Lung Edema Clearance: 20 Years of Progress
Invited Review: Lung edema clearance: role of Na\textsuperscript{+}-K\textsuperscript{+}-ATPase

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Sznajder, J. I., P. Factor, and D. H. Ingbar. Invited Review: Lung edema clearance: role of Na\textsuperscript{+}-K\textsuperscript{+}-ATPase. J Appl Physiol 93: 1860–1866, 2002. 10.1152/japplphysiol.00022.2002.—Acute hypoxemic respiratory failure is a consequence of edema accumulation due to elevation of pulmonary capillary pressures and/or increases in permeability of the alveolocapillary barrier. It has been recognized that lung edema clearance is distinct from edema accumulation and is largely effected by active Na\textsuperscript{+} transport out of the alveoli rather than reversal of the Starling forces, which control liquid flux from the pulmonary circulation into the alveolus. The alveolar epithelial Na\textsuperscript{+}-K\textsuperscript{+}-ATPase has an important role in regulating cell integrity and homeostasis. In the last 15 yr, Na\textsuperscript{+}-K\textsuperscript{+}-ATPase has been localized to the alveolar epithelium and its contribution to lung edema clearance has been appreciated. The importance of the alveolar epithelial Na\textsuperscript{+}-K\textsuperscript{+}-ATPase function is reflected in the changes in the lung’s ability to clear edema when the Na\textsuperscript{+}-K\textsuperscript{+}-ATPase is inhibited or increased. An important focus of the ongoing research is the study of the mechanisms of Na\textsuperscript{+}-K\textsuperscript{+}-ATPase regulation in the alveolar epithelium during lung injury and how to accelerate lung edema clearance by modulating Na\textsuperscript{+}-K\textsuperscript{+}-ATPase activity.

RESOLUTION OF PULMONARY EDEMA occurs as the result of active Na\textsuperscript{+} transport across the alveolar epithelium via apical amiloride-sensitive Na\textsuperscript{+} channels and basolateral Na\textsuperscript{+}-K\textsuperscript{+}-ATPases (19, 41, 57). This active vectorial Na\textsuperscript{+} flux produces a transepithelial osmotic gradient that causes water to passively move from the air spaces to the alveolar interstitium. In some models of acute lung injury and in patients with acute respiratory distress syndrome (ARDS), the lung’s ability to clear edema is impaired (3, 14, 16, 27, 49, 65, 72, 83, 89). Parallel studies in alveolar epithelial cells suggest that this impairment may be due to decreased function of epithelial Na\textsuperscript{+}-K\textsuperscript{+}-ATPase (13, 40, 49, 65). For example, severe lung injury in rats exposed to acute hyperoxia (100% O\textsubscript{2} for 64 h) reduces active Na\textsuperscript{+} transport in lungs and alveolar epithelial cells. However, moderate hyperoxic injury may lead to increased Na\textsuperscript{+}-K\textsuperscript{+}-ATPase mRNA levels, with variable functional effects (16, 35, 62). Similarly, subacute hyperoxia (85% O\textsubscript{2} for 7 days) is associated with proliferation of alveolar epithelial cells, upregulation of Na\textsuperscript{+}-K\textsuperscript{+}-ATPase in the alveolar epithelium, and increased lung edema clearance (63). Hypoxia also can inhibit the function of both alveolar epithelial Na\textsuperscript{+}-K\textsuperscript{+}-ATPase and the Na\textsuperscript{+} channel (17, 54, 55, 90). These observations suggest a paradigm in which during the acute phases of lung injury active Na\textsuperscript{+} transport may be impaired while edema accumulates due to changes in permeability of the alveolocapillary barrier. This acute phase is followed by a proliferative response associated with increased edema clearance (6, 63). Support for this model has been demonstrated in studies of patients with lung
injury and ARDS in whom impairment of the lung’s ability to clear edema correlated with worse outcomes (14, 38). There is also ample experimental evidence from healthy animal models and during lung injury that upregulating the Na\(^{+}\)-K\(^{-}\)-ATPases increases active Na\(^{+}\) transport across the alveolar epithelium and thus edema clearance (4, 8, 24, 27, 71, 75, 76).

**Na\(^{+}\)-K\(^{-}\)-ATPase STRUCTURE AND BIOCHEMISTRY**

The Na\(^{+}\) pump consists of two major subunits, α and β, which typically form a heterodimer in the plasma membrane enzyme (79). In some tissues, there is a third γ-subunit that modifies the functional activity of the enzyme, but its significance in lung is undefined. The essential role of Na\(^{+}\)-K\(^{-}\)-ATPase in cellular function has been recognized for more than 30 yr; more recently, its importance in the lung has been reported (6, 12, 27, 44, 62, 63). The α- and β-subunits have multiple isoforms; to date, four α- and five β-isoforms have been described (10, 61). These isoforms are expressed in a tissue-specific and developmentally regulated manner. The α- and β-subunits have >70% homology between isoforms within each subunit; there also is significant homology of the Na\(^{+}\)-K\(^{-}\)-ATPase coding and promoter DNA sequences across species (50–52).

The Na\(^{+}\)-K\(^{-}\)-ATPase α-subunit. The catalytic α-subunit exchanges intracellular Na\(^{+}\) for extracellular K\(^{+}\) in a 3:2 ratio and contains ouabain binding and phosphorylation sites. It has many transmembrane domains and forms the cationic pore. Transcriptional regulation studies of the rat Na\(^{+}\)-K\(^{-}\)-ATPase α1-subunit have identified a major transcription initiation site 262 bp upstream from the translation initiation site that is preceded by a TATA box at position −32. Included in this 5′-flanking region are two highly conserved SP1 transcription factor-binding sites, two glucocorticoid response element half-consensus sequences (96), a consensus cAMP response element, and a positive regulatory region located at −155/−49 bp from the transcription initiation site. An “Atp1a1” regulatory element (or ARE) at −94/−69 bp binds both common and at least seven cell type-specific transcription factors, which could account for differential, cell-specific expression of this subunit (81). The human α1-gene promoter has a TATA box, five SP1-like elements (77), and three potential thyroid hormone response elements (29).

In the lungs, the α2-subunit is expressed in alveolar epithelial cells and appears to have a role in alveolar fluid clearance (5). Polymorphisms of the human α2-subunit have been linked to increased susceptibility to seizures (15), but lung abnormalities have not been associated with polymorphisms of any of the subunits or isoforms. Polymorphisms at one α2-locus decreases cardiorespiratory endurance (maximal oxygen consumption) with training by as much as 40% (68).

The Na\(^{+}\)-K\(^{-}\)-ATPase β-subunit. The smaller β-subunit has a single transmembrane-spanning domain and, unlike the α-subunit, is glycosylated (59). The precise function of the β-subunit is controversial, but it appears to have a role in the assembly and trafficking of the Na\(^{+}\)-pump heterodimer to the correct domain of the cell membrane, as well as membrane-associated half-life. In different cell types and tissues, the relative quantities of α and β mRNA and protein are variable. In rat alveolar type II cells and in the rat lung, the quantities of β-subunit seem to limit the functional activity of the Na\(^{+}\) pump enzyme (5, 27). Transcriptional regulation of the β1-subunit gene is less defined than for α1, but it is likely to be as or more important in the lung (37). Genomic clones of the rat β1-subunit promoter contain a potential TATA box at position −31, four GC-rich boxes, and two sites with half consensus sequences for thyroid hormone response elements (53). Intron I of the rat β1-gene has a positive effect on basal transcription (C. H. Wendt and D. H. Ingbar, unpublished observations), but the specific regulatory elements involved are not defined. In the 5′-upstream region, two major and three minor transcription initiation sites have been identified. There is also a positive regulatory region (−650 to −630 bp) that is required for mineralocorticoid receptor or glucocorticoid receptor activation (21). The NH2-terminal region of the mineralocorticoid receptor inhibits GC stimulation of Na\(^{+}\)-K\(^{-}\)-ATPase β1-subunit transcription (46). Hyperoxia stimulates transcription of this subunit through an increase in the binding of SP1 transcription factor to the proximal promoter region (93).

**REGULATION OF ALVEOLAR EPITHELIAL Na\(^{+}\)-K\(^{-}\)-ATPase**

**Short-term regulatory mechanisms.** Several reports have suggested that basal Na\(^{+}\)-K\(^{-}\)-ATPase activity in intact cells is one-third of its maximal capacity (78). Thus recruitment of this reserve capacity represents a mechanism by which cellular Na\(^{+}\)-K\(^{-}\)-ATPase activity can be rapidly upregulated. Short-term increases in Na\(^{+}\)-K\(^{-}\)-ATPase function can be regulated via three pathways: 1) changes in the number of molecules in the cell plasma membrane, 2) changes in the catalytic property of enzymes already present at the plasma membrane, and 3) changes in enzyme affinity for Na\(^{+}\). Recent data indicate that Na\(^{+}\)-K\(^{-}\)-ATPase activity can be rapidly increased via at least two mechanisms. First, both dopamine and β-adrenergic agonists increase lung edema clearance within 1 h (1, 4, 6, 27). The β-adrenergic agonists increase the pump’s affinity for Na\(^{+}\) and recruit Na\(^{+}\)-pump subunit proteins to the basolateral plasma membrane from intracellular endosomal compartments (30) (see Fig. 1). In lung alveolar epithelial cells, activation of G protein-co coupled receptors, via either dopaminergic or adrenergic stimuli, rapidly (30 s to 15 min) increases Na\(^{+}\)-K\(^{-}\)-ATPase activity by insertion of Na\(^{+}\)-pump proteins from intracellular compartments into the plasma membrane (4, 48, 69) (see Fig. 2). These effects are dependent on a
dynamic interaction between protein-transporting vesicles, microtubulae, and the actin cytoskeleton as pretreatment with colchicine, brefeldine, or phallacidin prevents this recruitment. Interestingly, the short-term regulation of Na\(^+-\)K\(^+-\)ATPase in alveolar type 2 epithelial cells by dopamine has been associated with D\(_{1a}\) but not D\(_2\)-receptor stimulation. These highly regulated processes occur via simultaneous, phosphorylation events regulated by novel protein kinases and dephosphorylation events regulated by protein phosphatase 2A (48, 69). A second, rapid mechanism by which β-adrenergic agonists stimulate transepithelial Na\(^+\) transport and Na\(^+-\)K\(^+-\)ATPase is via cAMP-dependent activation of apical Cl\(^-\) channels in alveolar epithelial cells (42).

**Long-term regulatory mechanisms.** Long-term regulation of Na\(^+-\)K\(^+-\)ATPase occurs via transcriptional and posttranscriptional mechanisms, including changes in membrane enzyme-specific activity, and increases in plasma membrane Na\(^+\) pump proteins due to trafficking of heterodimers to the plasma membrane from intracellular pools, translation, protein degradation

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**Fig. 1.** Schematic representation of the pathway of Na\(^+-\)K\(^+-\)ATPase traffic from the plasma membrane to intracellular endosomal and lysosomal compartments and recruitment back of the Na\(^+\) pumps into the basolateral membranes on cathecholamine stimulation in the alveolar epithelium.

**Fig. 2.** Schematic representation of the dopaminergic-receptor (D2R) and β\(_2\)-adrenergic-receptor (β\(_2\)-AR) pathways leading to transcriptional and posttranscriptional regulation of the Na\(^+\) pump protein in alveolar epithelial cells. ERK, extracellular regulated kinase; mTOR, mammalian target of rapamycin; PKA, protein kinase A; PKC, protein Kinase C.
rates, mRNA stability, and transcription (reviewed in Refs. 9, 41, 86).

Transcriptional regulation of the Na\(^{+}\) pump subunit genes is an important component of the multifaceted response to growth hormones, hormonal stimulation, hyperoxia, and cellular stress. The triggers for increased Na\(^{+}\)-K\(^{+}\)-ATPase expression in the lung just before birth and the specific transcription factors and signaling pathways that initiate transcription in response to stress or stimulation are being defined. Unequal amounts of \(\alpha\) and \(\beta\) mRNA and protein concentrations are present in many tissues, although the final Na\(^{+}\) pump \(\alpha\)- and \(\beta\)-subunit stoichiometry is 1:1. Because the subunit genes are on different chromosomes, transcription may be independently regulated. Increased transcription of the Na\(^{+}\)-K\(^{+}\)-ATPase subunit genes in the lung may be mediated by hormones such as dexamethasone, insulin, and aldosterone (5, 37, 39, 41, 43, 45, 47, 94, 95). Aldosterone increases both transcription and plasma membrane insertion of preformed pump molecules (25, 64). Both functional enzyme activity and gene transcription are increased by low intracellular K\(^{+}\) concentration or high Na\(^{+}\) concentration or by various hormones, including thyroid hormone (59), and in the lung by aldosterone (64) and glucocorticoids (41). Corticosteroids, dexamethasone, 3,5,3’-triiodothyronine (T\(_3\)), and aldosterone, as well as keratinocyte growth factor and epidermal growth factor, increase Na\(^{+}\) reabsorption in mammalian lungs (11, 18, 27, 28, 31–34, 58, 66, 73, 74, 84, 85). Similar to steroids and growth factors, the commonly used drugs dopamine (via D\(_2\) receptors) and \(\beta\)-adrenergic agonists can activate Na\(^{+}\)-K\(^{+}\)-ATPase gene transcription and translation in alveolar epithelial cells (37) (67). Dopaminergic D\(_2\)-receptor-mediated stimulation of Na\(^{+}\)-K\(^{+}\)-ATPase mRNA and protein synthesis occurs via mitogen-activated protein kinases and a Ras-Raf-mitogen-activated protein kinase pathway (37). For example, terbutaline stimulated rat alveolar epithelial cell Na\(^{+}\)-K\(^{+}\)-ATPase function after several days (60). A more recent study reported that \(\beta\)-adrenergic stimulation of serum-starved alveolar epithelial cells regulated Na\(^{+}\)-K\(^{+}\)-ATPase translation via extracellular regulated kinase-rapamycin pathways independent of changes in Na\(^{+}\)-K\(^{+}\)-ATPase transcription (67).

Translation of Na\(^{+}\)-K\(^{+}\)-ATPase mRNA is an important locus of regulation in a variety of settings. For example, similar increases in steady-state levels of mRNA result in different activity levels of the Na\(^{+}\) pump, indicating that posttranscriptional steps play a role in the regulation of Na\(^{+}\)-K\(^{+}\)-ATPase (36, 67). In vitro studies of translation demonstrated that untranslated mRNA regions can affect subunit translation. The mRNA for \(\alpha\)\(_1\) is translated less efficiently than that for \(\beta\)\(_1\) because of \(\alpha\)\(_1\) mRNA’s 3’ untranslated mRNA region being extremely GC rich and folded in a complex fashion and because translational efficiency may be altered by glucocorticoids (22).

**OVEREXPRESSION OF Na\(^{+}\)-K\(^{+}\)-ATPase IN THE ALVEOLAR EPITHELIUM**

In several models of lung injury and most ARDS patients, lung edema clearance is impaired (7, 82, 91, 92). Thus methods that improve lung edema clearance might offer a therapeutic option for these patients with acute respiratory failure. For many years, it was believed that the Na\(^{+}\) channel was the locus of control for Na\(^{+}\) reabsorption, but recent data indicate that up-regulation of Na\(^{+}\)-K\(^{+}\)-ATPase alone is sufficient to increase alveolar fluid clearance (2, 26, 27). Many of the agents discussed above can stimulate fluid clearance by regulating the alveolar epithelial Na\(^{+}\)-K\(^{+}\)-ATPase, including dopamine, \(\beta\)-adrenergic agonists, glucocorticoids, T\(_3\), keratinocyte growth factor, and epidermal growth factor. Because in several models of lung injury models and in many ARDS patients lung edema clearance is impaired, a clinical goal is the augmentation of fluid clearance in patients with decreased or normal levels edema clearance, through increased Na\(^{+}\)-K\(^{+}\)-ATPase and/or Na\(^{+}\) channel function. The proof-of-concept experiments that augmentation of Na\(^{+}\)-K\(^{+}\)-ATPase is a valid approach and can be physiologically beneficial are based on gene transfer experiments. The two gene transfer approaches have been direct transfer of Na\(^{+}\)-K\(^{+}\)-ATPase genes and overexpression of the \(\beta\)-adrenergic receptor gene to promote the increase of both Na\(^{+}\) channels and Na\(^{+}\)-K\(^{+}\)-ATPase.

Adenoviral-mediated gene transfer has been utilized to transduce the alveolar epithelium of rats to study the role of alveolar Na\(^{+}\)-K\(^{+}\)-ATPase in lung edema clearance (2, 26, 27). First-generation (E1a /E3\(^{-}\)) replication-incompetent human type 5 adenoviruses that express rat Na\(^{+}\)-K\(^{+}\)-ATPase \(\alpha\)1- or \(\beta\)1-subunit cDNA were used to transduce lung epithelial cells. Overexpression of a \(\beta\)1-subunit, but not an \(\alpha\)1-subunit, increased Na\(^{+}\)-K\(^{+}\)-ATPase function in adult rat alveolar epithelial cells and rat fetal distal lung epithelial cells (87). Conversely, Na\(^{+}\)-K\(^{+}\)-ATPase function in a human lung cell line (A549) was increased only after overexpression of an \(\alpha\)1-subunit gene (28). These studies were extended to in vivo models by transducing the alveolar epithelium of normal adult rats using a surfactant-based delivery system that increased alveolar fluid reabsorption by >100% in rat lungs overexpressing a \(\beta\)1-subunit gene.

As described above, adult rats exposed to 100% \(O_2\) develop acute lung injury characterized by increased alveolar permeability, edema accumulation, and impairment of lung liquid clearance (16, 20, 65). A recent study reported the results of adenoviral-mediated overexpression of a Na\(^{+}\)-K\(^{+}\)-ATPase \(\beta\)1-subunit gene in the alveolar epithelium of adult rats before exposure to hyperoxia (100% \(O_2\) for 64 h). Rats overexpressing the Na\(^{+}\)-K\(^{+}\)-ATPase \(\beta\)1-subunit gene in the alveolar epithelium tolerated hyperoxia better, had no pleural effusions, and had lung liquid clearance rates that were 300% greater than hyperoxic controls or rats infected
with an α1-subunit-expressing virus. In addition, β1-subunit overexpression was associated with 100% survival through 14 days of hyperoxia, suggesting that augmentation of lung liquid clearance may confer protection from a severe experimental lung injury. Similarly, Stern et al. (80) reported that mice (C57BL/6) transduced with a chicken α3-gene fused to a β1 cDNA have increased whole lung Na\(^+\)-K\(^+\)-ATPase activity and less thiourea-induced edema than controls treated with a plasmid vector that encoded an irrelevant cDNA. Recently, in a model of increased left atrial pressures, it was reported that the lung's ability to clear edema was decreased by 50% as left atrial pressure was increased from 0 to 15 cmH\(_2\)O in isolated rat lungs (3, 72). Overexpression of Na\(^+\)-K\(^+\)-ATPase β1-subunit 7 days before measurement of lung liquid clearance improved clearance in this model of increased hydrostatic pulmonary circulation pressures (2).

β-Adrenergic-receptor overexpression. β-Adrenergic agonists increase active Na\(^+\) transport in alveolar epithelial cells and normal and injured animal lungs by increasing the function of both apical Na\(^+\) entry pathways via the epithelial Na\(^+\) channels and Na\(^+\)-K\(^+\)-ATPases. These effects result from the stimulation of both β1- and β2-adrenergic receptors (70, 75, 88), leading to upregulation of Na\(^+\) channels and Na\(^+\)-K\(^+\)-ATPases in the lung epithelium (8, 56, 60, 67). Overexpression of a β2-adrenergic receptor in rat alveoli with recombinant adenovirus that expresses a human β2-adrenergic-receptor cDNA increased lung liquid clearance by ~100% compared with sham-infected rats. The increased lung liquid clearance was associated with increased abundance in peripheral lung of both α1-subunit of the epithelial Na\(^+\) channels in apical membrane fractions and Na\(^+\)-K\(^+\)-ATPase protein abundance in basolateral cell membranes (23).

SUMMARY

Alveolar epithelial Na\(^+\)-K\(^+\)-ATPases are highly regulated proteins that contribute substantively to the active Na\(^+\) transport necessary to maintain a dry alveolar air space. A growing body of research indicates that downregulation of alveolar Na\(^+\)-K\(^+\)-ATPases is associated with pulmonary edema in experimental models of lung injury as well as in patients with high- and low-pressure pulmonary edema. Thus methods that counterbalance the inhibition of edema clearance during lung injury and improve the lungs ability to clear pulmonary edema are needed. As such, mechanisms that increase Na\(^+\)-K\(^+\)-ATPase function, (i.e., activation of dopaminergic or adrenergic receptors, corticosteroids, gene transfer) represent the rationale for investigation toward the development of therapeutic strategies to regulate the Na\(^+\)-K\(^+\)-ATPase function and increase edema clearance. During these first 20 yr since the demonstration of Matthey et al. (58a) that alveolar edema is cleared by active Na\(^+\) transport, the importance of alveolar Na\(^+\)-K\(^+\)-ATPases has been clearly established. The mechanisms responsible for regulating the Na\(^+\) pump are now being actively studied. New experimental data are broadening our understanding of this crucial protein to lung biology and pathophysiology.

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