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Na,K-ATPase Expression Is Increased in the Lungs of Alcohol-Fed Rats

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Abstract

Background—Alcohol abuse independently increases the risk of developing the acute respiratory distress syndrome (ARDS), a disease characterized by diffuse alveolar epithelial damage, lung edema, and consequent severe hypoxemia. Chronic alcohol abuse increases alveolar epithelial permeability both in vitro and in vivo, in part due to altered tight junction formation. However, both alcohol-fed animals and otherwise healthy alcoholic humans do not have pulmonary edema at baseline, even though their lungs are highly susceptible to acute edematous injury in response to inflammatory stresses. This suggests that active fluid transport by the alveolar epithelium is preserved or even augmented in the alcoholic lung. Chronic alcohol ingestion increases expression of apical sodium channels in the alveolar epithelium; however, its effects on the Na,K-ATPase complex that drives sodium and fluid transport out of the alveolar space have not been examined.

Methods—Age- and gender-matched Sprague–Dawley rats were fed the Lieber–DeCarli liquid diet containing either alcohol or an isocaloric substitution (control diet) for 6 weeks. Gene and protein expression of lung Na,K-ATPase α1, α2, and β1 subunits were quantified via real-time PCR and immunobiological analyses, respectively. Alcohol-induced, Na,K-ATPase-dependent epithelial barrier dysfunction was determined by calculating lung tissue wet:dry ratios following an ex vivo buffer-perfused challenge for 2 hours in the presence of ouabain (10^{-4} M), a Na, K-ATPase inhibitor.

Results—Chronic alcohol ingestion significantly increased gene and protein expression of each Na,K-ATPase subunit in rat lungs. Immunohistochemical analyses of the alcoholic lung also revealed that protein expression of the Na,K-ATPase α1 subunit was increased throughout the alveolar epithelium. Additionally, lungs isolated from alcohol-fed rats developed more edema than comparably treated lungs from control-fed rats, as reflected by increased lung tissue wet:dry ratios.

Conclusions—These findings indicate that chronic alcohol ingestion, which is known to increase alveolar epithelial paracellular permeability, actually increases the expression of Na,K-ATPase in the lung as a compensatory mechanism. This provides a potential explanation as to why the otherwise healthy alcoholic does not have evidence of pulmonary edema at baseline.

Keywords

Alcohol; Acute Respiratory Distress Syndrome; Edema; Lung

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The acute respiratory distress syndrome (ARDS) is a severe form of acute edematous lung injury characterized by noncardiogenic pulmonary edema and flooding of the alveolar airspaces with proteinaceous fluid. ARDS develops in response to inflammatory stresses including sepsis, trauma, gastric aspiration, pneumonia and massive blood transfusions (Ware and Matthay, 2000). Originally described in 1967 (Ashbaugh et al., 1967), ARDS is characterized by alveolar epithelial and endothelial barrier disruption, surfactant dysfunction, and intense inflammation that, in concert, produce profound derangements in gas exchange and severe respiratory failure. Although a great deal has been learned about the underlying pathophysiology of this syndrome in the past four decades, our treatment remains essentially supportive and despite aggressive ICU care and mechanical ventilation, the mortality rate for ARDS remains unacceptably high at 40–60% (Rubenfeld et al., 2005;Ware and Matthay, 2000).

An important clue to which patients are at greater risk for developing ARDS has been uncovered in recent epidemiologic studies demonstrating a link between alcohol abuse and acute lung injury (ALI), including the landmark study in 1996 that first identified an independent connection between alcohol abuse and ARDS (Moss et al., 1996). A more recent study confirmed those initial findings and determined that in individuals with septic shock, the relative risk of ARDS in alcoholic patients versus nonalcoholic patients was 3.7:1 (Moss et al., 2003). If these findings are extrapolated to the population at large, then alcohol abuse contributes to the development of ARDS in tens of thousands of patients in the U.S. each year.

Alveolar epithelial barrier dysfunction is a prominent feature of ARDS. The maintenance of a fluid-free alveolar space is critical for facilitating normal gas exchange. A pathological hallmark of ALI/ARDS is heterogeneous damage of the alveolar epithelium, with complete loss of the epithelial surface in some areas but with other alveoli relatively intact. Therefore, at a cellular level the extent of the alveolar epithelial damage may not be as widespread or as uniform as the chest radiograph might suggest, and preservation and repair of the alveolar epithelium are keys to survival. In fact, patients with impaired alveolar epithelial fluid clearance are three times more likely to die from ALI/ARDS than patients with a maximal ability to clear lung fluid (Sznajder, 2001; Ware and Matthay, 2001). However, more than 85% of patients with ALI/ARDS have at least a partial defect in lung fluid clearance (Ware and Matthay, 2001).

The ability of the lung to clear fluid away from the alveolar space and into the pulmonary circulation is largely dependent on active Na^+ transport by the alveolar epithelium; for review, see (Morty et al., 2007). Na^+ uptake at the apical side of the alveolar epithelium is mediated by amiloride-sensitive Na^+ channels (ENaC), whereas Na^+ is pumped out of the epithelial cells at the basolateral side by the Na,K-ATPase. The Na,K-ATPase belongs to the family of P-type ATPases and the functional enzyme consists of α and β subunits (Lingrel et al., 1994a–c; Sweadner, 1989). Multiple isoforms of both the α and β subunits have been reported (Lingrel et al., 1994b; Therien and Blostein, 2000). The catalytic α subunit contains binding sites for ATP as well as the Na,K-ATPase inhibitor, ouabain; for review, see (Kaplan, 2002). The alveolar epithelium expresses both $\alpha 1$ and $\alpha 2$ subunits (Borok et al., 2002; Ridge et al., 1997, 2003). The β subunit regulates activity and proper membrane localization of the α subunit (Factor et al., 1998; Geering, 1991; Kaplan, 2002; Therien and Blostein, 2000; Thome et al., 2001).

Chronic alcohol ingestion increases ENaC density on the apical side of alveolar epithelial cells (Guidot et al., 2000b), and acute alcohol administration (a single dose) to rats decreases Na,K-ATPase activity (Aytacoglu et al., 2006; Rodrigo et al., 2002). However, to our

knowledge the effects of chronic alcohol ingestion on the expression of Na,K-ATPase in the lung has not been determined. This critical evidence is necessary to support the aforementioned explanation as to why the otherwise healthy alcoholic lung is not edematous at baseline. Therefore, in this current study we sought to determine whether or not chronic alcohol ingestion increased the expression of Na,K-ATPase within the lung.

MATERIALS AND METHODS

Alcohol Feeding

AdultMale Sprague–Dawley rats (150–200 g, Charles River Laboratory, Wilmington, MA) were fed the Lieber–eCarli liquid diet (Research Diets, New Brunswick, NJ) containing either ethanol (36% of total calories) or an isocaloric substitution with maltose–dextrin ad libitum for 6 weeks as previously published (Guidot et al., 2000b; Velasquez et al., 2002). All work was performed with the approval of our Institutional Care and Use of Animals committee at the Atlanta VAMC.

RNA Isolation and Real-Time PCR

Total RNA isolation, real-time PCR, and gene expression analyses were performed as previously described (Otis et al., 2007). Total RNA was isolated from whole lung tissues from either control-fed or alcohol-fed rats using Trizol Reagent (Life Technologies, Gaithersburg, MD) following the manufacturer's protocol. Total RNA (1 μg) was reverse transcribed in a 20 μl final reaction volume using random primers and M-MLV reverse transcriptase (Invitrogen, Carlsbad, CA). The reverse transcription reaction was incubated at 65°C for 10 min, 80°C for 3 min, and 42°C for 60 min. cDNA (5 μl of a 1:10 dilution) was amplified using 400-nm gene-specific primers in a 25 μl reaction containing iQ SYBRgreen Supermix (Bio-Rad). Primers were as follows: Na,K-ATPase α1 (185 bp), 5′-CTTTGCTAGGACCTCTCCTCAA-3′ and 5′-GAAGAATCATGTCAGCAGCTTG-3′; Na,K-ATPase α2 (300 bp), 5′-GGGAACATGCAGAATCAGTGTA-3′ and 5′-CTTTCATATTCCCACCTCAAGC-3′; Na,K-ATPase β1 (234 bp), 5′-TGGAGACTTACCCTCTGACGA-3′ and 5′-GGATTTCAGTGTCCAAGGTGA-3′. Samples were incubated at 95°C for 15 min, followed by 40 cycles of denaturation, annealing, and extension at 95°C, 58°C, and 72°C, respectively. As a control, RT-PCR was also performed on 2 μl of each RNA sample to confirm absence of contaminating genomic DNA. Fluorescence was recorded at the end of each annealing and extension step. All reactions were performed in triplicate and the starting quantity of the gene of interest was normalized to 18S rRNA for each sample.

Western Blot Analyses of Na,K-ATPase Expression

Frozen lung tissues were homogenized in a solution containing: 50 mM Tris–HCl (pH 7.8), 150 mM NaCl, 5 mM EDTA, 15 mM $MgCl_2$, 0.5% Nonidet P-40, 0.3% Triton X-100, 1 mM dithiothreitol, and complete mini protease inhibitor cocktail tablets (Roche, Indianapolis, IN). Total protein (30 μg) from each sample was loaded onto a 12% acrylamide gel and electrophoresed at 160 volts for 90 min. The separated proteins were transferred to a 0.45 μM polyvinylidine difluoride (PVDF) membrane at 15 volts for 70 min. The PVDF membrane was incubated in blocking buffer (Tris-buffered saline with 0.2% Tween 20 (TBS-T) containing 5% nonfat dry milk) for 1 hour at room temperature and then probed overnight at 4°C with a primary antibody for either the α1 or the α2 subunit of the Na,K-ATPase complex (1:200 in blocking buffer, Santa Cruz Biotechnology, Santa Cruz, CA). The membrane was subsequently washed and then incubated with a horseradish peroxidase-labeled anti-rabbit IgG secondary antibody (1:4000 in blocking buffer, Santa Cruz Biotechnology) for 2 hours at room temperature. The bands on the membrane were enhanced by chemiluminescence (ECL: Amersham, Arlington Heights, IL), detected using a

BioRad Imaging System, and normalized to the expression of the housekeeping protein GAPDH.

Quantitative Immunostaining for the α1 Subunit of Na,K-ATPase

Briefly, lung sections (6 μm) were fixed in 4% formaldehyde for 10 min, washed with PBS, and endogenous peroxidase activity was quenched with 3% H_2O_2. Sections were permeabilized with 0.05% Tween-20 (PBS-T), blocked with 5% donkey serum, and incubated overnight at 4°C with anti-Na,K-ATPase α1 IgG (1:200; Santa Cruz Biotechnology). Sections were incubated with biotinylated donkey anti-goat IgG (Jackson ImmunoResearch, West Grove, PA) followed by horseradish peroxidase-streptavidin (Vectastain kit; Vector Laboratories, Burlingame, CA). Color was developed with 3,3′-diaminobenzidine tetrahydro-chloride substrate (Vector), counterstained with hematoxylin, and coverslipped. Goat IgG was used to control for nonspecific antibody binding. Multiple high-power photomicrographs were obtained using a Leica DM4000B microscope. To quantify the amount of immunohistochemical staining in sections, multiple photo-micrographs were imported into Adobe Photoshop 7.0 (Adobe Systems Inc.). A color sampler tool was used to gate representative shades of brown immunostaining. To determine the stain density of each field, the area of the field containing the selected color stain was selected, the remaining background was removed, and the resulting image was imported into Scion Image beta 4.02 (Scion Corporation, Frederick, MD). The density of staining was averaged and calculated for each section.

Isolation and Perfusion of Lungs

The lungs of control-fed and alcohol-fed rats were isolated and perfused as we have previously described (Holguin et al., 1998; Lois et al., 1999). Briefly, rats were anesthetized with pentobarbital (60 mg/kg IP) and a tracheostomy cannula was placed and secured with 2-O ligature, and the lungs and heart were excised en bloc and placed in an isolation chamber. Lungs were ventilated with a tidal volume of 3 cc at a rate of 60/min with 2.5 cm of H_2O positive end-expiratory pressure with a gas mixture containing 5% CO_2, 21%O_2 and 74% N_2, and perfused at a rate of 40 cc/kg body wt/min with Earle's Balanced Salt Solution (Sigma) to which was added sodium bicarbonate (2.2 g/l) and Ficoll-70 (40 mg/ml, Sigma), and the final pH adjusted to 7.4. After a 20 min equilibration period, ouabain at a final concentration of 10^{-4} M was added to perfused lungs for 2 hours. At the end of this perfusion period, the right lung was removed and weighed (wet weight) and then re-weighed after desiccation overnight at 70°C (dry weight). The ratio of the wet weight to the dry weight was calculated and expressed (wet:dry) for each experimental determination.

Statistics

Data are presented as mean ± SEM. Data analyses were done by ANOVA with Student–Newman–Keuls post-hoc tests for group comparisons and were considered statistically significant at a p value of <0.05.

RESULTS

Chronic Alcohol Ingestion Increased the Gene Expressions of the α1, α2, and β1 Subunits of Na,K-ATPase in the Lung

Using real-time PCR, we show that chronic alcohol ingestion significantly increased gene expressions of the α1, α2, and β1 of the Na,K-ATPase complex in whole lung tissues from rats compared with controls (Fig. 1A–C, respectively).

Chronic Alcohol Ingestion Increased the Protein Expression for Both the α1 and the α2 Subunits of Na,K-ATPase in the Lung

Because chronic alcohol ingestion increased gene expressions of both the α1 and the α2 subunits of the Na,K-ATPase complex, we next assessed whether or not the corresponding protein expressions for each of these subunits were increased. Consistent with the gene expression, chronic alcohol ingestion significantly increased the lung protein expression of both the α1 and the α2 subunits compared with control-fed animals (Fig. 2A and 2B, respectively). Protein expressions were normalized to GAPDH and values are expressed as mean ± SEM of 3–4 determinations. A representative band from the western blot for each protein is shown below the summary data.

Chronic Alcohol Ingestion Increased the Protein Expression of Na,K-ATPase Throughout the Alveolar Epithelium

To determine whether or not the increased Na,K-ATPase subunit expressions were associated with an actual increase in the complex within the alveolar epithelium, we next performed immunostaining of lung tissue for the relative expression of the α1 subunit of the Na,K-ATPase complex in the alveolar epithelium. Consistent with the expression data presented in Figs 1 and 2, chronic alcohol ingestion significantly increased the relative expression of the α1 subunit throughout the alveolar walls (Fig. 3). In Fig. 3B, each value represents the mean ± SEM of three (alcohol-fed) or four (control-fed) rat lungs.

Lungs From Alcohol-Fed Rats Are More Sensitive to Na,K-ATPase Inhibition by Ouabain During ex Vivo Perfusion

The results shown in Figs 1–3 were consistent with the original hypothesis that chronic alcohol ingestion increases expression of the Na,K-ATPase complex in the lung. To test whether or not this increased expression was associated with an increased capacity to clear fluid from the airway, we next assessed the relative sensitivity of lungs from alcohol-fed versus control-fed rats to inhibition of the Na,K-ATPase complex by ouabain. Although we did not measure sodium and fluid transport directly, we reasoned that if the alcoholic lung is more dependent on active fluid transport to remain dry because of the increased paracellular leak, than they should develop more edema when perfused in the presence of an inhibitor of the Na,K-ATPase complex. Consistent with this prediction, lungs from alcohol-fed rats had significantly higher wet:dry ratios (reflecting lung edema) compared with control-fed rats (Fig. 4). The average baseline (nonedematous) wet:dry ratios of control-fed and alcohol-fed rat lungs was 4.5 (as we have published previously (Pelaez et al., 2004)), and therefore the wet:dry ratios in each experimental group are expressed relative to that baseline.

DISCUSSION

In this study, we determined that chronic alcohol ingestion in rats for 6 weeks increased gene and protein expression of the α1, α2, and β1 subunits of the Na,K-ATPase complex in the lung. In parallel, Na,K-ATPase protein expression, as reflected by the intensity of immunostaining for the α1 subunit, was increased throughout the alveolar epithelium. This increased expression appeared to be functionally relevant, in that lungs from alcohol-fed rats developed more edema when perfused with the Na,K-ATPase inhibitor, ouabain, than lungs from control-fed rats. These findings complement our previous study that demonstrated increased expression of apical sodium channels in alveolar epithelial cells isolated from alcohol-fed rats (Guidot et al., 2000b). Taken together, these studies suggest that chronic alcohol ingestion up-regulates the ability of the lung to transport fluid out of the alveolar space. When viewed in the context that chronic alcohol ingestion increases paracellular permeability in the alveolar epithelium (Guidot et al., 2000b), this study provides a logical explanation as to why the otherwise healthy alcoholic lung is not edematous at baseline.

Specifically, although paracellular permeability and therefore fluid leak into the alveolar space is increased in the alcoholic lung, a parallel and perhaps compensatory increase in the ability to actively transport fluid via the transcellular route keeps the alveolar space relatively dry. Unfortunately, this new equilibrium leaves the alcoholic lung very little ability to maximally respond to an acute stress (Guidot et al., 2000b). Consequently, when challenged with an acute inflammatory insult such as sepsis, the alcoholic lung is far more susceptible to acute edematous injury (Holguin et al., 1998; Velasquez et al., 2002).

Although long recognized as a major risk factor for pneumonia and other serious illnesses that predispose to ALI, the independent association between alcohol abuse and ARDS has only been verified within the past decade (Licker et al., 2003; Moss et al., 1996, 2003). We and others have investigated the mechanisms underlying this association in both animal models and in clinical studies. In both experimental models and in otherwise healthy alcoholic subjects, chronic alcohol ingestion causes severe oxidant stress within the lower airways as reflected by dramatic decreases in the levels of the anti-oxidant, glutathione (Holguin et al., 1998; Moss et al., 2000). In parallel, alcohol ingestion impairs alveolar epithelial surfactant production (Guidot and Brown, 2000a; Holguin et al., 1998), barrier function (Guidot et al., 2000b), and renders the lung more susceptible to acute edematous injury in response to endotoxemia or sepsis (Holguin et al., 1998; Lois et al., 1999; Velasquez et al., 2002). Alcohol-induced oxidant stress and glutathione depletion appear to mediate many of the defects in alveolar epithelial function, as dietary supplementation with glutathione precursors prevents the glutathione depletion, alveolar epithelial barrier function, and sepsis-mediated ALI in the rat model (Guidot et al., 2000b; Holguin et al., 1998; Lois et al., 1999; Velasquez et al., 2002).

Lung fluid balance is regulated by the concerted actions of the pulmonary epithelial and endothelial barriers (Mehta et al., 2004). Although the basement membrane and capillary endothelium contribute to the barrier between the alveolar lumen and blood, 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 (Mutlu and Sznajder, 2004). Within the alveolar epithelium, there are two major factors that affect the ability of the alveolus to clear fluid from airspaces. One factor is the active clearance of water and ions by specific transporters expressed by alveolar epithelial cells (Chen et al., 2004; Dada and Sznajder, 2003; Dobbs et al., 1998; Mutlu and Sznajder, 2004; Sznajder et al., 2002). The second factor is the extent of permeability between alveolar epithelial cells (i.e., paracellular leak) (Wilson et al., 2001). Paracellular permeability between alveolar epithelial cells is controlled by tight junctions that create a barrier to free diffusion (Koval, 2006). Previously, we determined that alcohol-fed rats had decreased net vectorial fluid transport and increased bi-directional protein permeability across the alveolar epithelium compared with control-fed rats (Guidot et al., 2000b). In parallel, when alveolar epithelial cells were isolated from alcohol-fed rats and placed in primary culture for 8 days, they still formed a more permeable cell layer (measured as permeability of ^{14}C-inulin) than cells from control-fed rats (Guidot et al., 2000b). Cells from alcohol-fed rats also had more sodium-permeable channels in their apical membranes than cells isolated from control-fed rats, and when challenged with intratracheal saline in vivo they increased fluid transport in response to epinephrine stimulation, indicating that there still was transcellular salt and water transport in the alcoholic lung (Guidot et al., 2000b). Taken together, these findings suggested that although the alveolar epithelial paracellular permeability is increased by chronic alcohol ingestion, this defect may be compensated for by an up-regulation in active sodium transport. This would explain why alcohol ingestion alone does not cause lung edema in animal models and why otherwise healthy alcoholics have no evidence of lung edema by clinical criteria (Burnham et al., 2003; Holguin et al., 1998).

Chronic alcohol ingestion has systemic and lung-specific manifestations (reviewed in Guidot and Hart, 2005; Joshi and Guidot, 2007), and therefore, the alveolar epithelium is not its only target. However, as loss of the alveolar epithelial barrier and consequent flooding of the airspaces is a cardinal feature in ARDS, defects in alveolar epithelial barrier function very likely contribute to the increased risk of ALI in alcoholics. However, the findings that chronic alcohol ingestion increases alveolar epithelial paracellular permeability even in the absence of an inflammatory stress must be reconciled with the experimental and clinical observations that alcohol abuse alone does not cause lung edema. In our previous study in which we first characterized the alcohol-induced paracellular leak in the alveolar epithelium both in vitro and in vivo (Guidot et al., 2000b), we also determined that freshly isolated alveolar epithelial type II cells from alcohol-fed rats had increased expression of apical sodium channels (ENaC). In parallel, although the alcohol-fed rat lung had a decrease in its net liquid clearance in vivo, it nevertheless responded to catecholamine stimulation and was able to augment liquid clearance when stimulated with epinephrine (Guidot et al., 2000b). At that time, our interpretation was that the ability of the alveolar epithelium to transport sodium and therefore water via the transcellular mechanism compensated for the increased paracellular leak in the alcoholic lung. This was consistent with the fact that although the alcoholic lung had a decrease in its absolute capacity to clear a liquid challenge (Guidot et al., 2000b), this defect was due to a increase in leak and not a decrease in active fluid transport. However, the active transcellular transport of sodium and water requires the generation of a sodium gradient by the actions of the Na,K-ATPase complex on the basolateral surface of the alveolar epithelium, and the consequent passive movement of sodium through ENaC which is localized on the apical surface. Therefore, if we are to invoke augmented fluid transport by the alcoholic alveolar epithelium, there must be evidence that the expression and/or the activity of the Na,K-ATPase complex is increased.

Previous studies have shown that acute alcohol administration (a single dose) to rats decreased Na,K-ATPase activity after 3 hours (Aytacoglu et al., 2006; Rodrigo et al., 2002). Although seemingly contradictory to the findings in this study, these data may be explained in part by acute oxidative stress events in response to alcohol. In contrast, chronic exposure to lower doses of alcohol probably allows for a compensatory up-regulation of pathways that modulate expression of components of the Na,K-ATPase complex. Here, we provide the first evidence that chronic alcohol ingestion increases the expression of the Na,K-ATPase complex in the lung. In parallel, although we do not measure Na,K-ATPase activity directly, we determined that the alcoholic lung is more susceptible to Na,K-ATPase inhibition by ouabain. Overall, these results are consistent with our previous findings that ENaC expression is increased in the alveolar epithelial cells of alcohol-fed rats and, when taken together, support the hypothesis that chronic alcohol ingestion actually enhances transcellular sodium and fluid transport.

In summary, we report for the first time that chronic alcohol ingestion in an experimental rat model increases the expression of the Na,K-ATPase complex within the lung. These findings complement our previous study showing that alcohol likewise increased ENaC expression within the alveolar epithelium. Therefore, these two key components of active transcellular fluid transport are increased in the alcoholic alveolar epithelium. This appears to lead to an up-regulation of active sodium and fluid transport that can compensate for the increased paracellular permeability within this same alcoholic alveolar epithelium. Although the net result is that the otherwise healthy alcoholic lung is not edematous at baseline, it is nevertheless more susceptible to acute edematous injury in response to acute insults such as sepsis or trauma. Although the discrete molecular mechanisms underlying these changes need to be elucidated, this study provides new insights into how chronic alcohol abuse alters alveolar epithelial function.

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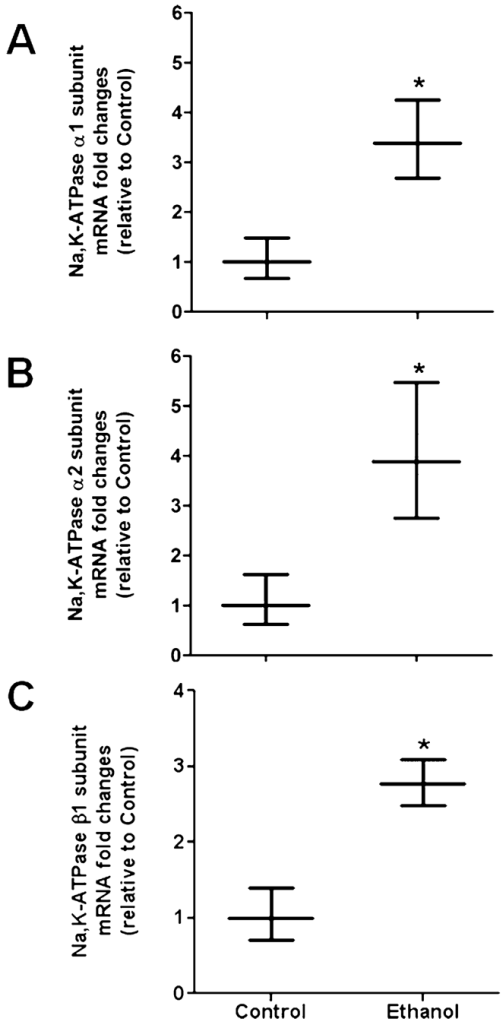

Fig. 1.

Chronic alcohol ingestion increases the gene expressions of the α1, α2, and β1 subunits of Na,K-ATPase in the lung. Real-time PCR was performed on whole lung homogenates for mRNA expression levels of the Na,K-ATPase α1 (panel A), α2 (panel B), and β1 subunits (panel C). Chronic alcohol ingestion increased the expression levels of each subunits relative to control-fed animals. Data are represented as the mean relative quantity and the range of possible quantities (i.e., delta delta Ct) as calculated from the standard errors of the delta Ct values. *$p < 0.05$ compared with control.

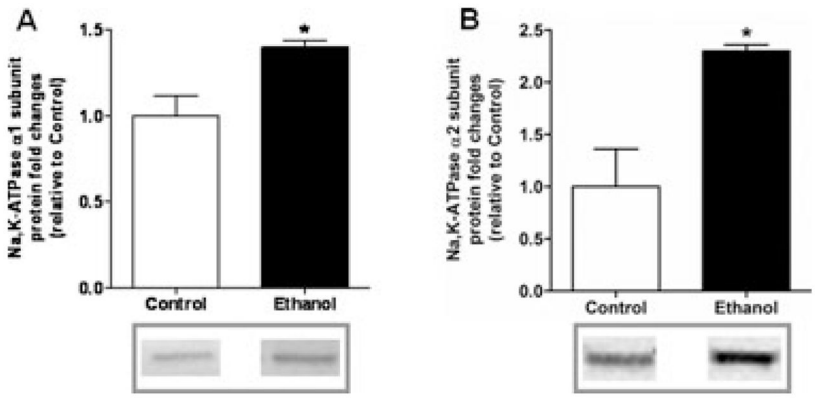

Fig. 2.

Chronic alcohol ingestion increases the protein expression for both the α1 and the α2 subunits of Na,K-ATPase in the lung. Western blot analyses were performed on whole lung homogenates to assess the relative protein expression of the Na,K-ATPase α1 (panel **A**) and α2 (panel **B**) subunits. Chronic alcohol ingestion increased the expression of both subunits relative to control-fed animals. Protein expression in each determination was normalized to expression of the housekeeping protein GAPDH and each value shown is the mean ± SEM of 3–4 determinations. A representative band for each protein is shown below the summary data. *$p < 0.05$ compared with control.

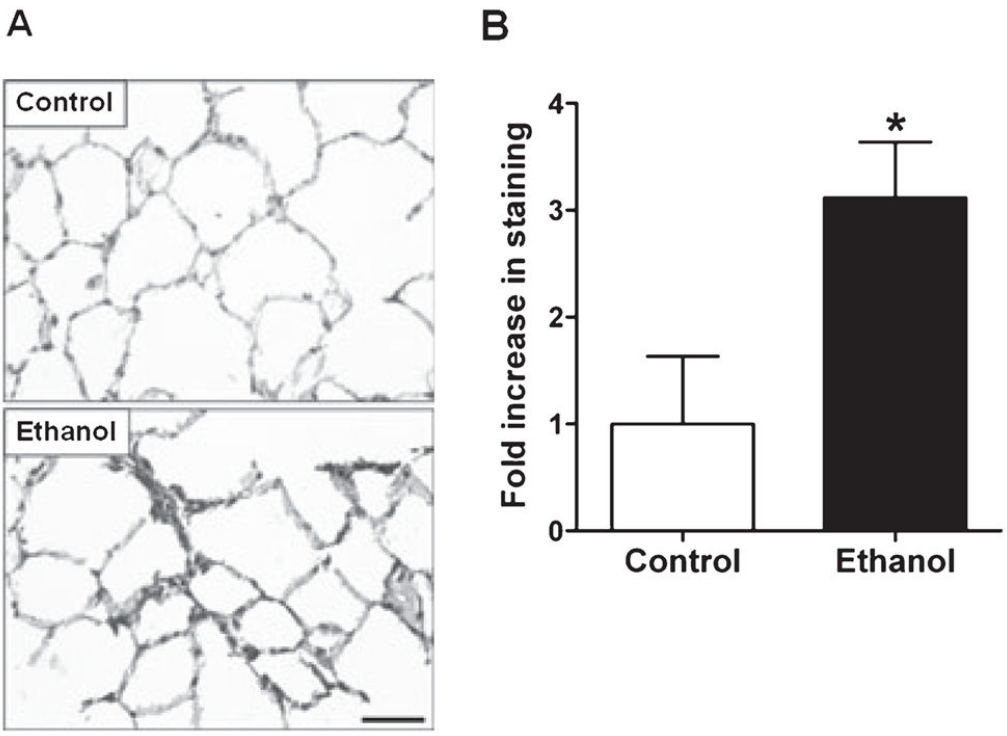

Fig. 3.

Chronic alcohol ingestion increases the protein expression of Na,K-ATPase throughout the alveolar epithelium. Lung tissue was immunostained for the α1 subunit of the Na,K-ATPase complex and the relative expression in the alveolar epithelium of alcohol-fed versus control-fed rats was determined by quantifying the intensity of immunostaining. Shown in panel **A** are representative lung images from a control-fed and an alcohol-fed rat. The summary data for the relative immunostaining intensity in each group are shown in panel **B**. Each value represents the mean ± SEM of three (alcohol-fed) or four (control-fed) rat lungs. *$p < 0.05$ compared with control.

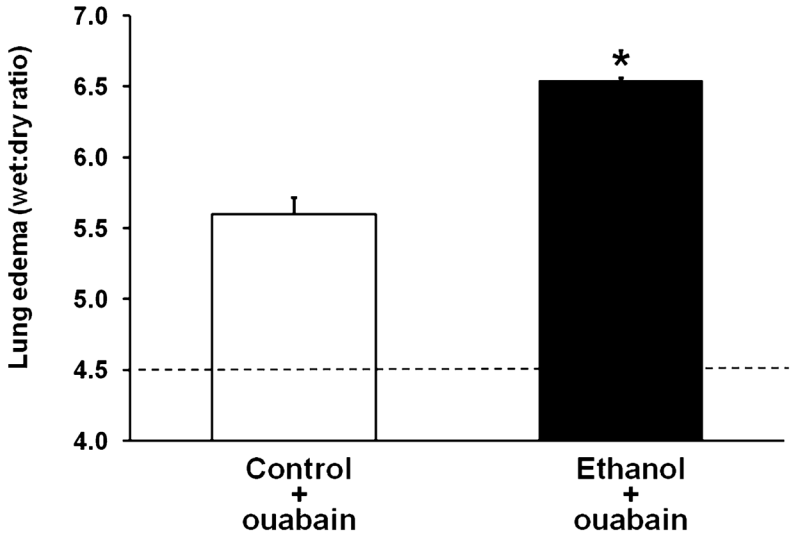

Fig. 4.

Lungs from alcohol-fed rats are more sensitive to Na,K-ATPase inhibition by ouabain during ex vivo perfusion than lungs from control-fed rats. Lungs were isolated and perfused in a buffer solution containing ouabain (10^{-4} M) for 2 hours. Then, the lung wet:dry ratios were determined. As noted by the dashed line, the average baseline (nonedematous) wet:dry ratios of control-fed and alcohol-fed rat lungs was 4.5. Therefore, wet:dry ratios in each experimental group are expressed relative to this baseline. *$p < 0.05$ compared with control.