β-Adrenergic Receptor Stimulation and Adenoviral Overexpression of Superoxide Dismutase Prevent the Hypoxia-mediated Decrease in Na,K-ATPase and Alveolar Fluid Reabsorption*

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Hypoxia has been shown to cause lung edema and impair lung edema clearance. In the present study, we exposed isolated rat lungs to pO\textsubscript{2} = 40 mm Hg for 60 min or rats to 8% O\textsubscript{2} for up to 24 h and then measured changes in alveolar fluid reabsorption (AFR) and Na,K-ATPase function. Low levels of oxygen severely impaired AFR in both ex vivo and in vivo models. The decrease in AFR was associated with a decrease in Na,K-ATPase activity and protein abundance in the basolateral membranes from peripheral lung tissue of hypoxic rats. β-Adrenergic agonists restored AFR in rats exposed to 8% O\textsubscript{2} from 0.02 ± 0.07 ml/h to 0.59 ± 0.03 ml/h, which was associated with parallel increases in Na,K-ATPase protein abundance in the basolateral membrane. Hypoxia is associated with increased production of reactive oxygen species. Therefore, we examined whether overexpression of SOD2, manganese superoxide dismutase, would prevent the hypoxia-mediated decrease in AFR. Spontaneously breathing rats were infected with a replication-deficient human type 5 adenovirus containing cDNA for SOD2. An otherwise identical virus that contained no cDNA was used as a control (Adnull). Hypoxic Adnull rats had decreased rates of AFR (0.12 ± 0.1 ml/h) as compared with hypoxic AdSOD2 and normoxic control rats (0.47 ± 0.04 ml/h and 0.49 ± 0.02 ml/h, respectively), with parallel changes in Na,K-ATPase.

Severe hypoxia can occur during ascent to high altitude (1) and in patients with acute respiratory distress syndrome and pulmonary edema. One of the primary defense mechanisms in the lung against alveolar fluid accumulation is the active transport of sodium out of the air spaces, which generates a transepithelial osmotic gradient that leads to alveolar fluid reabsorption (AFR). Sodium enters the apical membrane of alveolar epithelial cells through amiloride-sensitive Na\textsuperscript{+} channels (2, 3) and is then transported out across the basolateral membrane by the ouabain-inhibitable Na,K-ATPase (4–7). Hypoxia has been shown to impair AFR and may contribute to alveolar fluid accumulation (8, 9). However, the mechanisms by which hypoxia impairs AFR and alveolar epithelial sodium transport proteins has not been fully elucidated.

A mechanism by which hypoxia might impair AFR is by altering the function of either apical epithelial sodium channels and/or basolateral Na,K-ATPase proteins. Several in vitro studies using cultured alveolar epithelial cells have demonstrated that exposure to hypoxia results in the decrease in epithelial sodium channels and Na,K-ATPase protein abundance (10–12), which was reversed upon reoxygenation. Other investigators have reported various mechanisms associated with the decrease in alveolar fluid reabsorption in animals exposed to hypoxia in vivo (9, 11, 13).

In the current study, we provide evidence that exposure to hypoxia results in decreased Na,K-ATPase activity and protein abundance at the plasma membrane, which contributes to a decrease in alveolar fluid reabsorption in both in vivo and ex vivo models of hypoxia. These data suggest that (a) hypoxia decreases Na,K-ATPase activity by promoting the endocytosis of the Na,K-ATPase molecules, which is associated with a decrease in alveolar fluid reabsorption, (b) treatment with the β-adrenergic receptor agonist, isoproterenol, recruits functional Na,K-ATPase molecules to the plasma membrane following exposure to hypoxia and restores alveolar fluid reabsorption in rat lungs, and (c) overexpression of the reactive oxygen species scavenger, SOD2, prevents the hypoxia-mediated decrease in alveolar fluid reabsorption and Na,K-ATPase function.

EXPERIMENTAL PROCEDURES

Isolated Perfused Lungs—The isolated perfused lung preparation used in our laboratory has been described (4). Briefly, lungs were isolated from anesthetized rats (65 mg/kg Nembu- nal, intraperitoneal) following a 10-min ventilation with 100% O\textsubscript{2}. The pulmonary artery and left atrial appendage were cannulated and perfused with a solution of 3% bovine serum albumin in buffered physiological salt solution. Fluorescein isothiocyanate (FITC)-labeled albumin was added to the perfusate to monitor leakage of protein from the vascular space into the airways. The recirculating volume of the constant pressure per-
fusion system was 90 ml; arterial and venous pressures were set at 8 and 0 cm H₂O, respectively. The lungs were excised from the thoracic cavity and placed in a “pleural” bath (100 ml) filled with the same bovine serum albumin solution. The entire system was maintained at 37 °C in a water bath. The lungs were then instilled via the tracheal catheter with 5 ml of bovine serum albumin containing Evans blue dye-labeled albumin, [⁴²Na⁺] and [³H]mannitol. Samples were taken from the instilled, perfusate, and bath solutions following a 10-min equilibration period and 60 min later. Absorbance at 620 nm (for Evans blue dye-albumin), fluorescence (excitation 487 nm; emission 520 nm; for FITC-albumin), and scintillation counting (for [⁴²Na⁺] and [³H]mannitol) were measured in centrifuged samples from each compartment.

Calculations—The derivation of all equations involved in the mathematical model of edema clearance has previously been described in detail (4, 14). Concentration of Evans blue dye-albumin was used to estimate airspace volume. As virtually all Evans blue dye-albumin remains in the airspace, instillate volume (V) at a given time can be calculated from the increase in airspace protein concentration. The total unidirectional out-flux of Na⁺ from the alveolar space, a result of active transport and passive movement, was calculated from the rate of loss of [⁴²Na⁺] 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-albumin that appears in the alveolar instillate during the experimental protocol. For comparison, fluxes are reported as volume fluxes (volume/time) by using the appropriate solute concentrations.

Perfusing with Low pO₂ Levels—The perfusate and the instillate were bubbled with 95% oxygen, 5% CO₂ and the final pO₂ was measured using a NOVA Biomedical blood gas analyzer. pO₂ levels were checked every 15 min during the 60-min experiment.

Exposure to Hypoxia—Animals were placed in a Ruskinn INVIVO; 400 Hypoxia chamber with a 12:12-h light-dark cycle. Food and water were available ad libitum. Oxygen and carbon dioxide levels in the chamber were continuously monitored while maintaining the chamber temperature between 18 and 22 °C.

Adenovirus Infection Protocol—Rats were anesthetized with 40 mg/kg Nembutal intraperitoneally and orally intubated with a 14-gauge plastic catheter prior to adenoviral infection (15). Three experimental groups were studied: Sham-surfactant (n = 6), Adnull (n = 8), and AdSOD2 (n = 10). A mixture of adenovirus in 50% surfactant, 50% dialysis buffer vehicle was administered in four aliquots of 200 µl. Rats were rotated 90° between instillations given at 5-min intervals. Immediately before instillation, a forced exhalation was achieved by circumferential compression of the thorax. Compression was relinquished after endotracheal instillation of 200 µl of virus/vehicle followed by 800 µl of air. Rats were allowed to recover before exubation. Infected animals were maintained in separate isolator cages for 7 days prior to conducting experimental protocols.

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Immunohistochemistry—Longitudinal sections from each fixed lung were embedded in paraffin for immunohistochemical analysis. Five-micrometer sections were deparaffinized in xylene and rehydrated through serial ethanol solutions. Using previously described methods, specimens were then treated with 3% H₂O₂ to reduce endogenous peroxidase activity prior to blocking of background (nonspecific) immunoreactivity with nonimmune goat serum. Primary antibody (1:500 dilution, Na,K-ATPase α1 Ab) was added for 1 h at room temperature. Sections were washed with phosphate-buffered saline prior to the addition of goat anti-mouse secondary antibody. Immunodetection was accomplished via 3,3-diaminobenzidine staining for immunoperoxidase activity (Vector Elite ABC kit; Vector Laboratories, New Castle-upon-Tyne, UK). Sections were photographed (without counterstaining) using a digital photomicroscopy system (Spot II; Diagnostic Instruments, Sterling Heights, MI).

Whole Cell Lysate and Basolateral Cell Membrane Isolation and Western Blot Analysis—Approximately two millimeters of peripheral lung tissue was collected from each lobe and homogenized to obtain whole cell lysates and basolateral membranes as previously described (16, 17). Briefly, cell lysates were prepared by the addition of lysis buffer (20 mM Tris, pH 7.5, 150 mM NaCl, 1 mM EDTA, 1 mM EGTA, 1% Triton X-100, 2.5 mM sodium pyrophosphate, 1 mM Na₃VO₄, 1 µg/ml leupeptin, 1 mM phenylmethylsulfonyl fluoride) and centrifugation at 14,000 × g to eliminate the insoluble material. Basolateral membranes (BLM) were prepared using Percoll gradient centrifugation as described (17, 18). Briefly, peripheral lung tissue was homogenized in a buffer containing 300 mM mannitol in 12 mM Tris-HEPES, pH 7.6, and protease inhibitors as described above, then centrifuged twice to discard the nuclear and mitochondrial pellets. Supernatant was centrifuged at 48,000 × g for 30 min, and the BLM fraction was recovered after the membrane pellet was centrifuged in a 16% Percoll gradient at 48,000 × g for 30 min. Equal amount of proteins from cell lysates or BLMs were resolved by 10% SDS-PAGE and analyzed by immunoblotting with specific antibodies.

Na₉,K-ATPase Activity—Na₉,K-ATPase activity was determined by [³²P]ATP hydrolysis as described before (17, 23). Briefly, basolateral cell membrane proteins isolated from the peripheral lung were placed on ice, and aliquots (~10 µg of protein) were transferred to the Na₉,K-ATPase assay medium (final volume 100 µl) containing NaCl 50 mM, KCl 5 mM, MgCl₂ 10 mM, EGTA 1 mM, Tris-HCl 50 mM, Na₃ATP 7 mM, and [γ-³²P]ATP (specific activity 3000 Ci/mmol) in tracer amounts (3.3 nCi/µl). The samples were then incubated at 37 °C for 30 min, and the reaction was terminated by addition of 700 µl of trichloroacetic acid/charcoal (5/10% w/v) suspension and rapid cooling to 4 °C. After separating the charcoal phase (12,000 × g for 5 min) containing the unhydrolyzed nucleotide, the liberated [³²P] was counted in an aliquot (200 µl) from the supernatant. Na₉,K-ATPase activity was calculated as the difference between test samples (total ATPase activity) and samples assayed in the same medium but devoid of Na⁺ and K⁺ and in the presence of 4 mM ouabain (ouabain-insensitive ATPase activity). The results are expressed as mean nmol of Pᵢ/mg of protein.
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protein/h of triplicate measurements from three animals per group.

Data Analysis—When comparisons were made between two experimental groups, an unpaired Student’s t test was used. When multiple comparisons were made, a one way analysis of variance and Duncan’s means comparison test were used. Results were considered significant when \( p < 0.05 \).

RESULTS

Effect of Low \( pO_2 \) on Alveolar Fluid Reabsorption in an ex Vivo Model—We determined the effect of low levels of oxygen on AFR by perfusing, in different experiments, decreasing levels of oxygen through the pulmonary circulation of the isolated, perfused rat lung. We observed that when \( pO_2 \) levels were decreased from 100 mm Hg to either 85 or 60 mm Hg for 60 min there was a 60 and 40% reduction in AFR, respectively. When \( pO_2 \) levels were reduced to 40 mm Hg for 60 min there was an even greater reduction in AFR (Fig. 1A).

The passive movement of small solutes (\(^{3}H\)mannitol and \(^{22}Na^+\)) and FITC-albumin across the epithelial barrier of the rat lung did not significantly change when \( pO_2 \) levels were decreased in the pulmonary circulation of the isolated rat lungs as compared with control (data not shown), which validates the use of the model in assessing the role of low oxygen tension on AFR. The pulmonary circulation flow was measured periodically during the experiments and was similar (~12 ml/min) in all groups (data not shown).

The effect of low \( pO_2 \) on the Na,K-ATPase was assessed in basolateral membranes isolated from the peripheral lung tissue, as we have previously described (16). The Na,K-ATPase activity decreased from 64.1 ± 9.5 to 17.5 ± 3.4 nmol of ATP/h/mg of protein in the basolateral membrane of rat lungs perfused with a \( pO_2 \) of 40 mm Hg as compared with control (Fig. 1B). The decrease in activity paralleled a decrease in the Na,K-ATPase \( \alpha_1 \) protein abundance in the basolateral membrane isolated from rat lungs perfused with a \( pO_2 \) of 40 mm Hg (Fig. 1C). There was no decrease in the abundance of Na,K-ATPase protein in total cell lysates (Fig. 1C) suggesting that there was endocytosis of Na,K-ATPase but no degradation of the protein.

FIGURE 1. A, the effect of low levels of oxygen on AFR were determined by perfusing, in different experiments, decreasing levels of oxygen for 60 min through the pulmonary circulation of the isolated, perfused rat lung model. As \( pO_2 \) levels were decreased from 120 to 40 mm Hg there was a concomitant decrease in alveolar fluid reabsorption. The bars represent mean ± S.D., * \( p < 0.05 \) as compared with control; **, \( p < 0.001 \) as compared with control. B, Na,K-ATPase activity in BLM. BLMs were isolated from peripheral lung tissue of lungs perfused with \( pO_2 \) 40 mmHg for 60 min and compared with control lungs. Na,K-ATPase activity was measured by \(^{32}P\)ATP hydrolysis in BLM incubated in presence or absence of ouabain. Each bar represents the mean ± S.D. of four determinations performed independently (separate BLM isolations) and in triplicate. +, \( p < 0.05 \). C, Na,K-ATPase \( \alpha_1 \) protein expression. Representative Western blot analyses for Na,K-ATPase \( \alpha_1 \) subunit protein abundance in BLMs (10 \( \mu \)g of protein/lane) and total lysates (lobes, 40 \( \mu \)g of protein/lane) isolated from peripheral lobes of lungs perfused with \( pO_2 \) 40 mmHg for 60 min and compared with control lungs.
In hypoxia there is a decrease in Na,K-ATPase protein abundance. This decrease in protein abundance may be a reflection of increased degradation, presumably via the ubiquitin-proteasome pathway (28) or decreased transcription/translation of the protein.

**Effect of β-Adrenergic Agonists on Alveolar Fluid Reabsorption in Hypoxic Rat Lungs**—We have previously reported that stimulation of the β-adrenergic receptor with isoproterenol (ISO) increases AFR in rat lungs (15, 20–22) via the translocation/recruitment of pre-existing Na,K-ATPase molecules from internal/endosomal compartments to the basolateral membrane (23). We sought to determine whether these “internalized” Na,K-ATPase molecules were available for recruitment to the basolateral membranes of rats exposed to 8% O2 for 24 h and then treated with ISO (10 μM, 60 min perfused through the pulmonary circulation) and whether this would restore AFR in hypoxic rat lungs. In normoxic control lungs, ISO (10 μM) increased the rate of AFR by 100% (from 0.51 ± 0.05 to 1.05 ± 0.07 ml/h) and restored AFR to control levels in the lungs of hypoxic rats (from 0.02 ± 0.07 to 0.59 ± 0.03 ml/h) (Fig. 4A). The increase in AFR was associated with an increased Na,K-ATPase protein abundance in the basolateral membrane of ISO-treated hypoxic rat lungs as determined by Western blot analysis (Fig. 4B).

**Mitochondrial ROS Mediate Hypoxia-induced Decrease in Alveolar Fluid Reabsorption**—During hypoxia reactive oxygen species are initially produced as superoxide, which subsequently are converted to H2O2 in the mitochondria by superoxide dismutase (SOD2). To determine whether overexpression of the ROS scavenger SOD2 would prevent the hypoxia-induced decrease in AFR, spontaneously breathing rats were infected with an adenovirus expressing SOD2 (AdSOD2, 2–4 × 10^9 plaque-forming units) and compared with rats infected with a null virus (Adnull, 2–4 × 10^9 plaque-forming units) or sham-infected rats. Seven days postinfection, rats were exposed to normoxia or hypoxia (21 or 8% O2, respectively) for 24 h. Overexpression of SOD2 prevented the hypoxia-mediated decrease in AFR. Adnull- or sham-infected hypoxic rats had a significant decrease in AFR as compared with normoxic control rats (Fig. 5A). Lung homogenates were prepared, and proteins were separated by SDS-PAGE and immunoblotted with anti-SOD2. There was an abundant expression of SOD2 in AdSOD2-infected rats compared with Adnull-infected rats (Fig. 5B). Additionally, Na,K-ATPase activity and protein abundance in basolateral membrane isolated from the peripheral lung tissue of AdSOD2-infected rats exposed to hypoxia was not different from normoxic control rats (Fig. 5, C and D).
DISCUSSION

This study demonstrates that hypoxia decreases AFR, in both an in vivo and ex vivo model of exposure to low oxygen levels, which is associated with a decrease in Na,K-ATPase activity. These functional changes are associated with a significant decrease in Na,K-ATPase protein expression in total cell lysates, as well as in the basolateral membrane prepared from peripheral lung tissue. The hypoxia-mediated decrease in AFR and protein expression could be abrogated by treatment with either isoproterenol or the overexpression of SOD2.

The present study also provides evidence that the effects of hypoxia on alveolar ion transport proteins (10–12) is not a tissue culture phenomenon but also occurs in vivo. The regulation of alveolar transport proteins is vital in the maintenance of alveolar fluid balance in patients (24). A reduction in the number of copies of these transporters could reduce the capacity of alveolar fluid reabsorption, which would have significant deleterious effects (25).

Several studies have reported that exposure to hypoxia can reduce the ability of the lung to clear liquid from the air spaces, although the mechanisms by which the impairment in alveolar fluid reabsorption has not been fully elucidated (9, 13, 26, 27).
Vivona et al. (9) exposed rats to hypoxia and observed a significant decrease in alveolar fluid reabsorption with no change in expression of Na,K-ATPase protein prepared from crude cell lysates. In another study, animals exposed to hypoxia had a decrease in nasal potential difference that was associated with a decrease in Na,K-ATPase activity, but no change in Na,K-ATPase protein expression (13). In vitro studies of alveolar epithelial cells exposed to hypoxia clearly demonstrate a decrease in Na,K-ATPase activity that is associated with the endocytosis of the Na,K-ATPase from the basolateral membrane (12). Dada et al. (12) reported that during exposure to hypoxia, mitochondrial ROS activate PKC-ζ, which phosphorylates Na,K-ATPase and triggers it for endocytosis. This results in fewer Na,K-ATPase pumps at the plasma membrane and decreased Na,K-ATPase activity.

We report that in both the ex vivo and in vivo models of hypoxia, low Po2 levels caused a decrease in the Na,K-ATPase activity (Figs. 1A and 3A, respectively), which was associated with a decrease in protein abundance at the basolateral membrane in both models (Figs. 1C and 3B). However, there was no change in the Na,K-ATPase protein abundance in total lysates prepared from the lungs exposed to short term hypoxia (pO2 = 40 mm Hg, 60 min) (Fig. 1C), whereas there was a significant decrease in Na,K-ATPase protein abundance in total lysates prepared from lungs exposed to long term hypoxia (8% oxygen, 24 h) (Fig. 3C). These results suggest that Na,K-ATPase activity is regulated during hypoxia, in both short and long term hypoxia models, by altering the number of functional Na,K-ATPase molecules in the basolateral membrane through the endocytosis of Na,K-ATPase. Prolonged exposure to low levels of oxygen (see Fig. 3C) results in a decrease in the Na,K-ATPase protein abundance, which may reflect the degradation of Na,K-ATPase, presumably via the ubiquitin-proteasome pathway (28). Alternatively, hypoxia could affect the transcription and/or translation of Na,K-ATPase proteins thus altering the levels of the protein abundance in total cell lysates. However, steady-state levels of Na,K-ATPase α1 mRNA were either unchanged following exposure to hypoxia (9, 13) or significantly decreased during exposure to hypoxia (11, 29).

The decreased abundance of Na,K-ATPase molecules in the basolateral membrane was associated with decreased AFR in both the in vivo and ex vivo models of hypoxia. Conversely, when AFR was restored to control levels within 24 h of recovery in room air (Fig. 2A), this was associated with parallel increases in Na,K-ATPase activity and protein abundance in the basolateral membrane and in total lysates (Fig. 3). We speculate that AFR could be restored by increasing the abundance of the Na,K-ATPase molecules at the basolateral membrane by 1) increased rates of transcription and/or translation of Na,K-ATPase; 2) recruitment/translocation of internally stored functional Na,K-ATPase molecules. It has been previously reported that steady-state levels of Na,K-ATPase α1 mRNA were either unchanged following exposure to hypoxia (9, 13) or significantly decreased during exposure to hypoxia and then increased during the reoxygenation phase (11, 29). Our data suggests that extended exposure to hypoxia results in a decrease in Na,K-ATPase protein abundance, which returns to normal levels upon reoxygenation (Fig. 3C). The disparity in these reports will require additional investigations to determine the role of transcription and/or translational regulation of the Na,K-ATPase during exposure to hypoxia.

We have previously reported that catecholamines increase AFR in normal and injured rat lungs (4, 8, 20–22, 30) and that catecholamine-mediated increases in AFR are dependent upon expression levels of Na,K-ATPase proteins in the basolateral membrane. In the present study, we examined whether the β-adrenergic agonist ISO when perfused through the pulmonary circulation could improve active Na+ transport and AFR in hypoxic rats. The data show that ISO restored the ability of the lung to clear edema in rats exposed to hypoxia for 24 h (Fig. 4A). We reason that the reduced capacity of the lung to clear edema in hypoxic animals was probably not so severe as to preclude the ability of the lung to respond to β-adrenergic stimulation. Further support for this notion is the data in Fig. 4B showing that ISO increased the Na,K-ATPase protein abundance in the basolateral membrane in hypoxic lungs. There are several mechanisms by which ISO may increase the number of Na,K-ATPase molecules in the BLM, including changes in the rate of Na,K-ATPase protein synthesis. In fact, we have previously demonstrated that activation of the β-adrenergic receptor was associated with increased Na,K-ATPase abundance and enzymatic activity (19, 22, 23). However, translational regulation of the Na,K-ATPase via β-adrenergic receptor agonist generally occurs over a period >24 h, much longer than the 60-min time course of our experimental conditions. Therefore, we reasoned that Na,K pumps are stored in intracellular compartments and can be recruited for insertion in the basolateral membrane. As shown in Fig. 4, ISO-treated lungs had increased Na,K-ATPase protein abundance in the BLM of both normoxic and hypoxic rat lungs. These results suggest that ISO can be used to increase the Na,K-ATPase and restore AFR in hypoxic rat lungs (Fig. 4).

Hypoxia has been shown to increase the generation of mitochondrial ROS at the semiquinone site, where an electron can be transferred to O2 to produce superoxide (O2−). Superoxide generated within the mitochondrial matrix is converted to H2O2 by manganese SOD (SOD2), and H2O2 can then be degraded by mitochondrial glutathione peroxidase. In the present study, we infected rats with adenoviral vectors expressing SOD2 (AdSOD2). Animals treated with SOD2 adenoviral vectors had increased expression levels of SOD2 as demonstrated in Fig. 5B. We hypothesized that overexpression of SOD2 would detoxify ROS generated within the mitochondria and prevent the down-regulation of the Na,K-ATPase expression and AFR. Indeed, in animals that were infected with AdSOD2 and then subsequently exposed to hypoxia there was no change in AFR as compared with normoxic control animals. Further, the Na,K-ATPase activity and expression of the Na,K-ATPase α1 protein in the basolateral membrane of AdSOD2-infected hypoxic animals was also unchanged.

In summary, we provide evidence that β-adrenergic receptor agonists improve alveolar fluid reabsorption in hypoxia-exposed lungs. Increased rates of AFR by β-adrenergic stimulation were probably mediated by the recruitment and translocation of Na,K-ATPase from intracellular pools to the cell plasma membrane in alveolar epithelium. The overexpression of the ROS scav-
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engr SOD2 had a protective effect on animals that were subsequently exposed to low levels of oxygen, probably by preventing ROS-mediated activation of signal transduction cascades. The outcomes of patients with acute lung injury and pulmonary edema are related to the ability of the lung to clear edema. Conceivably, β-adrenergic agonists and/or ROS scavengers could be utilized as a therapeutic strategy to improve functional recovery of patients prone to high altitude pulmonary edema and patients with acute hypoxemic respiratory failure and pulmonary edema.

REFERENCES

1. Sartori, C., Allemann, Y., Duplaine, H., Lepori, M., Egli, M., Lipp, E., Hutter, D., Turini, P., Hugli, O., Cook, S., Nicod, P., and Scherrer, U. (2002) N. Engl. J. Med. 346, 1631–1636
2. Eaton, D. C., Chen, J., Ramosevac, S., Matalon, S., and Jain, L. (2004) Proc. Am. Thorac. Soc. 1, 10–16
3. Matalon, S., Lazrak, A., Jain, L., and Eaton, D. C. (2002) J. Appl. Physiol. 93, 1852–1859
4. Azzam, Z. S., Adir, Y., Crespo, A., Comellas, A., Lecuona, E., Dada, L. A., Krivoy, N., Rutschman, D. H., Sznajder, J. I., and Ridge, K. M. (2004) Am. J. Respir. Crit. Care Med. 170, 730–736
5. Ware, L. B., and Matthay, M. A. (2000) N. Engl. J. Med. 342, 1334–1349
6. Dumasius, V., Sznajder, J. I., Azzam, Z. S., Boja, J., Matalon, S., Maron, M. B., and Factor, P. (2001) Circ. Res. 89, 907–914
7. Saldias, F. J., Azzam, Z. S., Ridge, K. M., Yeldandi, A., Rutschman, D. H., Schraufnagel, D., and Sznajder, J. I. (2001) Am. J. Physiol. 281, L591–L597
8. Suzuki, S., Noda, M., Sugita, M., Ono, S., Koike, K., and Fujimura, S. (1999) J. Appl. Physiol. 87, 962–968
9. Vivona, M. L., Matthay, M., Chabaud, M. B., Friedlander, G., and Clerici, C. (2001) Am. J. Resp. Cell Mol. Biol. 25, 554–561
10. Planes, C., Friedlander, G., Loiseau, A., Amiel, C., and Clerici, C. (1996) Am. J. Physiol. 271, L70–L78
11. Wodopja, R., Ko, H. S., Billian, J., Wiesner, R., Bartsch, P., and Mairbaur, H. (2000) Am. J. Physiol. 279, L1110–L1119
12. Dada, L. A., Chandel, N. S., Ridge, K. M., Pedemonte, C., Bertorello, A. M., and Sznajder, J. I. (2003) J. Clin. Invest. 111, 1057–1064
13. Carpenter, T. C., Schomberg, S., Nichols, C., Stenmark, K. R., and Weil, J. V. (2003) Am. J. Physiol. 284, L77–L83
14. Rutschman, D. H., Olivera, W., and Sznajder, J. I. (1993) J. Appl. Physiol. 75, 1574–1580
15. Ridge, K. M., Olivera, W. G., Saldias, F., Azzam, Z., Horowitz, S., Rutschman, D. H., Dumasius, V., Factor, P., and Sznajder, J. I. (2003) Circ. Res. 92, 453–460
16. Azzam, Z. S., Dumasius, V., Saldias, F. J., Adir, Y., Sznajder, J. I., and Factor, P. (2002) Circulation 105, 497–501
17. Ridge, K., Dada, L., Lecuona, E., Bertorello, A., Katz, A., Mochly-Rosen, D., and Sznajder, J. I. (2002) Mol. Biol. Cell 13, 1381–1389
18. Barnard, M. L., Ridge, K. M., Saldias, F., Friedman, E., Guarrera, C., Lecuona, E., Bertorello, A. M., Katz, A. I., and Sznajder, J. I. (1999) Am. J. Resp. Crit. Care Med. 160, 982–986
19. Pesce, L., Comellas, A., and Sznajder, J. I. (2003) Am. J. Physiol. 285, L802–L807
20. Saldias, F., Lecuona, E., Friedman, E., Barnard, M. L., Ridge, K. M., and Sznajder, J. I. (1998) Am. J. Physiol. 274, L694–L701
21. Saldias, F. J., Comellas, A., Ridge, K. M., Lecuona, E., and Sznajder, J. I. (1999) J. Appl. Physiol. 87, 30–35
22. Saldias, F. J., Lecuona, E., Comellas, A. P., Ridge, K. M., Rutschman, D. H., and Sznajder, J. I. (2000) Am. J. Resp. Crit. Care Med. 162, 282–287
23. Bertorello, A. M., Ridge, K. M., Chibalin, A. V., Katz, A. I., and Sznajder, J. I. (1999) Am. J. Physiol. 276, L20–L27
24. Ware, L. B., and Matthay, M. (2001) Am. J. Resp. Crit. Care Med. 163, 1376–1383
25. Sznajder, J. J. (2001) Am. J. Resp. Cell Mol. Biol. 163, 1293–1294
26. Clerici, C., and Matthay, M. A. (2000) Am. J. Physiol. 288, 1890–1896
27. Sartori, C., Duplaine, H., Lepori, M., Egli, M., Maggiorini, M., Nicod, P., and Scherrer, U. (2004) Eur. Respir. J. 23, 916–920
28. Comellas, A. P., Dada, L. A., Lecuona, E., Pesce, L. M., Chandel, N. S., Quesada, N., Budinger, G. R. S., Strous, G. J., Ciechanover, A., and Sznajder, J. I. (April 13, 2006) Circ. Res. published ahead of print PMID: 16614303, 2006
29. Planes, C., Escoubet, B., Blot-Chabaud, M., Friedlander, G., Farman, N., and Clerici, C. (1997) Am. J. Respir. Cell Mol. Biol. 17, 508–518
30. Azzam, Z. S., Saldias, F., Comellas, A., Ridge, K., Rutschman, D., and Sznajder, J. I. (2001) J. Appl. Physiol. 90, 1088–1094