Na,K-ATPase Overexpression Improves Alveolar Fluid Clearance in a Rat Model of Elevated Left Atrial Pressure

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Background—Acute elevation of left atrial pressure (LAP) increases extravascular water and impairs active Na⁺ transport in rat lungs. We have reported that overexpression of Na,K-ATPase subunit genes in the alveolar epithelium increases alveolar fluid clearance (AFC) in normal and injured rat lungs with normal LAP. We reasoned that adenovirus-mediated transfer of an Na,K-ATPase β-subunit gene to the alveolar epithelium could improve AFC in rat lungs in the presence of acutely elevated LAP.

Methods and Results—Normal rats were infected with 4 × 10⁹ plaque-forming units of E1a⁻¹/E3⁻ recombinant adenoviruses that contained a cytomegalovirus promoter coupled to a rat Na,K-ATPase β₁-subunit cDNA (adβ₁) or no cDNA (adNull) 7 days before study. Na,K-ATPase α₁- and β₁-subunit abundance in basolateral cell membranes isolated from the peripheral lung was significantly increased in adβ₁-infected lungs compared with sham and adNull-infected controls. In all groups, elevation of LAP reduced membrane-bound Na,K-ATPase abundance; however, abundance in adβ₁-infected lungs remained greater than in controls. AFC, measured with a fluid-filled isolated lung preparation in the presence of elevated LAP (15 cmH₂O), in Na,K-ATPase β₁-subunit–overexpressing lungs was up to 100% greater than in controls and was not different from rats studied at normal LAP (0 cmH₂O).

Conclusions—These data suggest that alveolar overexpression of an Na,K-ATPase β₁-subunit can counteract downregulation of membrane-bound solute transporters owing to elevated pulmonary vascular pressures and can restore active Na⁺ transport and AFC in this rat model of acute hydrostatic pulmonary edema. (Circulation. 2002;105:497-501.)

Key Words: epithelium ● genes ● viruses ● heart failure ● edema

Pulmonary edema is absorbed from the alveolar airspace as a consequence of vectorial (transcellular) Na⁺ transport by apical Na⁺ channels and basolateral Na,K-ATPases that are located in alveolar epithelial cells.¹ Recently, we reported that acute ex vivo elevation of left atrial pressure (LAP) reduces active Na⁺ transport and alveolar fluid clearance (AFC) in isolated rat lungs.²,³ Accumulating data from experimental models of lung injury ⁴⁻⁵ and patients with respiratory failure ⁶⁻⁷ similarly indicate that AFC is reduced in some forms of pulmonary edema.

We have reported that adenovirus-mediated transfer of Na,K-ATPase subunit genes increases Na,K-ATPase expression and function in human and rat lung epithelial cells and the alveolar epithelium of rats.⁸⁻¹⁰ We have also reported that overexpression of a β₁ but not an α₁-Na,K-ATPase subunit gene increases AFC by more than 100% in normal rats,⁹ mitigates oxidant-mediated decreases in active Na⁺ transport in rat fetal distal lung epithelial cells,¹¹ and increases AFC and survival of rats exposed to 100% oxygen.⁸ These studies demonstrate the importance of Na,K-ATPases to vectorial Na⁺ transport in the lung and suggest that augmentation of Na,K-ATPase function may be useful for the treatment of pulmonary edema.

Our prior studies of the effects of Na,K-ATPase subunit gene overexpression and elevated LAP caused us to hypothesize that adenovirus-mediated gene transfer could be used to improve AFC in the setting of increased pulmonary vascular hydrostatic pressures in rats. To test this hypothesis, we infected rats with a recombinant adenovirus that expresses a rat Na,K-ATPase β₁-subunit cDNA. We then measured membrane-bound Na,K-ATPase expression and active, alveolar Na⁺ transport in isolated lungs with increased LAP. Herein, we report the results of the use of gene transfer to improve the ability of the lung to clear alveolar fluid in a model of acute hydrostatic pulmonary edema.

Methods

Adenovirus Delivery to Rat Lungs

Replication-incompetent (E1a⁻¹/E3⁻) human type 5 adenoviruses containing expression cassettes with a human immediate/early cyto-
megavirus promoter/enhancer element and either a rat Na,K-ATPase β-subunit cDNA (adβ1) or no cDNA (adNull) were propagated, purified, and tiered as described previously.9,10 The use of animals for the present study was approved by the Northwestern University Institutional Animal Care and Use Committee. Specific pathogen-free male Sprague-Dawley rats (weight 275 to 325 g, Harlan Inc, Indianapolis, Ind) were infected with 4×10^3 plaque-forming units of adβ1 or adNull in a 50% surfactant vehicle delivered endotracheally as described previously.9 These animals were compared with uninfected (control) and sham-infected (vehicle only) controls. After infection, animals were maintained in isolator cages for 7 days until study. A total of 91 rats were used for these studies.

**Isolated Lung Experiments**

The isolated lung preparation was performed as described previously.2,12,13 Briefly, lungs were isolated from anesthetized rats (pentobarbital sodium 65 mg/kg IP) after a 10-minute ventilation with 100% O2 via a catheter placed through a midline tracheotomy. The pulmonary artery and left atrial appendage were cannulated and perfused with a solution of 3% BSA in buffered physiological salt solution, FITC-tagged albumin was added to the perfusate to monitor leakage of protein from the vascular space into the airways. The lungs were excised from the thoracic cavity and placed in a "pleural" bath (100 mL) filled with the same BSA solution without FITC-tagged albumin and maintained at 37°C. The lungs were then instilled via the tracheal catheter with 5 mL of BSA containing Evans blue dye (EBD)–labeled albumin, [22Na] and [3H]-mannitol. Absorbance at 620 nm (for EBD-labeled albumin), fluorescence (excitation 487 nm, emission 520 nm; for FITC-labeled albumin), and scintillation counting (for [22Na] and [3H]-mannitol) were measured in centrifuged samples from the instillate, perfusate, and bath solutions after a 10-minute equilibration period and 60 minutes later.

The derivation of all equations involved in the mathematical model of edema clearance has been described previously.13 Concentration of EBD-labeled albumin was used to estimate airspace volume. Because virtually all EBD-labeled albumin remains in the airspace, instillate volume at a given time can be calculated from the increase in airspace protein concentration. The total unidirectional flux of Na+ from the alveolar space, a result of active transport and passive movement, was calculated from the rate of loss of [22Na] from the airspaces. Passive sodium flux was calculated by subtracting the active sodium flux, calculated from the rate of net fluid clearance, from the total. Similarly, the unidirectional volume flux of mannitol was calculated from the rate of loss of [3H]-mannitol from the airspaces. Albumin flux from the pulmonary circulation into the alveolar space was determined from the fraction of FITC-labeled albumin that appeared in the alveolar instillate during the experiment.

Alveolar Fluid Clearance

AFC was measured 7 days after infection of rats with vehicle alone (sham) or 4×10^3 plaque-forming units of adβ1 or adNull in presence and absence of ouabain (5×10^{-4} mol/L) in vascular perfusate. Control rats received no virus or vehicle. Data are mean±SD. *P<0.02 vs LAP=0 cmH2O control.

**Na,K-ATPase Function in BLMs**

Triplicate samples of BLM protein (20 μg) from sham-, adNull-, and adβ1-infected rats studied at normal or elevated LAP (n=6 animals/group) were resuspended in a high [Na+] solution buffer (50 mmol/L NaCl, 5 mmol/L KCl) with [γ-32P]-ATP as described previously.14 Na,K-ATPase activity was calculated as the difference in liberation of 32P from ATP between the test samples (total ATPase activity) and samples assayed in reaction buffer with 2.5 mmol/L ouabain and devoid of Na+ and K+ (ouabain-insensitive ATPase activity). Results were expressed as nanomoles of inorganic phosphate per milligram of protein per hour.

**Statistical Analysis**

Data are presented as mean±SD. One-way ANOVA (DataDesk, Data Description, Inc) was used when multiple comparisons were made. Results were considered significant at P<0.05.
Alveolar Epithelial Permeability

The passive movement of $^{22}\text{Na}^+$, $^3\text{H}$-mannitol from and FITC-albumin into the alveolar airspace was measured to generate an index of alveolar barrier function. Passive $^{22}\text{Na}^+$ and mannitol flux were increased in all elevated-LAP groups compared with LAP/0 cmH$_2$O controls (Figure 2A). Movement of the larger-molecule albumin (Figure 2B) was minimally increased in all LAP/15 cmH$_2$O groups compared with LAP/0 cmH$_2$O controls. Elevation of LAP did not affect pulmonary circulation flow rates or $^{22}\text{Na}^+$ concentration ($^{22}\text{Na}^+=135$ mEq/mL) in the instillate, perfusate, or pleural bath in any of the isolated lung studies.

Na,K-ATPase Protein Abundance

To test whether acute elevations of LAP affect membrane-bound Na,K-ATPase abundance, cell membrane fractions enriched for the BLM domain were prepared from peripheral lung tissue after 60 minutes of perfusion of the pulmonary vasculature at LAPs of 15 or 0 cmH$_2$O. Semiquantitative (Western) analysis from 6 to 9 animals per group revealed higher levels of both Na,K-ATPase $\beta_1$- and $\alpha_1$-subunits in ad$\beta_1$-infected lungs than in similarly perfused sham- and adNull-infected lungs (Figure 3). Intragroup comparison indicated that LAP elevation was associated with reductions of $\alpha_1$-subunit levels in all groups compared with similarly infected normal LAP specimens (Figure 3B). Acute LAP elevation reduced $\beta_1$-subunit protein expression in the sham and ad$\beta_1$ groups (Figure 3A). AdNull-infected lungs demonstrated a trend toward reduction of $\beta_1$-subunit abundance that did not reach statistical significance.

Membrane-bound Na,K-ATPase activity was quantified by measurement of ouabain-sensitive ATP hydrolysis (Na,K-ATPase activity) by BLMs isolated from the peripheral lung of rats studied at LAPs of 0 and 15 cmH$_2$O for 60 minutes (Figure 4). Activity was measured in the presence of low [K$^+$]/[Na$^+$] and ATP to maximize ATPase function per molecule ($V_{max}$) and produce a quantitative index of membrane-bound receptor number. These experiments revealed that Na,K-ATPase activity fell by $\sim$35% to 45% in the setting of elevated LAP in all groups (Figure 4). Na,K-ATPase function in BLMs from ad$\beta_1$-infected lungs studied at normal or elevated LAP was greater than that in all other groups.

Discussion

Acute LAP elevation decreases AFC in fetal lambs, adult sheep, and isolated rat lungs.$^{15-17}$ We have reported that adenovirus-mediated gene transfer of an Na,K-ATPase $\beta_1$-
subunit gene increases active Na\(^+\) transport in rat alveolar epithelial cells and AFC in normal and hyperoxic rat lungs.\(^9\) The present study was designed to test whether overexpression of an Na,K-ATPase gene could improve AFC in a rat model of increased pulmonary vascular pressures that resembles acute left heart failure.

In the present study, we observed that acute elevation of LAP was associated with diminution of both Na,K-ATPase activity and abundance in BLMs isolated from peripheral lung tissue (Figures 3 and 4). Data from our group and other investigators indicate that bidirectional movement of assembled Na,K-ATPase heterodimers from intracellular pools contributes to the short-term regulation of Na,K-ATPase function.\(^{18-20}\) Given these prior data and the results of the present study, we speculate that acute elevation of pulmonary vascular pressures (left atrial hypertension) decreases the number of Na,K-ATPases in the plasma membrane and that this loss of transporters impairs fluid reabsorption and contributes to the pathophysiology of hydrostatic pulmonary edema. This hypothesis is supported by the observation that increasing Na,K-ATPase abundance in BLMs normalizes AFC. Taken together, our data suggest that Na,K-ATPase gene transfer improves AFC in this model of acute lung injury by increasing functional Na,K-ATPase levels in the cell membrane. Interestingly, clearance rates in the ad\(\beta\) lungs, although not different from those in normal LAP controls, were less than that measured in our prior studies of \(\beta\sub{1}\) subunit overexpression (1.09 ± 0.09 mL/h) in normal rats. We speculate that this may be due to continued movement of fluid along hydrostatic pressure gradients out of the vascular space, although we cannot exclude that other epithelial transport proteins may be dysfunctional in this model. In addition, our model only allows insight into the acute effects of LAP elevation on alveolar active Na\(^+\) transport. Sustained elevation of LAP, as would be expected in chronic congestive heart failure, might be associated with markedly different changes in alveolar transport protein expression and function.

We used an established surfactant-based delivery system and viral dose that is capable of widespread gene transfer to the alveolar epithelium of rats.\(^9\) The duration of recovery (7 days) was based on past studies and was intended to maximize transgene function while allowing time for vector-induced host responses to subside.\(^9\) Alveolar permeability in all of the elevated-LAP groups in the present study was increased to a level similar to that in our prior reports of the effects of increased LAP on AFC.\(^2,3\) Alveolar barrier function in the adNull- and ad\(\beta\)\sub{1}–infected lungs was not different from similarly treated uninfected and sham-infected controls (Figure 2), which suggests that adenoviral infection does not compound changes in permeability in this model. We believe that the changes in permeability seen with elevated pulmonary capillary hydrostatic pressures in the present study may be due to stretch-induced pore formation and/or capillary stress failure.\(^{21,22}\) Similar findings of increased permeability in a rabbit model of hydrostatic pulmonary edema have been reported by Bachofen et al.\(^{23,24}\) Importantly, the mild increases in alveolar permeability noted in the present study were less than those noted in our prior studies of lung injury\(^4,8\) and did not impede assessment of AFC.

Prior data indicate that Na,K-ATPases are predominantly expressed in the basolateral plasma membranes of alveolar epithelial cells\(^{25,26}\) and that our adenovector delivery system preferentially targets the alveolar epithelium.\(^{6,9}\) The BLMs used in the present study were isolated from peripheral lung tissue and included membranes from all polarized cells in the distal lung (ie, alveolar epithelium and endothelium). The inclusion of proteins from this heterogeneous mix of cells may dilute alveolar epithelial proteins and lead to underestimation of the abundance and function of alveolar epithelial solute transport proteins. An alternate approach would be to isolate alveolar type 2 epithelial cells. However, doing so would exclude any contribution of type 1 epithelial cells and would preclude isolation of BLMs because of loss of cell polarity during cell isolation. We believe that the significant increases in Na,K-ATPase function and \(\alpha\sub{1}\) and \(\beta\sub{1}\) subunit expression in BLMs from the peripheral lung of ad\(\beta\)\sub{1}–infected animals corroborate our AFC data (Figure 1) and support the notion that peripheral lung tissue can be used to gain useful insights into the regulation of alveolar epithelial solute transport proteins.

We have previously shown\(^9,27\) that adenovirus-mediated gene transfer of a rat \(\beta\)\sub{1}-CDNA to rat lungs significantly increases \(\beta\) subunit protein expression in the alveolar epithelium. In the present study, we observed increases in \(\beta\)\sub{1}–protein expression in BLMs from ad\(\beta\)\sub{1}–infected lungs, which indicates that gene transfer increases \(\beta\) subunit levels in the appropriate domain of the cell membrane. Our prior studies showed that \(\alpha\) subunit overexpression does not increase active transport in rat alveolar cells or lungs, which caused us to hypothesize that the \(\beta\) subunit is rate-limiting in the rat lung. The finding of increased \(\alpha\) subunit expression in BLMs from ad\(\beta\)\sub{1}–infected lungs in the present study and prior in vitro data allow us to speculate that \(\beta\) subunit overexpression increases membrane-bound Na,K-ATPase levels via recruitment of \(\alpha\)/\(\beta\) heterodimers from intracellular pools.\(^{18}\) Whether \(\beta\) subunit overexpression affects \(\alpha\) subunit gene transcription or translation rates was not tested in the present study.

The data presented in this report support the paradigm that acute elevation of LAP impairs active Na\(^+\) transport in part by downregulating transport proteins in the cell membrane. The results of the present study show for the first time that gene transfer can increase Na,K-ATPase transport protein abundance and function and restore AFC in this model of acute hydrostatic pulmonary edema. The ability of adenovectors to transduce the alveoli of acutely injured edematous rat lungs\(^{28,29}\) allows us to propose the use of genetic therapies to counterbalance the pathophysiological impairment of alveolar active Na\(^+\) transport seen in the setting of acute left atrial hypertension.

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References


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