lambs with subsequent lung injury compared to the 16 lambs without lung injury.

These results suggest that inflation pressure may influence lung vascular protein permeability and edema in lambs that receive mechanical ventilation after premature birth, and that the degree to which circulating neutrophils decrease soon after birth may help to predict subsequent development of lung vascular injury and edema in preterm lambs.

REFERENCES

Alveolar Type II Cell Na,K-ATPase is Upregulated During Mechanical Ventilation-induced Pulmonary Edema*

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Pulmonary edema formation occurs by two mechanisms: increased hydrostatic pressures in the pulmonary circulation, as seen in patients with congestive heart failure, and/or increased permeability, as seen in patients with adult respiratory distress syndrome.1-3 Regardless of the etiology, once lung edema is present, its clearance occurs mainly by active epithelial Na+ transport.4,6 Previous studies have shown that lung liquid clearance stops completely when active metabolic processes for solute transport are nonspecifically inhibited by hyperthermia, and that clearance is partially inhibited by amiloride, ouabain, and atrial natriuretic factor.3,4 Although other mechanisms may have a role, the clearance of pulmonary edema appears to be mostly effected by a combination of alveolar apical Na+ channels and the basolaterally located Na,K-ATPases.8,10 Specifically, alveolar Na,K-ATPase has been proposed to play an important role in effecting edema clearance by the active transport of Na+.

Recently, it has been shown that mechanical ventilation with high tidal volumes in rats produces a form of barotrauma characterized by increased lung permeability and alveolar edema accumulation.11 Other forms of injury have been shown to upregulate protective mechanisms against lung injury.10,12 Thus, we tested whether alveolar epithelial Na,K-ATPase is upregulated as a protective mechanism against lung edema in this model of acute lung injury.

EXPERIMENTAL DESIGN AND RESULTS

We studied 12 adult, pathogen-free Sprague Dawley male rats (weight ~ 300 g). Two groups of four rats were mechanically ventilated for 25 min with the following: (a) low tidal volumes (3 to 5 ml) to a peak airway pressure of 10 cm H2O; and (b) high tidal volumes (12 to 15 ml) to a peak airway pressure of 35 cm H2O and compared to (c) four control nonventilated rats.

Immediately following mechanical ventilation, alveolar type II (ATII) cells were isolated and Na,K-ATPase hydrolytic activity was measured by preincubating microsomal membrane fractions with and without 1 mM ouabain for 45 min. The results were corrected for spontaneous hydrolysis of Na,K-ATPase, and the inorganic phosphate was measured by the method of Fiske and Subbarow. We measured 3H-ouabain binding in these ATII cells in the presence of 10^-6 M 3H-ouabain. We isolated RNA from these ATII cells and measured the α1 Na,K-ATPase mRNA with a cRNA probe and corrected for the lane loading with 18S. We also measured

![Graph](http://journal.publications.chestnet.org/pdfaccess.ashx?url=/data/journals/chest/21692/ on 05/30/2017)
the expression of Na,K-ATPase protein by immunocytochemistry in ATII cells, utilizing an isofom specific polyclonal antibody for rat Na,K-ATPase α1, α2, or β1 subunits. Immunogold-stained Na,K-ATPases were quantified on an image analysis system (Quantimet 520 system).

We observed that rats ventilated with the high tidal volumes had increased lung edema estimated by wet/dry lung weight ratio as compared to the other two animal groups: high tidal volumes (6.0 ± 0.4), control (4.6 ± 0.3), and low tidal volumes (4.7 ± 0.6). The Na,K-ATPase activity from ATII cells isolated following mechanical ventilation was increased in the rats ventilated with high tidal volumes (168 ± 17 μmol Pi/h/mg protein) as compared to rats ventilated with low tidal volumes (122 ± 13 mol Pi/h/mg protein) and to control rats (102 ± 14 μmol Pi/h/mg protein). As shown in Figure 1, 3H-ouabain binding increased in ATII cells harvested from rats ventilated with high tidal volumes as compared to the other two groups, indicating more Na,K-ATPases in the plasma membrane. Also, by immunocytochemical analysis the Na,K-ATPase β1 protein increased in ATII cells from rats ventilated with high tidal volumes as compared to the other two groups, whereas no change was observed in the Na,K-ATPase α1 and α2 proteins. We also observed that in ATII cells from rats ventilated with high tidal volumes, the Na,K-ATPase α1 mRNA expression was fourfold higher than in the other two groups (n = 2).

**Discussion**

Previous studies in a hyperoxic model of lung injury found that edema is cleared more rapidly than in normoxic rats, in association with upregulated Na,K-ATPase in ATII cells.10,11 These observations support the notion that Na,K-ATPase is upregulated in the lungs as a protective mechanism against lung injury, possibly contributing to the resolution of the lung edema. A different model of lung injury causes significant pulmonary edema after only 25 min of mechanical ventilation in rats with high tidal volumes.11

Our results confirm that short-term high tidal volume ventilation produces lung edema. This is associated with increased Na,K-ATPase activity, an increase in 3H-ouabain binding, and Na,K-ATPase β1 protein expression in ATII cells isolated from these rats as compared to rats ventilated with low tidal volumes and control rats. Also, we observed an increase in the α1 Na,K-ATPase mRNA expression following high tidal volume mechanical ventilation. These data suggest that short-term high tidal volume ventilation triggers a rapid protective mechanism (eg, Na,K-ATPase) against pulmonary edema.

In summary, our results suggest that more than one mechanism may participate in the upregulation of Na,K-ATPase during high tidal volume ventilation. It is unlikely for the Na,K-ATPase activity to be increased as a result of new protein translation, and thus, we reason that the increased Na,K-ATPase activity is due to more efficient "pumping" and/or to translocation of Na,K-ATPase protein from inner to plasma membrane. Another less likely possibility is increased stabilization of Na,K-ATPase protein by the cytoskeleton polarizing proteins. Also, transcriptional regulation may be important in the long term. Thus, it is possible that the combined effects of all these mechanisms for Na,K-ATPase upregulation may be responsible for the very rapid edema clearance observed after mechanical ventilation-induced lung edema.13

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