies using primary cultures of fetal and adult lung cells (77, 299, 348), but only $\beta$- and $\gamma$-ENaC mRNA in the human alveolar epithelial cell line A549 (194). This latter observation is in contrast to the recent evaluation of the human $\alpha$-ENaC promoter showing the presence of glucocorticoid responsive element in the 5’-flanking re-
gion in human (315) and in rat (77, 276). Interestingly, one study indicated that the differential regulation of mRNA of ENaC subunits correlated with similar changes in the quantity of protein and with a change in selectivity of the sodium channel from essentially nonselective to highly selective (194). Also, glucocorticoids differentially regulate Na\(^{+}\)-K\(^{+}\)-ATPase subunit expression in fetal and adult rat lungs (20, 77, 158). In adult alveolar type II cells, dexamethasone increased \(\beta_{1}\) but not \(\alpha_{1}\)-Na\(^{+}\)-K\(^{+}\)-ATPase mRNAs (20, 77), while both \(\alpha_{1}\) and \(\beta_{1}\)-Na\(^{+}\)-K\(^{+}\)-ATPase proteins were increased. Because Na\(^{+}\)-K\(^{+}\)-ATPase activity increased only when the proteins of \(\alpha_{1}\) and \(\beta_{2}\)-Na\(^{+}\)-K\(^{+}\)-ATPase were upregulated, it was suggested that posttranslational events may be involved in the regulation of Na\(^{+}\)-K\(^{+}\)-ATPase by glucocorticoids (20).

2. Mineralocorticoids

The lung is a potential target organ for aldosterone because it expresses mineralocorticoid receptors and the enzyme 11\(\beta\)-hydroxysteroid dehydrogenase that converts the glucocorticoid corticosterone into receptor-inactive 11-dehydro-corticosterone, thereby allowing preferential access of aldosterone to mineralocorticoid receptors (186, 339, 341). There is excellent evidence that aldosterone plays a major role in the regulation of sodium transport in epithelial tissues, which has led to the hypothesis that aldosterone might also regulate salt transport in the lung. In vitro, overnight incubation of alveolar type II cells with aldosterone concentrations ranging from \(10^{-7}\) to \(10^{-4}\) M did not increase the \(I_{sc}\) (72). On the other hand, in isolated alveolar type II cells, aldosterone showed increased mRNA expression of \(\beta_{1}\)-Na\(^{+}\)-K\(^{+}\)-ATPase and increased amount of \(\alpha_{1}\) and \(\beta_{1}\)-Na\(^{+}\)-K\(^{+}\)-ATPase proteins, suggesting that aldosterone regulates \(\beta_{1}\)-subunit at the transcriptional/translational level while the \(\alpha_{1}\)-subunit is probably recruited from intracellular pool to the basolateral membrane (271). This effect was associated with a fourfold increase in the Na\(^{+}\)-K\(^{+}\)-ATPase hydrolytic activity and ouabain-sensitive \(^{86}\)Rb uptake (271). The in vivo relevance of these observations was substantiated by the finding that 24 h after aerosolized delivery of aldosterone there was a 50% increase in fluid clearance in rats (271). Similarly, rats treated with a low-sodium diet that developed a hyponatremiaism have an increase in fluid clearance related to a stimulation of amiloride-sensitive component (339). The effect of aldosterone on ENaC mRNA expression has not been studied. However, a new study found that alveolar type II cells cultured in the presence of aldosterone (10 \(\mu\)M) had an increase in the frequency of highly selective channels than cells cultured in the absence of aldosterone (163). These results suggest that aldosterone may regulate ENaC subunits as demonstrated in other epithelial cells (52, 87, 203–205, 231, 277, 368) and indicate that further studies will be useful to determine the molecular and cellular effect of aldosterone on ENaC in alveolar epithelial cells.

Also, there are data that estrogen may modulate mRNA levels and amiloride-sensitive \(I_{sc}\) in adult rat lung and cultured alveolar type II cells (342).

3. Growth factors

Several studies have reported that growth factors can upregulate sodium uptake and net fluid transport across the distal airspaces of the lung by several different mechanisms. This section considers the effects of three growth factors that have been studied.

A) EPIDERMAL GROWTH FACTOR. In vitro studies demonstrated that incubations of alveolar epithelial type II cells with epidermal growth factor (EGF; 20 ng/ml) for 4–5 days resulted in a significant increase in both \(\alpha_{1}\) and \(\beta_{2}\)-Na\(^{+}\)-K\(^{+}\)-ATPase mRNA and protein concentrations with no changes in ENaC subunit mRNA. These transcriptional effects were paralleled by an increase in benzamil-inhibitable \(I_{sc}\) (79). Subsequently, the same research group reported that alveolar type II cells cultured with EGF induced a decrease in mRNA for all three subunits of ENaC, and patch-clamp studies of these cells showed only 25-pS channels that were amiloride sensitive and relatively nonselective for cations (\(P_{Na}/P_{K} = 1.0:0.48\)). Channel density was increased with EGF. Thus the authors concluded that EGF induced the formation of NSC channels in cultured type II cells (172). Also, when EGF was aerosolized to rats, there was a marked increase in fluid clearance 24 h later. Because EGF has mitogenic properties, it is possible that some of the EGF effect in vivo could have occurred by stimulating type II cell proliferation, although there was no evidence for a mitogenic effect in the rat studies (344).

B) TRANSFORMING GROWTH FACTOR-\(\alpha\). EGF has been reported to increase sodium uptake and transport in isolated alveolar epithelial type II cells after direct in vitro exposure (37) as well as after prior in vivo exposure (161). EGF and transforming growth factor-\(\alpha\) (TGF-\(\alpha\)) have also been shown to stimulate intracellular accumulation of cAMP in several epithelial cell lines, heart cells, parotid gland cells, and embryonic cells (12, 245). Epithelial sodium transport can be regulated by tyrosine kinase in kidney epithelial cells (222), which is stimulated by both EGF and TGF-\(\alpha\).

Interestingly, TGF-\(\alpha\) can upregulate fluid clearance rapidly, apparently by direct membrane effects. One study in rats demonstrated a marked increase in fluid clearance within 1 h of instillation into the living rat lung (118). There was no increase in cAMP levels, and the effect was not inhibited by \(\beta\)-adrenergic blockade. However, genistein prevented the effect, suggesting that the effect might be mediated by tyrosine kinase activity. This observation may have relevance to patients with pulmonary
edema, since measurable quantities of TGF-α have been reported in patients with acute lung injury (62).

c) KERATINOCTYE GROWTH FACTOR. It is well know that keratinocyte growth factor (KGF) is a potent mitogen for alveolar epithelial type II cells. Administration of KGF (5 mg/kg) into the distal airspaces of the rat lung upregulated fluid clearance by 66% over baseline levels (373), an effect that was sustained for 120 h. Also, the magnitude of the increase in fluid clearance was comparable to the effect previously reported in rats with short-term upregulation from β-adrenergic agonists. There was a good correlation between the increase in the number of alveolar type II cells and the effect of KGF on upregulating fluid clearance (373). Although there was a modest increase in the expression of α-ENaC 72 h after administration of KGF, the predominant effect seemed to be related to the type II cell hyperplasia. This sustained upregulation of fluid clearance suggested potential therapeutic value of an epithelial-specific mitogen-like KGF. Furthermore, the addition of a β2-adrenergic agonist further upregulated fluid clearance in rats so that the combination of KGF treatment plus terbutaline resulted in a net clearance of 50% of the instilled fluid in 1 h in rats compared with control rats with fluid clearance rates of 23%/h. Other investigators have shown that KGF can enhance sodium and fluid transport in normal and injured rat lungs (137, 140). In vitro studies of the effect of KGF in cultured alveolar epithelial cells indicated that KGF might also work by enhancing the expression of α1-Na⁺-K⁺-ATPase subunits (39), thereby enhancing sodium transport independent of an effect on the number of alveolar type II cells.

5. Serine proteases

The activity of ENaC can be upregulated by serine protease activity (356). In one study, exposure of a kidney epithelial cells line (A6) to the protease inhibitor aprotinin reduced epithelial sodium transport, and sodium channel activity could be restored by exposure to a nonspecific protease trypsin. Expression of a serine protease in *Xenopus* oocytes along with ENaC increases the activity of the sodium channel by two- to threefold. This protease (CAP1) is expressed in lung as well as other organs. Thus it is possible that under normal and pathological conditions, ENaC activity could be regulated by the activity of proteases expressed at the surface of the same cell and thus may provide a mechanism for autocrine regulation of ENaC in the lung. Preliminary data in recent lung studies support this hypothesis (360).

IV. MECHANISMS THAT IMPAIR VECTORIAL FLUID TRANSPORT

Several mechanisms have been identified that can impair fluid transport from the distal airspaces of the lung. This section considers one hormonal factor, atrial natriuretic factor (ANF), and three other conditions that have relevance to human disease: hypoxia, anesthetics, and reactive oxygen and nitrogen species

A. Atrial Natriuretic Peptide

While several hormones are capable of upregulating epithelial fluid transport in the lung, as discussed in section III, atrial natriuretic peptide (ANP) is an example of a hormone that can downregulate fluid transport in the lung. ANP plays a major role in volume and electrolyte homeostasis, through potent biological effect including natriuresis, diuresis, and vasorelaxation. In addition to being a target organ for ANP from atrial origin, the lung is also a site of synthesis and release of bioactive ANP (142). The lung has the highest tissue concentration of ANP binding sites (295), and alveolar type II cells have been shown to express both guanylate cyclase (GC) receptor subtypes with a functional predominance of GC-A over the GC-B receptors but do not possess clearance receptors (49, 349). The GC receptors are present at the apical and at the basolateral sides, but cGMP expression is polarized at the basolateral side (349).

The functional role of ANP on salt and water transport in the lung is not clear. In one study in guinea pigs, ANP administration seemed to have a protective effect on the development of cardiogenic and noncardiogenic lung edema (157). However, in isolated perfused liquid-filled rat lungs, ANP increased alveolar epithelial permeability and decreased active sodium transport, thus decreasing...
alveolar liquid clearance (269). In sheep with left atrial hypertension, there was an increase in plasma levels of ANP that may have inhibited the normal upregulation of fluid clearance that should have occurred in the presence of a rise in endogenous catecholamines (49). In alveolar type II cells cultured on filters, ANP decreased the amiloride-sensitive component of $^{22}$Na influx (349) but did not change the basal Na$^{+}$-K$^{+}$-ATPase activity (49, 349). Interestingly, in the presence of β2-agonist stimulation, ANP inhibited Na$^{+}$-K$^{+}$-ATPase activity (49). Thus, on balance, it appears that ANP may impair fluid clearance by a direct effect on reducing sodium transport in alveolar type II cells.

### B. Hypoxia

Hypoxia may occur during residence or recreation at high altitudes and under a variety of pathological conditions associated with acute and chronic respiratory disease. Therefore, it is important to understand the effect of hypoxia on the ion and fluid transport capacity of the lung epithelium.

Both in vitro and in vivo studies clearly show that decreased O$_2$ tension reduces the capacity of alveolar epithelial cells to actively transport sodium across alveolar epithelium. In alveolar type II cells, hypoxia (0% and 3% O$_2$) inhibits domed formation (292), decreases both amiloride-sensitive $^{22}$Na influx and Na$^{+}$-K$^{+}$-ATPase activity, and decreases the amiloride-sensitive $I_{sc}$ (208, 209, 292, 293, 383), indicating that the transepithelial sodium transport is impaired. The mechanisms whereby hypoxia induces decreased sodium transport proteins activity depend on the severity and the length of hypoxic exposure. For long exposure times (>12 h), the decrease in amiloride-sensitive sodium channel and Na$^{+}$-K$^{+}$-ATPase activity was associated with a parallel decline in mRNA levels of the three subunits, α, β, and γ of rENaC and two subunits α$_1$- and β$_1$-Na$^{+}$-K$^{+}$-ATPase and the rate of α-rENaC protein synthesis, indicating a transcriptional or posttranscriptional regulation. For a short exposure time (3-h exposure), the decrease in $^{22}$Na influx and Na$^{+}$-K$^{+}$-ATPase activity preceded any detectable change in mRNA levels, findings that suggest other mechanisms may be involved in regulation, including decreased efficiency in the translation of rENaC mRNA or in apical membrane trafficking of rENaC subunits, abnormal degradation or internalization of the channel protein, or hypoxia-induced modification of intracellular signals (147, 292).

The decrease in gene expression of sodium proteins likely represents the direct effect of hypoxia and raised the question of whether gene expression for sodium channels and Na$^{+}$-K$^{+}$-ATPase is regulated by ambient P$_O_2$ in alveolar type II cells. In support of this hypothesis, an increase in O$_2$ tension upregulated the level of ENaC mRNA transcripts in alveolar type II cells. The transfer of fetal distal lung epithelial (FDLE) cells in culture from 3% O$_2$ to higher O$_2$ concentrations upregulated α, β, and γ-rENaC, mRNA subunits as well as sodium channel activity (10, 283, 296). Exposure of rats to sublethal hypoxia (85% O$_2$) increased alveolar type II cell α-rENaC mRNA level (387). In addition, it has been recently reported that α$_1$- and β$_1$-Na$^{+}$-K$^{+}$-ATPase mRNA subunit levels both increased in alveolar type II cells from rats exposed to hyperoxia (148). The mechanism whereby O$_2$ tension regulates sodium channels and Na$^{+}$-K$^{+}$-ATPase gene expression is thought to be transcriptional. Activation of ENaC by increased O$_2$ concentration is associated with NF-κB activation consistent with the identification of NF-κB transcription binding sites in the α-ENaC promoter region (294). In addition to the potential role for an O$_2$-responsive element in the ENaC promoter, it is also possible that there are other genes that are O$_2$ responsive and alter ENaC expression through their expressed protein or metabolic products.

The effect of hypoxia under in vivo conditions has been studied primarily in rats. In anesthetized rats, as well as in isolated perfused lungs, hypoxia decreased alveolar liquid clearance by inhibition of the amiloride-sensitive component (338, 369). In contrast to the in vitro studies, hypoxia increased α-rENaC and β$_1$-Na$^{+}$-K$^{+}$-ATPase mRNA transcripts with little increase or no change in protein amounts, suggesting a posttranslational mechanism such as a direct change of sodium transporter protein activity or protein internalization (369). This latter hypothesis was supported by the normalization of fluid clearance by a cAMP agonist (terbutaline), which is known to increase the trafficking of sodium transporter proteins from the cytoplasm to the membrane (48, 183, 328).

There is some limited data that ouabain-like substances may be released under conditions of hypoxia that could decrease epithelial fluid transport. A soluble factor that inhibits Na$^{+}$-K$^{+}$-ATPase activity is released from cultured rat alveolar type II cells after hypoxic exposure (293). Also, an endogenous ouabain-like compound has been found to be increased in high altitude in human plasma (80, 107, 108). One experimental study in rats that reported a reduction in airway epithelial sodium transport from hypoxia supported this hypothesis as well (352).

### C. Anesthetics

In alveolar epithelial cells, the halogenated anesthetics affect sodium and fluid transport at the physiological level as well as on a cellular level. In the rat, halothane and isoflurane decrease fluid clearance by inhibition of the amiloride-sensitive component. This effect was rapidly reversible after cessation of halothane exposure (301). Unlike the rat, the ability of the rabbit to clear fluid from the alveolar space through an amiloride-sensitive
pathway is not decreased by halothane (253). In vitro, exposure to a low concentration of halothane (1%) and for a short time (30 min) induced a reversible decrease in Na\(^+\)-K\(^+\)-ATPase activity and amiloride-sensitive \(^{22}\)Na influx in rat alveolar type II cells (242). The mechanisms whereby halothane induced a decrease in sodium transport protein activity have not been yet elucidated but are not related in vitro to a decrease in intracellular ATP content or to change in cytosolic free calcium. Also, some studies show that halothane increases permeability of alveolar capillary barrier to both water and protein as measured by the pulmonary clearance of \(^{99m}\)TcDTPA in both rabbit (384) and humans (58). Taken together, these observations suggest that halogenated anesthetics may interfere with the clearance of alveolar edema.

Lidocaine is widely used in patients with acute cardiac disorders and has also been recently implicated as a possible cause of pulmonary edema after liposuction. In experimental studies in rats, either intravenous or intralveolar lidocaine reduced fluid clearance in rats by 50% (189). Because lidocaine did not inhibit ENaC when expressed in oocytes, it seems that the inhibitory effect on vectorial fluid transport was primarily on the basal surface of alveolar epithelial cells, either through an effect on the activity of Na\(^+\)-K\(^+\)-ATPase or through an indirect effect through blockade of potassium channels, a well-known property of lidocaine (47, 156, 302, 357). The effect of lidocaine was completely reversible with \(\beta_2\)-agonist therapy (189).

D. Reactive Oxygen and Nitrogen Species

Under several pathological conditions, in response to proinflammatory cytokines, activated neutrophils and macrophages can localize in the lung and migrate into the airspaces of the lung and release reactive oxygen species by the membrane-bound enzyme complex NADPH oxidase and nitric oxide (NO) via the calcium-insensitive inducible form of NO synthase. NO decreased \(I_{sc}\) across cultured rat type II cells without affecting transepithelial resistance. NO also inhibited 60% of amiloride-sensitive \(I_{sc}\) across type II cell monolayers after permeabilization of the basolateral membrane with amphotericin B (141). NO reacted with superoxide (O\(_2\)\(^-\)) to form peroxynitrite (ONOO\(^-\)), a potent oxidant and nitrating species that directly oxidizes a wide spectrum of biological molecules, such as DNA constituents, lipids, and proteins (220). Boluses of peroxynitrite (0.5–1 mM) into suspensions of freshly isolated type II cells from rabbits decreased amiloride-inhibitable sodium uptake to 68 and 56% of control values without affecting cell viability (153). Some investigators reported that products of macrophages, including NO, can downregulate sodium transport in fetal distal lung epithelium stimulated with endotoxin (71, 83). Also, another study indicated that a generator of peroxynitrite (3-morpholinosydnonimine) inhibited the amiloride-sensitive whole cell conductance in \(Xenopus\) oocytes expressing the three cloned subunits of ENaC (90). The data indicate that oxidation of critical amino acids residues in ENaC protein is probably responsible for this effect. This evidence matches well with other studies that have shown that protein nitration and oxidation by reactive oxygen and nitrogen species have been associated with diminished function of a variety of important proteins present in the alveolar space, including \(\alpha_1\) proteinase inhibitor (243) and surfactant protein A (389–391).

V. EPITHELIAL FLUID TRANSPORT UNDER PATHOLOGICAL CONDITIONS

Fluid clearance from the distal airspaces of the lung has been measured in mechanically ventilated patients with acute respiratory failure from pulmonary edema as well as in several animal models designed to simulate clinically relevant pathological conditions (Fig. 5).

A. Clinical Studies

Studies of fluid clearance 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 airspaces of the lung with a standard suction catheter passed through the endotracheal tube into a wedged position in the distal airways of the lung (150, 230, 361, 374, 375, 391). This method for measuring fluid clearance in patients was adapted from the method for aspirating fluid from the distal airspaces of the lung in experimental studies in small and large animals (30, 228). The clinical procedure has been validated in patients by demonstrating that there is a relationship between fluid clearance and the improvement in oxygenation and the chest radiograph (230, 361).

1. Resolution of hydrostatic pulmonary edema

In 65 patients with severe hydrostatic pulmonary edema, predominantly from acute or chronic left ventricular dysfunction, there was net fluid clearance in the majority of the patients during the first 4 h after endotracheal intubation and the onset of positive pressure ventilation (361). The rate of fluid clearance in these patients varied between maximal (>14%/h) in 38% and submaximal (3–14%/h) in 37%. Overall, 75% of the patients had intact fluid clearance. There was no significant correlation between the levels of fluid clearance and endogenous plasma levels of epinephrine, although twice as many of the patients with intact fluid clearance received aerosolized \(\beta\)-adrenergic therapy as those with impaired fluid.
clearance, but this difference did not reach statistical significance, perhaps because the total number of studied patients was modest. The inability to transport edema fluid from the distal airspaces of the lung in 25% of the patients was not simply related to elevated pulmonary vascular pressures. Several mechanisms could downregulate fluid transport in these patients including elevated levels of ANF (49, 269) or the presence of ouabain-like substances in the circulation (80, 107, 108). Experimental studies have provided some insight into the mechanisms that may downregulate fluid transport from the distal airspaces of the lung in the presence of elevated pulmonary vascular hydrostatic pressures (see sect. V A2). Because hydrostatic pulmonary edema is associated with an uninjured epithelial barrier, the studies of hydrostatic pulmonary edema provide an important comparison group to the patients with pulmonary edema from acute lung injury because some degree of morphological or functional injury to the epithelial barriers probably occurs in most lung injuries (see sect. V B). Also, there is experimental evidence that accelerated fluid clearance can occur during the resolution of hydrostatic pulmonary edema in patients that is faster than in sheep, but similar to the fast rates recorded in rats (120) (Table 2). There is also recent data that very fast rates of fluid clearance can occur from the lungs of patients with pulmonary alveolar proteinosis by catecholamine-independent mechanisms (63).

2. Resolution of increased permeability edema from clinical acute lung injury

The majority of 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 (Fig. 6). In contrast, a minority of patients can remove alveolar edema fluid rapidly, and these patients have a higher survival rate (230, 374, 375). These results indicate that a functional, intact distal lung epithelium is associated with a better prognosis 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 may impair fluid clearance from the airspaces of the lung? Some patients have pathological (8) and biochemical (248) evidence of necrotic injury to the alveolar epithelium. There is also some clinical data that a decrease in 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 for epithelial function (63).
to the epithelial fluid transport may occur in some patients with lung injury, depressing their ability to remove alveolar edema fluid (391) (see sect. vB2)

B. Experimental Studies

This section discusses what has been learned from experimental studies of high-pressure, hydrostatic pulmonary edema as well as from animal models of acute lung injury with increased permeability pulmonary edema.

1. Hydrostatic stress

The first major study to evaluate the effect of acute hydrostatic pulmonary edema on fluid clearance was done in anesthetized, ventilated sheep in which left atrial pressure was elevated acutely to either 18 or 25 cmH 2O, done in anesthetized, ventilated sheep in which left atrial pressure of 24 cmH 2O. This finding provided direct evidence that an intact distal lung epithelium could actively remove fluid even though there was interstitial edema and a moderately elevated left atrial pressure. 

In an isolated perfused rat lung model, other investigators have found that 15 cmH 2O pulmonary venous pressure reduces fluid clearance from the airspaces by 50%, although elevations to 5 and 10 cmH 2O (control = 0 cmH 2O in this model) had no effect (310). The lower level at which vascular pressure impaired fluid clearance in this model compared with the sheep studies may be explained by the lack of functioning lung lymphatics in these isolated perfused lung studies. Interestingly, in this rat study, there was no evidence of morphological injury to the epithelial barrier, a finding that matches well with the finding that a reduction of vascular pressure was associated with a return of fluid clearance to a normal level (7). Thus, as in the sheep studies, there was no evidence of sustained injury to the epithelial barrier, a finding that fits well with the clinical studies of the resolution of hydrostatic pulmonary edema (361).

It is possible that there may be some mechanisms, other than elevated lung vascular pressures, that could depress vectorial fluid transport in hydrostatic pulmonary edema. For example, as already discussed, ANF is released with atrial stretch; this molecule can inhibit alveolar epithelial sodium uptake and fluid clearance (49, 269, 310, 349). Also, hypoxia may impair vectorial fluid clearance from the airspaces of the lung (see sect. vB for discussion of hypoxia and epithelial fluid transport).

Can the resolution of alveolar edema be accelerated in hydrostatic lung edema? In the initial sheep study, aerosolization of salmeterol, a potent lipid-soluble β2 agonist, did not increase fluid clearance over 4 h in the presence of left atrial pressure elevation to either 18 or 25 cmH 2O (120). When left atrial pressure was normalized (6 cmH 2O), then aerosolized salmeterol markedly increased fluid clearance (49). Studies in rats demonstrated that a β2 agonist could enhance the resolution of lung edema and improve oxygenation in the resolution phase of hydrostatic pulmonary edema (120). Also, in isolated perfused rat lungs (7), there was a significant increase in fluid clearance with administration of isoproterenol or dopamine.

2. Hypovolemic shock

Hypovolemic shock from blood loss is an important clinical problem after major trauma. Short-term studies in rats that simulated acute hemorrhagic shock with a 30% loss of blood volume resulted in a sharp rise in endogenous levels of plasma epinephrine, a finding that was associated with a doubling of fluid clearance from the distal airspaces of the lung (241, 285). The effect was inhibited by propranolol and partially inhibited by amiloride.

When hypovolemic shock, however, was prolonged for 4–5 h in rats, the results were markedly different. Under these conditions, there was no increase in fluid transport from the airspaces of the lung, even when β2-
adrenergic agonists were instilled into the distal airspaces. This result prompted a series of experiments to discover the mechanisms that downregulate fluid clearance after prolonged hemorrhagic shock in rats. The initial studies established that the mechanism was neutrophil dependent (240). Further studies established that the process involves a-adrenergic activity and release of oxidant radicals with interleukin-1β in the airspaces (190, 198), probably from neutrophils that accumulate in the lung after the onset of hemorrhagic shock. Finally, recent work has indicated that an increase in the expression of inducible NO synthase in the lung and release of NO, probably in part from alveolar macrophages, diminishes the capacity of the alveolar epithelium to actively transport fluid from the airspaces after severe hemorrhage. Second, NO inhibits the upregulation of alveolar epithelial fluid transport by cAMP-dependent mechanisms by directly affecting the function of the β2-adrenergic receptor and adenyl cyclase. Third, shock-mediated release of NO in the airspaces of the lung depends in part on the activation and nuclear translocation of NF-κB (288).

The results of these experimental studies may have important clinical implications for explaining the susceptibility to pulmonary edema in some patients after major trauma. Since, as already discussed, in vitro studies demonstrated that peroxynitrate can directly impair the function of sodium channels (24, 90, 141, 143, 153, 218), these in vivo studies fit well with one recent clinical study that reported an inverse relationship between elevated levels of nitrate and nitrite and the rate of fluid clearance in patients with pulmonary edema (391). Also, one prior study identified nitrotyrosine (the stable by-product of peroxynitrite reactions with tyrosine residues) in the lungs of patients with the acute respiratory distress syndrome (144) as well as in alveolar macrophages isolated from patients with the same cause of acute respiratory failure (11, 325).

3. Endotoxemia/bacteremia

The effect of endotoxemia and bacteremia on lung vascular permeability was well described in studies in sheep several years ago (43, 44). However, the impact on the function of the alveolar epithelial barrier was not addressed in those studies. More recent work has indicated that the acute shock produced by severe bacteremia in rats markedly increases plasma epinephrine levels, as in hemorrhagic shock, and the elevated epinephrine levels markedly upregulate the fluid transport capacity of the distal lung epithelium (290). Thus it is possible that in the short term, upregulation of fluid clearance may protect the airspaces against alveolar flooding when there is an increase in lung vascular permeability and accumulation of interstitial edema. In fact, one study in sheep demonstrated that lung vascular permeability can be augmented markedly with intravenous endotoxin with a rise in protein-rich lung lymph flow, but this effect was not associated with a change in lung epithelial permeability to protein and no change in the capacity of the alveolar epithelium to remove alveolar fluid (380). These studies were done over 4 and 24 h in sheep, and in some of the studies both intra-alveolar and intravenous endotoxin was administered, but in all cases the epithelial barrier remained intact and capable of transporting alveolar fluid normally.

However, when large doses of live bacteria (Pseudomonas aeruginosa) were given to sheep, there was an increase in both lung endothelial and epithelial permeability to protein in the sheep that had developed the most severe shock (291). These sheep had alveolar flooding, and their capacity to remove alveolar fluid was impaired, similar to the findings in humans who develop severe permeability pulmonary edema with septic shock (305). The mechanisms for injury to the epithelial barrier probably depend on both neutrophil-dependent release of injurious proteases and reactive oxygen species as well as the bacterial exoproducts. In one study, gram-negative bacteria that produced proteases increased alveolar epithelial barrier permeability to protein by altering basolateral surface permeability while the nonprotease-producing strains only increased lung vascular permeability (287).

4. Pneumonia

In sharp contrast to intra-alveolar endotoxin, live bacteria increased alveolar epithelial barrier permeability and decreased fluid transport in sheep (380). Further studies indicated that the products of P. aeruginosa were important in determining the extent of injury. For example, exoenzyme S and phospholipase C mediated injury to the epithelial barrier in rabbits with a decrease in vectorial fluid transport (381). Subsequent studies indicated that bacterial pneumonia may progress to septic shock when the infecting gram-negative organism generates proinflammatory cytokines in the airspaces of the lung that are released into the circulation when bacterial-mediated injury results in sufficient injury to the distal lung epithelial barrier (188). Several experimental studies have indicated that active and passive immunization against P. aeruginosa antigens can prevent epithelial injury in sheep (289) and in mice (314).

Recent data also indicate that influenza virus infection (A/PR/8/34) can specifically alter epithelial ion transport by inhibiting amiloride-sensitive sodium current across mouse tracheal epithelium (187). The inhibitory effect of the influenza virus was caused by binding the viral hemagglutinin to a cell-surface receptor, which then activated phospholipase C and protein kinase C. It is well known that protein kinase C can reduce ENaC activity so that influenza infections in the lung may inhibit the func-
tion of ENaC (138). Given the importance of sodium channels in vectorial transport of fluid in distal airway epithelia and in the alveoli, these results provide a new mechanism that may explain the accumulation of alveolar edema fluid in patients with viral pneumonia and acute lung injury.

Interestingly, in the process of studying *P. aeruginosa* pneumonia in rats, one group of investigators found that the rate of fluid clearance was upregulated in the rats that survived (300). The mechanism for this effect was secondary to release of tumor necrosis factor-α (TNF-α), which was surprising since TNF-α plays an important role in mediating the host inflammatory response to infection (1, 175, 200) as well as potentially contributing to the inflammatory response to infection (1, 175, 200). Based on the A549 human cell studies, the effect of TNF-α to increase fluid clearance was confirmed in a subsequent rat study (36) in which a neutralizing polyclonal anti-TNF-α antibody inhibited the upregulation of fluid clearance induced by intestinal ischemia-reperfusion. Also, the effect of TNF-α is amiloride inhibitable in both rats (36, 122) and isolated A549 cell patch-clamp studies (122). Based on the A549 human cell studies, the effect appears to be a receptor-mediated process. It is not clear, however, what signaling pathways are involved, since cAMP levels are not elevated by TNF-α (36).

5. Hyperoxia

Several investigators have used hyperoxia as a model to study the effect of acute lung injury on epithelial ion and fluid transport in the lung, in part because the injury develops over 2–5 days in rats and mice and pathologically resembles clinical acute lung injury with both endothelial and epithelial injury in association with an influx of neutrophils and protein-rich pulmonary edema.

However, the results of these studies have not been uniform, in part because of variations in the duration of O₂ exposure, the exact level of hyperoxia, and the use of rats or mice. In one rat study, administration of 85% O₂ for 7 days increased the level of α-ENaC protein, and both inward and outward sodium currents were stimulated in patch clamps of isolated alveolar type II cells (149). Subsequently, another study from the same group showed increased expression and activity of amiloride-inhibitable, sodium channels in alveolar type II cells of rats exposed to 85% O₂ for 7 days followed by 100% O₂ for 4 days. Both the number and the open probability of the L-type sodium channels (25 pS) were increased (387). Another group also studied the effect of 85% O₂ for 7 days in rats and found that amiloride-inhibitable sodium uptake was greater than in control rats and that ouabain decreased active sodium transport to a greater percentage in the hyperoxic rats, suggesting an upregulation of Na⁺-K⁺-ATPase activity after subacute hyperoxia (101, 128, 270, 343). In other studies, exposure to 100% O₂ for 48 h produced moderate interstitial lung edema but no impairment of basal or cAMP-stimulated fluid transport (125, 192, 311). However, when exposure was prolonged to 64 h, one group of investigators reported decreased transport in rats, an effect that seemed to be related to decreased gene expression of α₁-Na⁺-K⁺-ATPase subunits (272). Other studies found that there was rapid upregulation of mRNA for α₁- and β₁-subunits of Na⁺-K⁺-ATPase as well as antigenic protein shortly after prolonged exposure to >97% O₂ in rats for 60 h (250, 251), suggesting the induction of the Na⁺-K⁺-ATPase could occur as a protective mechanism. Similar findings were reported by Stern et al. (335) in a thiourea model of lung injury in which upregulation of Na⁺-K⁺-ATPase gene expression and protein occurred after initial injury and was associated with recovery from the pulmonary edema. Other studies have reported that hyperoxia does not seem to produce a clear change in sodium and fluid transport during the period of hyperoxia (40, 55, 56).

Interestingly, one group of investigators reported that pretreatment of rats with aerosolized adenoviral β₁-Na⁺-K⁺-ATPase upregulated fluid transport and also made the rats resistant to the lethal effects of hyperoxia (100). This represented the first evidence that gene therapy could potentially be used to produce a sustained upregulation of fluid transport in the lung in the presence of a pathological condition. Furthermore, gene transfer of both α₁- and β₁-Na⁺-K⁺-ATPase decreases edema formation induced by thiourea in rats (335). An earlier study had shown that administration of the adenoviral β₁-Na⁺-K⁺-ATPase gene, but not the α₁-Na⁺-K⁺-ATPase, would increase fluid transport in normal rats (102).

6. Subacute lung injury

The mechanisms that regulate fluid balance across the distal lung epithelium in the subacute phase after lung injury have been studied after bleomycin lung injury, a commonly used model for subacute lung injury that is associated with fibrosis (286). In one study, the rate of fluid clearance was doubled compared with normal lungs, a finding that correlated with a large increase in alveolar type II-like cells (115). However, these epithelial cells did not express α-ENaC, and when isolated and studied in vitro, the absolute rate of transport was only 50% of normal control type II cells, and all of the transport was amiloride insensitive. Also, β-agonist therapy did not up-regulate transport. Thus the provisional repair epithelium in this rat model lacked the normal sodium and fluid transport properties, although the extent of epithelial hyperplasia with type II-like cells appeared to compensate well for the impairment of transport of the isolated type II-like cells. By 60 days, the lungs had recovered their
normal architecture, and fluid clearance from the distal airspaces was normal.

VI. FUTURE DIRECTIONS

Important advances have been made in the understanding of the reabsorption of fluid and solutes by the distal epithelia with characterization of sodium transport and water pathways under both physiological and pathological conditions. However, several fundamental issues require additional study.

Alveolar type II cells and distal airway epithelial cells, such as Clara cells, are implicated in sodium and fluid transport, but their relative contributions in both physiological and pathological conditions are not well defined. The current in vivo models and cultured cell systems cannot determine the relative contribution of these cells to net fluid transport in the distal lung. Innovative approaches are needed. Another important issue is the relative contribution of alveolar type I cells in sodium, chloride, and fluid transport across the alveolar epithelium. Alveolar type I cells cover 95% of the alveolar surface area, and the recent demonstration of the presence of water channels and ENaC expression in those cells suggests a role for these cells in net fluid clearance. Improved models are needed to assess the differential contribution of alveolar type I and II cells to fluid transport.

Another important area of research is the characterization of the sodium transporters involved in sodium and fluid reabsorption and their regulation. Amiloride-sensitive sodium transport is one of the major pathways for sodium entry across distal epithelial cells, but several questions remain unsolved. For example, are the molecular and biophysical characteristics of these channels in vitro representative of their in vivo characteristics, and how are these channels regulated during physiological and pathological conditions? The mechanisms that regulate the trafficking of ENaC and Na\(^+\)-K\(^+\)-ATPases between the cytoplasm and the membrane need to be evaluated also in distal lung epithelia. Increased insertion of transport proteins may be one important mechanism for increasing sodium and fluid transport under pathological conditions and may potentially contribute to regulating the clearance of edema fluid from distal airspaces of the lung. In addition to amiloride-sensitive sodium transport, a characterization of ion transporters involved in amiloride-insensitive sodium transport needs to be defined. Also, the pathways for chloride reabsorption under basal and stimulated conditions need to be determined with particular attention to the role of CFTR under cAMP-stimulated conditions.

Recent advances have been made with transgenic mice models to define the role of sodium and water channels in the lung fluid balance. Knockout of the three subunits of ENaC has clearly established the preponderant role of \(\alpha\)-compared with \(\beta\)- and \(\gamma\)-ENaC in alveolar transepithelial sodium absorption. Similarly, the knockout mice for several aquaporin-type water channels have revealed that in the lung these channels are not essential for water transport. However, a genomic disruption of genes that are expressed during development or in multiple tissue types complicates the phenotypic analysis. A solution to this problem may be provided by conditional knockouts. This system permits control of the timing for cell-specific expression of specific proteins, thereby circumventing both embryonic lethality and confounding effects of complex adaptive responses that can occur when the physiological observations follow the gene knockout events by days or weeks. In this gene, expression is regulated temporally and spatially using cell-specific promoters, such as SP-C for alveolar type II cells, in combination with a regulatory on-off system. This approach may provide a major opportunity to advance the understanding of the role of sodium and fluid transport during physiological and pathological conditions during the reabsorption of edema from the distal airspaces of the lung.

Progress in the last decade demonstrates that it is feasible to quantify the rate of edema reabsorption from the distal airspaces of the lung in ventilated, critically ill patients with acute pulmonary edema. In conjunction with progress in experimental studies of lung fluid balance under clinically relevant pathological conditions, further studies should be done to test the potential role of catecholamine-dependent and -independent therapies that might enhance the resolution of clinical pulmonary edema.

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