REGULATION OF THE SODIUM PUMP IN HYPEROXIC LUNG INJURY

David H. Ingbar, Joseph M. Lasnier, O. Douglas Wangenstein, and Christine H. Wendt

Departments of Medicine, Physiology and Pediatrics
University of Minnesota School of Medicine
Minneapolis, MN 55455

INTRODUCTION

A major component of the early lesion in the adult respiratory distress syndrome (ARDS) is alveolar edema. Considerable data suggests that decreasing alveolar flooding improves the outcome from acute lung injury (ALI). Studies in humans with ARDS demonstrate that prognosis correlates with the capacity to resorb fluid (Matthay 1990). Retrospective studies indicate that patients with fluid balance out>in, lesser weight gain, lower pulmonary capillary wedge pressures or diuretic treatment have greater survival rates. It is not surprising that decreasing the degree of early alveolar flood could decrease the subsequent need for high pressure ventilation or high levels of oxygen - thus avoiding amplification of the injury.

Active resorption of alveolar sodium and fluid is critical for edema resolution and avoiding further amplification of lung injury (O'Brodovich 1995, Matthay 1996). It also is critical for the normal transition to postnatal breathing (O'Brodovich 1991). In the normal human and rodent lung many substances augment alveolar sodium and fluid resorption. Oxidant stress is a major mechanism of damage during ALI and this stress persists due to the neutrophilic inflammation and use of high levels of inspired oxygen in many patients. Oxidative injury can either inhibit or augment active sodium transport, depending on a number of variables (Ingbar 1997b, 1998). Recently we demonstrated that even the injured alveolar epithelium is capable of active fluid resorption and this can be stimulated by β -adrenergic agonists, such as terbutaline (Lasnier). This chapter examines the effects of oxidants on one component of the sodium resorption system, the sodium pump - Na,K-ATPase; the sodium channel is the other critical component of this system.

Clearance of sodium and water from the alveolar space is primarily mediated by a combination of an apical amiloride-sensitive sodium channel (Matalon 1996) and the basolateral sodium pump, Na,K-ATPase (Matthay 1996). The importance of this pathway is supported by inhibitor and ion substitution studies in living animals, isolated perfused lungs and cultured type II alveolar epithelial cell monolayers (Matalon 1991, Saumon). We have examined the mechanisms by which oxidant stress affects alveolar epithelial solute and fluid resorption, and particularly on the sodium pump, the major

basolateral pathway for sodium transport.

Na,K-ATPase STRUCTURE

Na,K-ATPase, is a ubiquitous heterodimeric transmembrane protein composed of $\alpha 1$ and $\beta 1$ subunits in a 1:1 ratio (Horisberger). The $\alpha 1$ subunit is a polypeptide of approximately 96-112 kD with 7 membrane spanning domains that catalyzes the movement of Na^+ and K^+ , is phosphorylated by ATP and binds the specific inhibitor ouabain (Skou). It has 3 mRNA isoforms whose expression varies in different tissues and in development (Jewell). Alpha-1 is the predominant form in epithelial tissues (Sweadner) including ATII cells (Nici, Schneeberger), but cultured type I-like cells may express $\alpha 2$ (Ridge). The β subunit has 3 isoforms and is a glycosylated polypeptide with a peptide core of ~35kD. It targets the assembled molecule to the plasma membrane (Geering) through the C-terminal extracellular domain of the β subunit. The β subunit also is necessary for K-dependent reactions (McDonough 1990a). The $\beta 1$ isoform is ubiquitous, whereas $\beta 2$ is in brain only.

Na,K-ATPase REGULATION

The Na,K-ATPase is regulated at multiple levels and by short and long term mechanisms (Bertorello 1995 a,b), as befits an enzyme that consumes up to 30% of cellular ATP. Regulation of the sodium pump can be achieved rapidly by altering the activity of the enzyme or by moving it to and from the plasma membrane. Pump activity is rapidly increased by lower K^+ or higher Na^+ intracellular ion concentrations, and by hormones such as glucocorticoids (GCs), mineralocorticoids, β -adrenergic agonists and thyroid hormone (McDonough 1990b). Beta agonists increase sodium resorption and Na,K-ATPase activity in alveolar type II (ATII) cells (Suzuki). In some cell types, Na,K-ATPase activity can be inhibited by dopamine, endothelin or PGE2 and stimulated by vasopressin, or growth factors (Berterello). Hypoxia decreased ATII cell sodium pump activity, presumably through impaired calcium homeostasis (Planes).

Post-transcriptional regulation is important at multiple levels (Gick). Unequal ratios of α and β mRNA and protein concentrations are present in many tissues (Lingrel), even though the functional sodium pump enzyme has 1:1 stoichiometry. The mRNA degradation rates can change with cell differentiation and this may contribute to changing ratios of the subunits (Chambers). Translation is regulated and this probably accounts for some of the differences in the ratios of subunit mRNAs and proteins. The mRNA for $\alpha 1$ is translated less efficiently than $\beta 1$ due to the $\alpha 1$ mRNA's 3' untranslated region (UTR) which is extremely G-C rich (Devarajan). Once the subunits are translated, they can not leave the endoplasmic reticulum without assembly into α - β heterodimers (Geering), glycosylation and disulfide bond formation (Beggah). After assembly, they undergo an ill-defined maturation and then are transported into the cytoplasmic membrane over 1-4 hours to generate active pump that can bind ouabain (Caplan, Mircheff). There is an inactive, endogenous pool of Na,K-ATPase that recycles from plasma membrane sodium pump. Movement of pump molecules between this intracellular pool and the plasma membrane can be stimulated, as by insulin in muscle, but the regulatory factors are not well understood. Mechanical ventilation or stretch may recruit Na,K-ATPase in ATII cells from this pool. The α subunit can be phosphorylated at several amino acids by either protein kinases C or A (Feschenko, Beguin, but the functional consequences vary depending on the cell type, the particular kinase and the stimulant (Berterello). Normally the pump molecules in the plasma membrane do not operate at peak capacity, providing an important mechanism for short term regulation. Hormones such as glucocorticoids (GCs), thyroid hormone, aldosterone and catecholamines stimulate both short term and long term (transcriptional) increases in pump function (Ewart). Degradation rates of the two subunits also can be differentially affected, changing the balance of alpha and beta subunits (Lescale-Matys). The wide repertoire of potential regulatory

mechanisms makes studying the response of the Na,K-ATPase to injury much more complex.

TRANSCRIPTIONAL REGULATION OF Na,K-ATPase

Transcriptional regulation is common (reviewed in Lingrel), and likely plays a role during lung development (Ingbar 1996). Pump transcription in cultured cells usually is upregulated by increased intracellular Na or lower K levels (McDonough 1990b) and hormones such as GCs, aldosterone and T3 (Horisberger). The Na,K-ATPase isoforms belong to a multigene family and they are expressed in a tissue-specific and developmentally regulated manner (reviewed in Lingrel). Since the subunit genes are on different chromosomes, transcription may be independently regulated. Both subunits have >70% homology between isoforms of each subunit and there is significant interspecies homology of the Na,K-ATPase coding and promoter sequences. For example, rat and sheep have 97% homology of the $\alpha 1$ isoform.

For the rat $\alpha 1$ subunit, the major transcription initiation site is 262 base pairs upstream from the translation initiation site and is preceded by a TATA box at position -32 (Kawakami 1993, 1996). The factors regulating $\alpha 1$ gene transcription are partially defined. Included in the 5' flanking region are 2 Sp1 TF binding sites, two putative GC response element (GRE) half consensus sequences (Yagawa 1990) and a consensus cAMP response element (CRE). The human gene has a TATA box and 5 Sp1-like elements (Shull 1990). Two positive regulatory regions in the rat $\alpha 1$ subunit promoter are (1) at -155 and -49 bp and (2) an "Atp1 $\alpha 1$ " regulatory element or "ARE" at -94 to -69 bp that binds both common (ARE C1 and C2) and cell type-specific transcription factors (ARE C3) (Yagawa 1992). This sequence is conserved across rat, horse and human genes, but is not present in other Na,K-ATPase subunit promoters. At least 7 ARE-binding factors have been identified by gel mobility shift assays and southwestern cloning (Watanabe). One stimulatory transcription factor (TF), AREC3, is a homologue of the *Drosophila sine oculis* (Six) gene product that is required for visual system development, but is distinct from other homeodomain proteins. It is present at low levels in adult mouse lung, but otherwise has not been assessed in lung tissue. AREB6 is a zinc finger protein that can regulate Atp1 $\alpha 1$ positively or negatively, depending on cell type and conformational state of the protein. It is a 5' extended version of Nil-2, a negative regulator of the interleukin-2 gene. Across multiple cell lines, activating transcription factor-1 (ATF-1)/ CRE binding protein (CREB) heterodimers bound to the -61/-72 region of the $\alpha 1$ promoter and were required for transcription (Kobayashi). Although these TFs are ubiquitous and probably are required for basal transcription of many genes, they can be regulated by cAMP, Ca, and transforming growth factor- α (TGF- α). Recent *in vivo* footprinting found that rat tissue-specific transcriptional control involved cooperation between a canonical GC box at -57/-49 that binds an Sp1-like TF and the ATF region (-72/-65) (Nomoto). There also were DNase I hypersensitive sites at -83/-80 in liver and kidney, but not brain. HeLa cells treated with aldosterone after mineralocorticoid receptor transfection had less cAMP-inducible $\alpha 1$ gene transcription of the -103/-57 promoter region (Ahmad), but little is known of mineralocorticoid regulation. The human $\alpha 1$ gene has three potential thyroid hormone response elements, but only one is functional (Feng). In summary a number of potential TFs have been identified, but their presence and function in the lung are virtually undefined.

Much less is known about transcriptional regulation of the $\beta 1$ subunit gene. The rat gene's promoter contains a potential TATA box at position -31, four GC rich boxes, a CRE-like sequence and two sites with putative half consensus thyroid response element sequences TRE-1 and TRE-2 (Liu). The human gene consists of six exons and has two major and three minor transcription initiation sites.

LUNG ION TRANSPORT AND Na,K-ATPase

Although present to some extent in all cells, the sodium pump is found in high density on the

basolateral membranes of epithelial cells specialized for Na transport. Net vectoral ion transport across the alveolus resorbs fluid and maintains a dry alveolus. The joint importance of Na,K-ATPase and the sodium channel in salt and water resorption across the alveolar epithelium is supported by studies of intact animals, intact lungs and cultured cells (Saumon, Matalon 1991, Matthay 1996).

The ion transport properties of adult type II cells recently were reviewed (Matalon 1991). Sodium may enter the apical membrane by at least 3 mechanisms: Na channels, Na-glucose cotransport or Na-H⁺ exchange, but Na channels are quantitatively predominant, at least in adult cells. There likely are several different types of Na channels in alveolar epithelial cells (AECs) (Matalon 1996), since the amiloride affinity and conductance properties differ between freshly isolated and cultured ATII cells. In alveoli, this ubiquitous enzyme is detected primarily on the basolateral surface of type II cells (Nici, Schneeberger), but has not been detected on type I AECs in vivo. One group detected increases in $\alpha 2$ isoform and decreases in $\alpha 1$ with time that ATII cells are in culture, suggesting that ATI cells may have $\alpha 2$ (Ridge), but this has not been established in vivo. The beta subunit has not yet been localized in lung. The density of sodium pump on type II cells supports their importance in maintaining a dry alveolus, although type I cells also may contribute to fluid clearance. The Na,K-ATPase is the primary sodium exit pathway via the basolateral membrane. Both sodium pump and channel are needed for Na transport, but it is not clear which is limiting under normal or pathologic circumstances - especially since their activity usually changes in parallel. In addition to clearing alveoli of fluid, the low intracellular sodium concentration maintained by Na,K-ATPase allows sodium-coupled co-transport of amino acids, glucose and other vital substrates into ATII cells.

REGULATION OF LUNG SODIUM TRANSPORT AND RESPONSE TO INJURY

Many factors can stimulate sodium and fluid resorption in the normal lung (reviewed in Matthay 1996), including catecholamines, dobutamine, dopamine, keratinocyte growth factor (KGF), aldosterone, hepatocyte growth factor (HGF), transforming growth factor (TGF α), cAMP, endotoxin, *Pseudomonas* exotoxin A, and tumor necrosis factor- α (TNF- α). It is not clear if these factors act directly or through indirect mechanisms and what transporters they influence. Whenever tested, inhibitors of sodium channel or Na,K-ATPase significantly reduced alveolar fluid resorption. In cultured ATII cells, catecholamines and epidermal growth factor (EGF) (Borok) augmented transepithelial Na transport and terbutaline stimulated Na,K-ATPase activity independent of changes in intracellular sodium concentration (Suzuki). Important recent studies demonstrated that β -adrenergic agonists increased alveolar fluid clearance several fold in ex vivo human and rat lungs (Sakuma).

The impact of lung injury on alveolar fluid clearance and sodium transport has been examined in several injury models (Matthay 1996). Sepsis, pneumonia and endotoxin augmented clearance, in part through increased TNF α . Endotoxin-stimulated macrophages impaired lung epithelial transport (Compeau) through a nitric oxide (NO)-dependent mechanism. Intravascular and intratracheal dibutyl cAMP attenuated edema formation in a phosgene-induced lung injury through activation of prostaglandins. In permeability pulmonary edema induced by naphthyl-thiourea, there is increased sodium pump activity and alveolar solute resorption during recovery (Zuege).

Na,K-ATPase AND REACTIVE OXIDANT SPECIES

The effects of oxidant stress on Na,K-ATPase and alveolar fluid resorption have varied in different models studied. This also has been demonstrated in many different cell types from other organs (Ingbar 1997). Severe oxidant stress may inhibit general protein synthesis, inhibit sodium pump function or increase protein degradation. Recently we reviewed the multiple reported effects of oxidants on Na,K-ATPase function in different systems (Ingbar 1997). The studies of oxidant effects by other groups will first be reviewed and then the results of our studies on the lung Na,K-ATPase

will be summarized.

Acute severe hyperoxia, an injury with little type II cell proliferation, has variable effects on fluid resorption depending on severity of injury. Moderate injury has normal or increased resorption (Zheng, Garat, Carter 1997b), but severe injury can decrease clearance (Olivera 1995). In spite of increased permeability, the lung can still resorb fluid actively (Lasnier, Garat). Short periods of hyperbaric hyperoxia increase rat lung Na,K-ATPase mRNA levels (Harris). KGF is a growth factor for ATII cells in vivo and protects lungs from HCl, hyperoxic, bleomycin and radiation-induced injuries (Panos). The mechanism of KGF protection is not clear - it may be due to type II cell proliferation, increased surfactant decreased apoptosis or, in part, increased fluid clearance.

Less severe or more subacute injury can stimulate type II cell proliferation and may increase resorption through this mechanism. For example, chronic hyperoxia causes ATII proliferation with increased Na channels (Yue 1995) and Na,K-ATPase (Olivera 1994), but fluid clearance increased only in some rats (Yue 1997).

Multiple methods of sodium pump inhibition by oxidants are possible (Boldyrev), including direct protein oxidation (such as sulphydryl oxidation), peroxidation of surrounding membrane lipid, depletion of intracellular energy stores, uncoupling of energy utilization and ion transport (Elmosehi) or changes in ionic gradients. ROS can aggregate or fragment many proteins and may provide denatured substrates for protease degradation (Davies, Zolotarjan). The sodium pump's sensitivity to ROS may be due to oxidation of critical cysteine residues and disulfide bonds that maintain the enzyme conformation (Gevondyan). While the enzyme functions after all the $\alpha 1$ subunit cysteines have been mutated (Lane), the $\beta 1$ disulfides are critical for α subunit catalytic activity (Ueno). Other amino acids, such as tyrosines, may undergo modification to nitrotyrosine or chlorotyrosine (Heinecke) that could alter function, but has not yet been demonstrated for Na,K-ATPase. The effects of hyperoxia and ROS on sodium pump activity likely vary with different reactive oxygen species, the severity of injury, and cell type. Reactive oxygen species inhibit Na,K-ATPase isolated from canine kidneys (Huang) and myocytes (Haddock) and activated neutrophils inhibit Na,K-ATPase *in vitro* (Julin), likely through oxidant mechanisms. Nitric oxide inhibits sodium pump activity in mouse proximal tubule epithelial cells. In the brain, lipid peroxidation is the likely mechanism of decreased activity due to modification of sodium pump binding sites for ATP, K, and Na (Mishra). In ATII cells, H₂O₂ or peroxynitrite can inhibit the pump with differential effects from the apical and basal sides (Clerici, Hu, Kim).

In contrast, in at least some circumstances oxidants can stimulate Na,K-ATPase. Reactive oxygen species increase the activity of the sodium pump in intact endothelial cells (Elliott, Meharg). While protein kinases are activated in this model, they do not seem to be causal (Charles). Nitrogen dioxide augments airway epithelial sodium pump (Robison). The apparent contradiction between these results and the inhibition noted above may be partially a function of when the effect is examined. In one study of ATII cells, high levels of hydrogen peroxide initially inhibited the pump, followed shortly by rebound upregulation (Gonzalez-Flecha) - again consistent with homeostatic defense of this vital cell function.

We analyzed the effects of hyperoxia on Na,K-ATPase mRNA, protein and activity in three lung injury models and have ion transport data for two of the three models. The basis for our experimental design was that this comprehensive approach would provide information about the important steps affected in the response to hyperoxia and control of Na,K-ATPase function in the lung. The models used were (1) *in vivo* hyperoxia of rats with analysis of peripheral lung biochemical parameters and isolated perfused lung or *in situ* sodium transport; (2) type II cells isolated from rats after 60 hours of *in vivo* hyperoxia; and (3) rat alveolar epithelial cells exposed *in vitro* to hyperoxia for 24-48 hours. We discovered that the Na,K-ATPase mRNA-protein-activity parameters did not change in parallel, but there were many consistent responses in the different models. For example, across each model, Na,K-ATPase gene expression consistently increased. The type II cell protein level

changes were complex, with maintained β -1, but decreased α subunit protein in acute hyperoxia, followed by return to normal levels. In terms of transport, we were surprised to discover heterogeneity of the response of unidirectional active sodium resorption in the rats (Carter 1997b). In intact type II cells (models 2 and 3) steady state sodium pump transport activity was normal, but the sodium pump Vmax of membrane preparations decreased. Thus these data indicate that sodium pump and transport are affected and regulated in at least several discrete ways during hyperoxia - increased gene expression; differential changes in relative expression of the α -1 and β -1 subunit proteins; and modification of peak enzyme capacity for pump molecules in the plasma membrane with maintained steady state functional activity. It is very likely that there is simultaneous oxidative injury to some pump molecules while there is a homeostatic increase in transcription accompanied by altered intracellular regulation.

In a standard in vivo acute hyperoxic model (60 hour >95% oxygen) (Thet, Crapo), α 1 and β 1 mRNA increased early and were 3-5 fold elevated at 60 hours when the alveolar septae were widened with interstitial and mild alveolar edema present, but AECs appeared normal (Nici). No new cell population with sodium pump subunit staining was seen. Levels of α 1 and β 1 subunit antigenic proteins increased significantly by day 1 after O₂ exposure ended and remained elevated through 7 days of recovery. We measured the active (amiloride-inhibitable) and total unidirectional sodium resorption from the alveolar space (Carter 1997b). We were surprised to discover heterogeneity in the degree of injury - as reported in chronic hyperoxia (Yue 1997). The more severely injured rats had augmented active Na resorption, whereas the mildly injured rats had decreased active Na resorption - consistent with upregulation with worsening injury.

ATII cells were studied from rats after 60 hours of in vivo 100% oxygen (Carter 1997a). Ouabain-sensitive Rb uptake into intact ATII cells was normal with hyperoxia, however, the sodium pump Vmax of ATII cell membranes was reduced to 75% of control. The ATII cell α 1 subunit protein decreased in hyperoxia while the β 1 was stable. The mRNA levels of both subunits increased. Thus levels of mRNA, protein and enzyme activity did not respond in parallel to hyperoxia and steady state activity of the intact cells correlated best with the amount of β 1 subunit protein.

We developed an in vitro hyperoxic ATII cell model to eliminate interactions with other cell types and hormonal influences, enabling us to study the effects of hyperoxia more directly (Carter 1994). One day after recovery from isolation, normal rat ATII cells were exposed to 95% O₂/5% CO₂ in vitro. The cells demonstrated ~25% death with increased Erythrosin B uptake and decreased DNA/plate at 48, but not 24, hours. Within 24 hours of hyperoxia, the type II cells increased levels of Na,K-ATPase mRNA for both subunits, with β 1 rising more than α 1, but the α 1 protein level was decreased and the β 1 level was unchanged. The sodium pump Rb uptake of the intact cells was normal, but the Vmax of cell membranes again was reduced significantly. Results in this model were almost identical to those in the ATII cells isolated after in vivo injury, supporting the direct effects of hyperoxia in effecting these changes in ATII cells in this much simpler system.

An important general scientific question is the mechanisms through which oxidants and oxygen tension affect gene expression in the lung (Fanburg). To study the detailed mechanism of Na,K-ATPase gene upregulation in hyperoxia, we screened for hyperoxic induction of Na,K-ATPase mRNA in other cell lines. Two alveolar epithelial cell (AEC) lines, A549 and MP48 cells did not increase Na,K-ATPase mRNA in response to hyperoxia; but they do not form tight junctions in cell culture. In contrast, the renal tubular MDCK (Madin-Darby canine kidney) epithelial cells increased Na,K-ATPase α 1 and β 1 mRNA in response to hyperoxia in vitro (Wendt). Hyperoxia increased the transepithelial resistance of MDCK cells cultured on Millicell HA (Millipore) filters, suggesting that increased functional Na,K-ATPase and consistent with increased dome formation (Dreher). We sought to determine the mechanism of increased Na,K-ATPase steady state mRNA levels. The mRNA half lives of the α 1 and β 1 subunit mRNAs did not significantly change with hyperoxia, with approximate half-lives for the α 1 subunit were 4.4 hrs in normoxia and 4.7 hrs. in hyperoxia and for the β 1 subunit

were 5.9 hrs. in normoxia and 6.0 hrs. in hyperoxia. In nuclear run-on assays on both MDCK and type II cells after normoxia or 12 hrs. of hyperoxia, hyperoxia-induced transcription of both subunits in both cell types: 1.7 fold for $\alpha 1$ subunit and ~3 fold for $\beta 1$ subunit in type II cells and 1.3 fold for $\alpha 1$ subunit and 3.0 fold for $\beta 1$ subunit in MDCK cells. Thus the increased sodium pump gene expression was primarily due to increased transcription.

To identify hyperoxia-sensitive regulatory regions of the gene promoters, MDCK cells were transfected with wild type and 5' deletions of $\alpha 1$ (1537bp) and $\beta 1$ (817 bp) subunit promoter-luciferase reporter constructs (gifts of K. Kawakami, Dept. Biology, Jichi Medical School and G. Gick, SUNY Brooklyn, respectively) (Wendt). Promoter activity of full length and multiple 5' deletion constructs of the $\alpha 1$ construct did not change with hyperoxia, suggesting that transcriptional control may lie outside of the 5' 1537 bp region. In contrast, hyperoxia increased $\beta 1$ luciferase activity two fold relative to normoxia. Transfection with two $\beta 1$ 5' promoter deletion constructs identified a region between -102 and -41 required for increased promoter activity with hyperoxia. Sequence analysis of this region did not identify consensus sequences for known oxidant-sensitive transcription factors, such as AP-1, ARE (Rushmore), or NF κ B. The region is GC rich and has partial sequence homology with an SP-1 site, but this redox-sensitive transcription factor usually is believed to be important for basal transcription..

We confirmed that this 61 bp $\beta 1$ promoter region (-102 to -41) was specific and sufficient for hyperoxia induction, by subcloning oligonucleotides spanning this region into a luciferase vector containing a minimal promoter of the mouse mammary tumor virus (MMTV) that alone was not inducible by hyperoxia. When transfected into MDCK cells, hyperoxia induced promoter activity for both the larger oligonucleotide (-157 to -38) and even more with the smaller dimer of -84 to -44. Thus a 40 bp region between -84 and -44 is necessary and sufficient for hyperoxic induction. The specific transcription factors in addition to SP1 that interact with this region are being determined.

PHYSIOLOGIC RELEVANCE OF ALTERING SODIUM PUMP IN LUNG INJURY

A major question concerning clinical strategies to increase fluid resorption is whether they still work in high permeability injury states. As discussed by Dr. Matthay, this now is being demonstrated in several lung injury models. We developed an in situ method for measuring alveolar fluid resorption in warm dead rats (Lasnier). Fluid absorption reflected changes in alveolar fluid volume. In this model, terbutaline stimulated fluid resorption approximately 2 fold in both normal rat lungs and lungs injured by 60 hours of hyperoxia. In both situations the terbutaline-induced increased absorption was prevented by ouabain. There also was a statistical trend towards greater resorption induced by hyperoxia itself.

SUMMARY

Oxidants have been intensively studied for many years because of their importance as a pathophysiologic mechanism of disease. However, relatively little is known about their impact on certain fundamental cellular processes, such as translation, and on the integrated response of transporting epithelia. A disadvantage of studying oxidants is their complex multiplicity of effects - as demonstrated by the non-parallel changes in Na,K-ATPase mRNA, protein and activity we have observed. Our studies indicate that multiple simultaneous effects likely occur, including inhibition of peak Na,K-ATPase capacity and increased gene transcription. Understanding the mechanisms by which oxidants impact ATII cell monolayer sodium transport and sodium pump oxidation, translation and transcription should significantly augment our ability to design therapeutic strategies likely to succeed in upregulating alveolar fluid resorption in ARDS and other conditions with alveolar flooding. It also will add knowledge about this critical enzyme, important for many cell functions in a variety of

organs.

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