Mechanical Forces Impair Alveolar Ion Transport Processes – A Putative Mechanism Contributing to the Formation of Pulmonary Edema

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1. Introduction

The aim of this chapter is to highlight the importance of transepithelial ion transport processes for lung function in general and to focus on the impact of mechanical forces on pulmonary ion transport in particular. Linking mechanical forces with pulmonary ion transport derives from the fact that the lung is a dynamic organ as well as from several studies providing evidence that the amount of mechanical forces as used during artificial ventilation correlates with mortality rates in patients with respiratory failure such as ALI (acute lung injury) and ARDS (acute respiratory distress syndrome) (ARDS Network Investigators, 2000). In these patients the formation of pulmonary edema is a characteristic symptom (Frank and Matthay, 2003; Ricard et al., 2003) and the basic rationale behind this is, that mechanical perturbations cause epithelial leakage in response to mechanically induced damage of the epithelial layer. This damage is suggested to be a major cause for the formation of pulmonary edema as well as the inability to reabsorb the edema fluid. However, little is known whether or not mechanical forces may directly interfere with pulmonary ion transport processes and this represents a putative mechanism that facilitates the formation of pulmonary edema – in addition to damages of the epithelial layer.

2. Air breathing and pulmonary ion transport

The water land transition of tetrapods represents a fundamental process within vertebrate evolution that was accompanied by the development of lungs as gas exchanging structures. The major advantage of air breathing is the almost infinite access to oxygen, although this bears also some risks. The gas-exchanging structures must be moistened to facilitate oxygen solubility and the architecture of these structures must be adapted to the requirement of efficient gas exchange by diffusion. These problems were basically fixed by invagination of the gas-exchanging surface, protecting them from desiccation and from mechanical damage. Therefore, invagination could be considered as a basic improvement allowing the development of a highly conserved architecture of the air blood barrier that can be found within all air-breathing vertebrates. This architecture is referred to as the "three ply design" (Maina and West, 2005). The three layers of the air-blood barrier are represented by the

pulmonary epithelium, the basal lamina and the endothelium that forms the pulmonary capillaries. Although invagination and the development of the three ply design fixed a lot of problems, other challenges arose. To ensure a constant supply of oxygen as well as a continuous replacement of the breathing medium, ventilation mechanisms were needed. This was achieved by different strategies within the different vertebrate classes. Early tetrapods and amphibians ventilate their lungs by a buccal pump (Brainerd and Owerkowicz, 2006). In higher vertebrates ventilation occurs by a costal pump (Roux, 2002) where the movement of the ribs ensures aspiration of air. But independent of the strategy how the exchange of the breathing medium is accomplished, the gas-exchanging surface is permanently exposed to pollutions and pathogens that are omnipresent in the air supplied to the lungs.

It is also well known that the entire pulmonary epithelium is covered by a thin fluid layer (PLL: pulmonary liquid layer) (Daniels and Orgeig, 2003), that is of particular importance for the function of the lung. The PLL extends from the distal parts of the lung to the upper airways and consists of mucus, surfactant and periciliary fluid (Rubin, 2002) (Fig. 1). On the one hand the PLL is the first physical border that is exposed to the environment and the first line of host defense that absorbs pathogens. This is of particular importance in the airways, where the PLL is part of the innate immune system and crucial for protecting the host from the permanent exposure to pollutions and pathogens due to their removal by the mucociliary clearance (Welsh, 1987; Davis and Lazarowski, 2008).
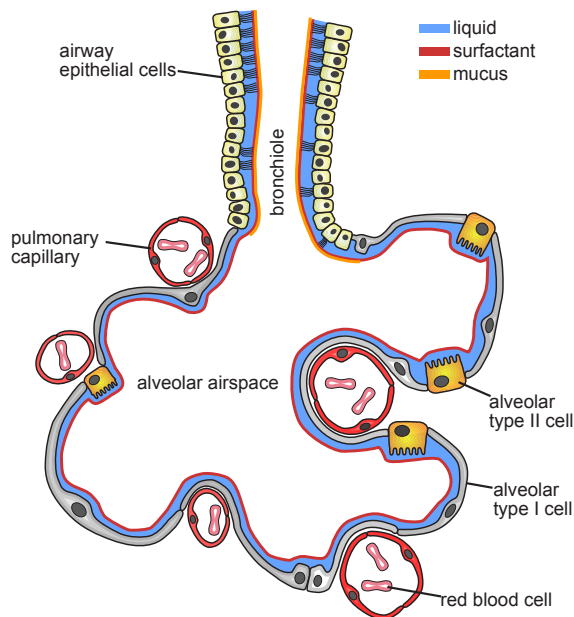

Fig. 1. Schematic drawing of the distal lung region. The distal lung is represented by respiratory bronchioles and terminal alveoli that are lined by a continuous layer of epithelial cells consisting of different cell types. The entire epithelium is further covered with a liquid film (PLL: pulmonary liquid layer) consisting of different layers. In the airways the PLL is composed from a liquid layer (periciliary fluid) and a layer of mucus and surfactant. In the alveolar regions the PLL consists of a liquid layer (alveolar fluid) and a surfactant layer.

On the other hand the height and volume of the PLL in the respiratory zone – primarily represented by the alveolar fluid – determines the distance of diffusion for the gases that is a limiting rate for the diffusion efficiency as defined by Fick's law of diffusion. Thus, increased fluid content in the alveolar region decreases oxygenation of the blood (Matalon and O'Brodovich, 1999; Matthay et al., 2000).

Therefore, the consistency as well as the volume of the PLL layer must be tightly regulated and controlled – in the airways as well as in the gas-exchanging region – to ensure effective host defense as well as effective gas exchange.

This principle becomes evident regarding pulmonary diseases that are associated with inappropriate fluid balance in the lung. For example hyperabsorption of water from the airspace increases the viscosity of the PLL and this interferes with the removal of inhaled pathogens due to impaired mucociliary clearance as observed in patients with cystic fibrosis (Fig. 2A) (Widdicombe et al., 1985; Riordan et al., 1989; Matsui et al., 1998). In contrast, too much fluid in the lung impairs gas diffusion and this can be observed in patients with pulmonary edema (Figure 2B) (Sznajder, 2001; Hoschele and Mairbaurl, 2003).

Thus, a defined content of water covering the pulmonary epithelia is a basic requirement for proper lung function and this depends on the development of appropriate ion transport processes accomplished by the pulmonary epithelia.
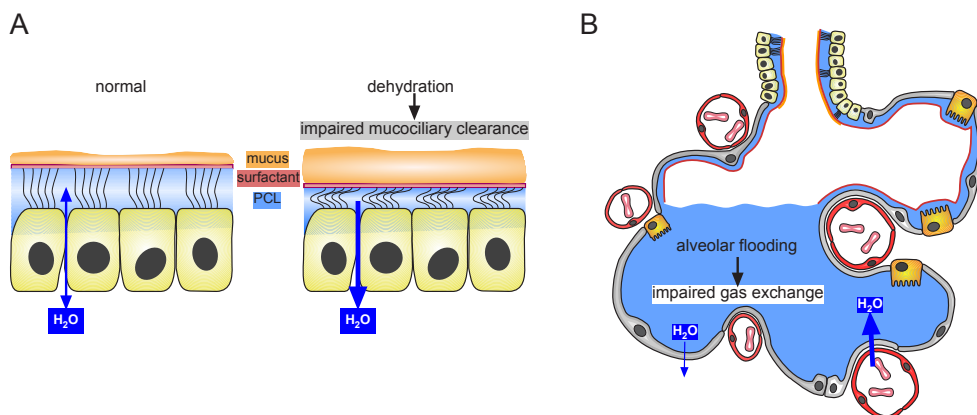

Fig. 2. Impaired epithelial fluid transport affects basic lung functions. A) Increased water reabsorption in the airways results in dehydration of the periciliary liquid layer (PCL) leading to an impaired mucociliary clearance and mucus accumulation within the airways. B) In the alveolar region an imbalance between fluid reabsorption and fluid infiltration leads to the formation of pulmonary edema that impairs the exchange of the breathing gases.

2.1 Transepithelial Na^+ and Cl^- transport are the main pathways to control the water content in the lung

Studying ion transport processes across pulmonary epithelia within the last decades improved our understanding how the fluid content in the lung is sustained. The basic principle of pulmonary water transport is ubiquitous – ions are transported across the epithelial layer and this generates transepithelial osmotic gradients that cause water diffusion across the epithelium (Sackin and Boulpaep, 1975). It is well accepted that

pulmonary epithelia are Na^+ reabsorptive epithelia where active Na^+ reabsorption represents the bulk of transepithelial ion transport. Na^+ transport occurs via two steps: 1) Na^+ ions are taken up through epithelial Na^+ channels (ENaC) at the luminal side of the epithelial cells and are pumped out from the cells at the basolateral side by the Na^+/K^+ATPase. This process is suggested to be the principle mechanism for water reabsorption from the airspace into the body (Matthay et al., 2002). Another major component with significant impact on transepithelial water movement is represented by the transepithelial transport of Cl^-. In the airways it is well accepted that Cl^- is secreted via luminal Cl^- channels (Smith et al., 1982; Willumsen et al., 1989; Chambers et al., 2007). However the particular role of Cl^- channels Cl^- in alveolar epithelial cells remains unclear since there is evidence that Cl^- is secreted (McCray et al., 1993; Tizzano et al., 1994; Lazrak et al., 2002; Sommer et al., 2007), as well as absorbed (Fang et al., 2002; Fang et al., 2006). There are at least two different Cl^- channels identified in the apical membrane of pulmonary epithelial cells – the Ca^{2+} dependent Cl^- channel (TMEM16a) (Caputo et al., 2008; Schroeder et al., 2008; Yang et al., 2008) and the cAMP dependent CFTR Cl^- channel (Riordan et al., 1989; Welsh and Smith, 1993). In addition it might be noted that alveolar epithelial cells are characterized by a high water permeability (Folkesson et al., 1994; Dobbs et al., 1998) although the role of these proteins in pulmonary fluid handling is uncertain since no significant impact of aquaporins on alveolar clearance has been detected in transgenic (aquaporin deficient) animals (Verkman et al., 2000; Verkman, 2007).

However, the crucial importance of defined and regulated ion transport processes in the lung to control the water content is beyond dispute, since this was impressively confirmed by several studies using transgenic animal models with impaired ion channel functions. For example deletion of the α ENaC subunit in mice leads to early death due to the inability to reabsorb the alveolar fluid from the lungs after birth (Hummler et al., 1996). Interestingly, rescuing α ENaC expression in transgenic mice that were derived from α ENaC deficient mice demonstrated that these animals expressed decreased levels of ENaC mRNA and that this resulted in an increased susceptibility to the formation of pulmonary edema (Olivier et al., 2002). In addition overexpression of the β ENaC subunit and hyperabsorption of Na^+ is associated with impaired mucociliary clearance resulting in a phenotype that is characteristic for cystic fibrosis (Mall et al., 2004). Recent studies established that mutations in the CFTR gene of pigs resulted in a cystic fibrosis like lung disease (Rogers et al., 2008; Stoltz et al., 2010).

3. Mechanical forces and breathing

Ventilation of the lungs due to the movement of the chest is associated with the appearance of physical forces. These forces are pressure (force per area), strain (deformation e.g. reasoned by the impact of pressure) and shear stress (movement of fluid at the cellular surface) (Fig. 3) (Wirtz and Dobbs, 2000). It might also be considered that due to the complex anatomy of the gas exchanging area it is difficult to estimate the distinct forces acting on individual cells (Liu et al., 1999) and that the local appearance of forces can be influenced by parameters like surfactants, focal adhesion molecules, the contractile machinery of the cells as well as the activity of molecular motor proteins within the cells (Fredberg and Kamm, 2006).

Although more or less all cell types within the lung are exposed to these physical stimuli the following section will focus on epithelial cells. Especially, the appearance and the reason for the stimuli as well as their impact on the pulmonary epithelial cells will be discussed.
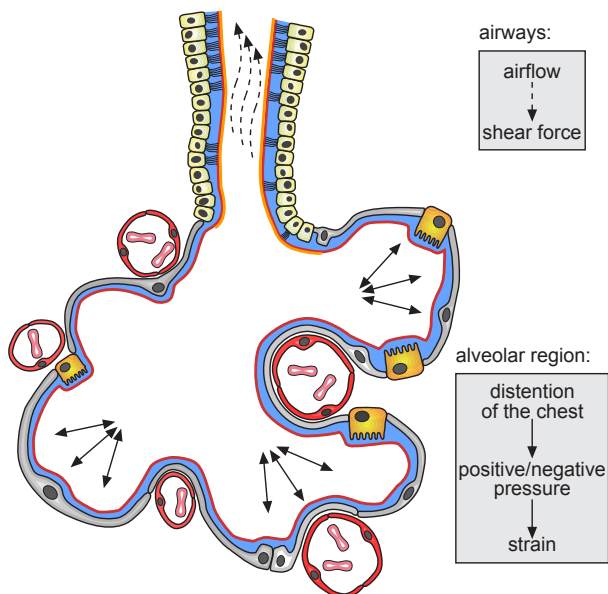
Fig. 3. Breathing is associated with the appearance of physical forces. Airway epithelial cells are primarily exposed to shear forces that are reasoned by the airstream flowing passing the surface of the airways. In the alveolar region the main physical force is strain due to the positive and negative pressure that are reasoned by the movement of the chest.

3.1 Pressure and strain in the lung

The appearance of pressure is the consequence of the movement of the chest. During inspiration and expiration epithelial cells are exposed to negative and positive pressures. This pressure is defined as transpulmonary pressure and resembles the pressure difference between the pressure in the pleural space and the atmospheric pressure (Fredberg and Kamm, 2006). During inspiration at rest the inflating pressure is approx. 5 cm H_2O and this can increase to approx. 30 cm H_2O at deep inspirations (Fredberg and Kamm, 2006). In the lung the appearance of pressure will always cause deformation of the cells due to the delicate anatomy and morphology of the alveolar structure. Therefore, increasing pressure is always associated with the appearance of strain. From this point of view, it seems obvious to consider that strain rather than pressure is the adequate stimulus acting on epithelial cells as a consequence of the breathing movements (Liu et al., 1999).

3.2 Shear stress in the lung

In addition to strain, the luminal surface of the epithelial cells is exposed to shear stress. Shear stress is defined by the tangential movement of particles (e.g. air or fluid) at the surface between different physical compartments. In the airways shear stress is primarily caused by the oscillating airflow passing the surface of the airway epithelial cells (Tarran et al., 2006). In the distal lung regions shear stress is a consequence of the movement due to distention, where the epithelial cell represents one compartment that is distended and the fluid of the PLL represents a static component of another compartment. Thus a relative

movement of the epithelial cell (with respect to the fluid) will cause shear stress at the luminal surface of the cells.

The next sections will focus on the impact of pressure and strain in particular on lung functions.

3.3 Strain is crucial for several functions of epithelial cells

It is well known that physical forces are important stimuli for distinct cellular functions of pulmonary epithelial cells (Fig. 4). An important indication for this is represented by the fact that development of the mammalian lung within the last third of gestation depends on breathing movements although the lungs are fluid filled (Kitterman, 1996). In animal models prevention of these breathing movements by spinal nerve sections resulted in retarded lung growth and development (Fewell et al., 1981). Further, *in vitro* studies demonstrated that distention of alveolar epithelial cells is an important trigger that affects alveolar cell differentiation. Mechanical distention of isolated and cultivated fetal alveolar cells promotes their differentiation to AT I cells whereas that lack of distention promotes the differentiation to ATII cells (Edwards, 2001).
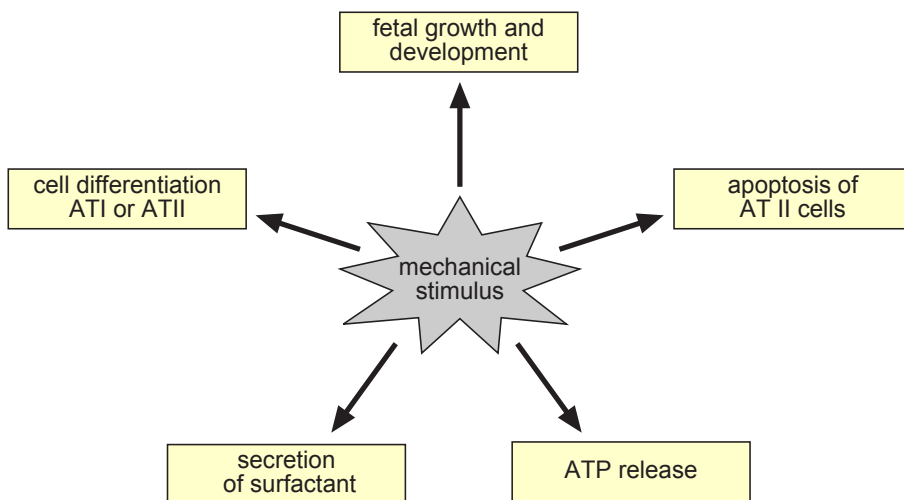

Fig. 4. Under normal breathing conditions mechanical stimuli are important for several functions of the lung.

Besides affecting cell differentiation and lung development there is also evidence for the contribution of physical stress as a factor that mediates production and secretion of surfactant proteins by ATII cells (Edwards et al., 1999) as well as by cultured H441 cells (Sanchez-Esteban et al., 1998). There is also evidence that mechanical forces induce apoptosis in ATII cells (Edwards et al., 1999; Hammerschmidt et al., 2007). In addition, different signaling mediators and ATP in particular are released in response to mechanical stress (Homolya et al., 2000; Okada et al., 2006; Button et al., 2007). The release of ATP offers the possibility to initiate a variety of different cellular reactions by acting on multiple purinergic receptors – including P2X, P2Y and P1 receptors (Leipziger, 2003; Bucheimer and Linden, 2004; Barth and Kasper, 2009). The relevance of purinergic signaling has been

implicated by a study identifying alterations of transepithelial ion transport processes in airway epithelia in response to adenosine as a factor contributing to cystic fibrosis lung disease (Tarran et al., 2005; Tarran et al., 2006).

3.4 Deleterious effects of mechanical forces in the lung

Although, there is more than sufficient evidence that identifies mechanical stimuli as an important mediator for normal lung functions, a main reason for studying their effect in the lung arises from the hazardous effects that are caused by mechanical forces during artificial ventilation (Frank and Matthay, 2003; Ricard et al., 2003) (Fig. 5).
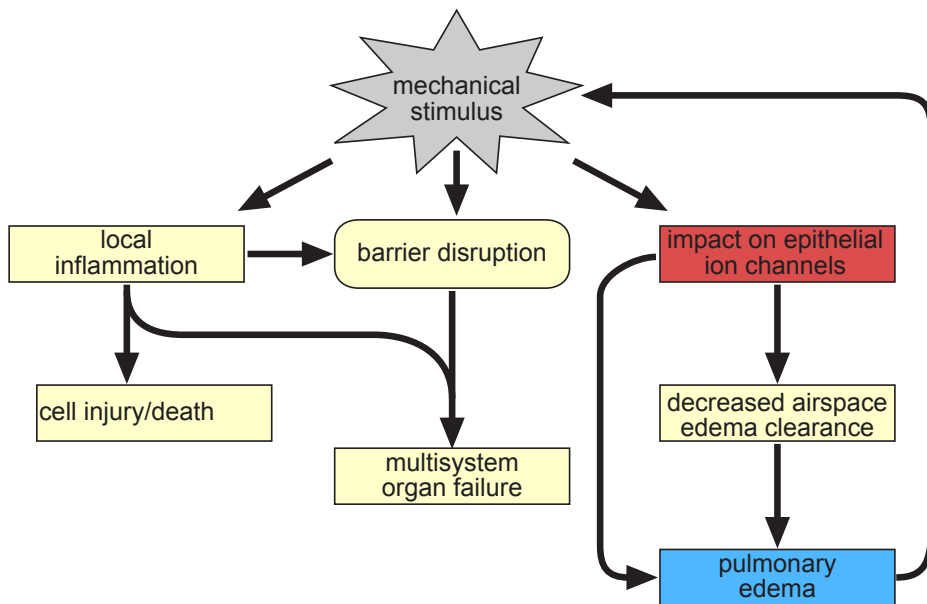

Fig. 5. Effect of deleterious physical forces related to ventilator induced lung injury. This scheme was modified from (Frank and Matthay, 2003) and extended by the potential interference of mechanical stimuli with the activity of epithelial ion channels. This represents a yet unidentified mechanism to improve the understanding how mechanical forces contribute to the formation and probably maintenance of pulmonary edema.

Artificial ventilation is a remedy of first choice for the treatment of patients with respiratory failure (e.g. acute lung injury (ALI); acute respiratory distress syndrome (ARDS)) (Ware and Matthay, 2000). However, the development of pulmonary edema due to fluid influxes into the airspace as well as the inability to resolve the edema fluid by decreased fluid reabsorption, is a major reason for the morbidity and mortality in these patients (Morty et al., 2007). The inability of the patients to resolve the edema fluid correlates with the mechanical ventilation duration times and mortality. High fluid clearance was associated with shorter durations of mechanical ventilation as well as with significantly decreased mortality rates (Sznajder, 2001; Ware and Matthay, 2001).

Considering the important role of pulmonary ion transport processes as discussed above, it is obvious that pulmonary edema are somehow related to alterations of pulmonary ion

transport processes (Matthay, 2002). Although changes of ion transport processes do not have to be causative for the development of pulmonary edema – there is sufficient evidence demonstrating that artificial ventilation worsens the situation in ALI/ARDS patients. This phenomenon has been termed ventilator induced lung injury (VILI) (Ricard et al., 2003) and is attributed to inappropriate ventilation strategies and thus the appearance of extensive physical forces (Fig. 5) (Plataki and Hubmayr, 2010).

This indication arises from the outcome of a multicenter trail study demonstrating a significantly reduced mortality in a group of patients that was ventilated with decreased tidal volumes (6 ml/kg) compared with high tidal volumes (12 ml/kg) (ARDS Network Investigators, 2000). Ventilation with decreased tidal volumes was also accompanied by the appearance of lower plateau pressures (ARDS Network Investigators, 2000) and this is associated with decreased mechanical forces.

However, regarding the symptoms observed in patients with ALI, ARDS and VILI there is an obvious connection between artificial ventilation and the development of pulmonary edema. There are two possible explanations reasonable for the development of pulmonary edema related to artificial ventilation:

1. Artificial ventilation causes ruptures and damages of the epithelial layer – enabling an uncontrolled influx of protein rich fluid into the airspace.
2. Mechanical forces as appearing during ventilation directly interfere with pulmonary epithelial ion transport processes

The first point has been extensively studied and there is no doubt that mechanical strain induced by artificial ventilation is a major reason for the development of pulmonary edema (Frank and Matthay, 2003). This is clearly indicated by the appearance of proteins in the edema fluid (Ware and Matthay, 2000). The second point is also obvious since a correlation between the ability to reabsorb alveolar fluid and the outcome of patients has been identified (Ware and Matthay, 2001). But for this instance, the mechanisms how artificial ventilation and thus mechanical forces interfere with ion transport processes are unknown.

3.5 Tools for studying the impact of mechanical forces on pulmonary ion transport

The first choice for studying functional epithelial ion transport is represented by electrophysiological Ussing chamber recordings. This technique was established by Hans Ussing during his studies on ion transport processes across the amphibian skin (Ussing and Zerahn, 1951). A major advantage as well as a prerequisite of this technique is the use of an intact epithelial layer consisting of differentiated polar cells. Although this technique is applicable for many freshly dissected epithelia including airway epithelia from mammals (Olver et al., 1975; Widdicombe and Welsh, 1980), the use of mammalian alveolar epithelium for elaborating this technique is not possible. This is due to the complex anatomy reasoned by miniaturization of the gas-exchanging region. Improved protocols and procedures for the isolation and cultivation of alveolar cells generally fixed this problem, but the use of isolated cells bears the risk of using cells with an artificial non-physiological phenotype. There is a bunch of studies published demonstrating that in isolated and cultivated cells the expression of ion transport proteins as well as their phenotype in terms of ion transport properties vary in dependence of the cultivation conditions used (Kunzelmann et al., 1996; Jain et al., 2001; Leroy et al., 2004; Dvorak et al., 2011). Further, these cells do not have their native surrounding (neighboring cells of different cell type, basal lamina etc.) that is important for the detection and transmission of forces. It remains also a challenge to expose these cultivated cells to defined physical forces (e.g. strain) to determine immediate changes

of ion transport processes because usually the cells are cultivated on rigid polyester or polycarbonate membranes.

On the other hand pioneer studies addressing the function and relevance of pulmonary ion transport were performed on anaesthetized animals (Egan et al., 1976; Matthay et al., 1982). These studies identified the basic mechanisms of water and electrolyte transport by the alveolar epithelium. However, from such studies it is difficult to disentangle the particular ion conductances and to identify the specific ion transporting molecules that are involved in those processes.

3.5.1 Using native lung preparations for studying the impact of strain on pulmonary epithelial ion transport

Our lab has therefore established a native model for investigations of pulmonary ion transport. In accordance to Krogh's principle (Krebs, 1975) we decided to use lung preparations derived from the South African Clawed Frog *Xenopus laevis*. The alveolar epithelium of the *Xenopus* lung consists of one cell type referred to as pneumocytes (Meban, 1973). The anatomy and morphology of these cells is reminiscent to that of alveolar type I cells (Fischer et al., 1989), but they expose functional properties of alveolar type II cells as represented by the presence of lamellar bodies (Fischer et al., 1989). Most important, the *Xenopus* pneumocytes expose a Na^+ reabsorptive phenotype (Fischer et al., 1989; Kim, 1990; Fronius et al., 2003). In addition, expression and function of the CFTR Cl^- channel has been detected (Sommer et al., 2007). Recent studies identified the presence of the $Na^+/K^+/2Cl^-$ cotransporter, the function of a HCO_3^-/Cl^- exchanger (Berger et al., 2010) and evidence for basolateral Cl^- channels (Berger et al., 2011). Thus, the *Xenopus* pneumocytes exhibit the basic repertoire of ion channels and transporters that are supposed to be important in mediating the volume of the PLL.

The main advantage of this amphibian organ is its relatively simple sac-like structure. This feature is a prerequisite for dissecting a preparation that is suitable for Ussing chamber recordings. Comparable studies with other native lung preparations from higher vertebrates – and mammals in particular - are not possible, because of the growing complexity and miniaturization of the gas-exchanging region. In addition to establishing the use of *Xenopus* lung preparations for electrophysiological recordings, an Ussing chamber was developed that enables the exposure of the mounted pulmonary epithelium to mechanical forces. Mechanical forces are applied via an increased hydrostatic pressure and this is achieved by changing the outflow-height from the compartments Fig. 6.

4. Impact of strain on pulmonary ion transport in the *Xenopus* lung

Although the connection between high tidal volumes and pulmonary edema has been well established, little is known whether or not the underlying mechanisms can be attributed – at least partly – to a direct interaction of the mechanical forces with ion transport processes. Studies have been published providing evidence that high volume ventilation resulted in a decreased Na^+ transport due to a decreased Na^+/K^+-ATPase activity (Lecuona et al., 1999). Other studies demonstrated an increased Na^+/K^+-ATPase activity in response to cyclic stretch (Fisher and Margulies, 2002). Although these studies identified interference of strain with Na^+/K^+-ATPase activity, changes were observed hours after exposure to mechanical forces. So far little is known about a direct short-term effect of mechanical forces on ion transport processes in the lung.

customized Ussing chamber

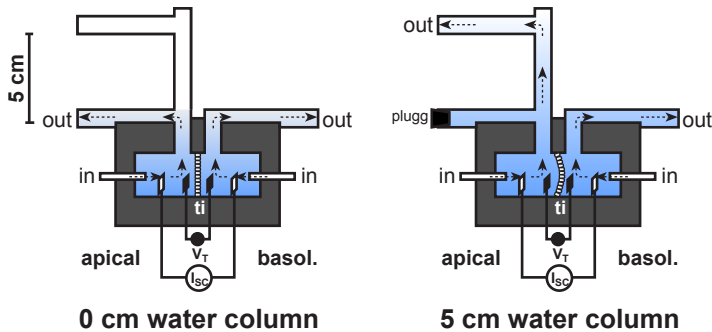**0 cm water column**   **5 cm water column**

Fig. 6. Drawing of the Ussing chamber used to study the effect of mechanical forces on pulmonary epithelial ion transport (modified from (Bogdan et al., 2008)). Mechanical forces were applied by increasing the water column (5 cm water column) of the outflow. Both chamber compartments (apical, basolateral) were continuously perfused and the transepithelial short-circuit current (I_{SC}) and potential (V_T) was permanently monitored (ti: mounted tissue).

Using *Xenopus* lung preparations in combination with a customized Ussing chamber (Fig. 6) 5 cm hydrostatic pressure was applied from the apical side to mechanically challenge the tissue. Application of hydrostatic pressure was accompanied by immediate changes of the measured transepithelial short-circuit current (I_{SC}). The net effect induced by 5 cm H_2O was characterized by a decreased transepithelial current (Bogdan et al., 2008). Interestingly, the application of 5 cm H_2O from the basolateral side induced exactly the same response whereas the application of 5 cm H_2O synchronously from the apical and basolateral side did not cause any effect of the I_{SC} (Bogdan et al., 2008). These observations clearly demonstrate that the effective mechanical stimulus is strain and that it does not matter from which side the tissue is deflected.

In this setup a decreased transepithelial ion current is an indication for a decreased net reabsorption of ions from the apical to the basolateral side of the epithelium. Further, a reduced ion reabsorption reasoned by changes in ion transport processes will also reduce the osmoticaly driven water reabsorption from the airspace. This means that the changes of ion transport in the pulmonary epithelium observed with hydrostatic pressure decrease water reabsorption from the airspace and this represents a mechanism to facilitate the development of pulmonary edema – without affecting the integrity of the epithelial barrier that was assessed by determining the transepithelial electrical resistance.

4.1 Strain induces short-term activation of Na$^+$, K$^+$ and Cl$^-$ channels

Further investigations using different ion channel inhibitors and substituting different ions from the perfusion solution revealed that the observed effect is reasoned by the activation of different ion channels and ion conductances resembled by:

1. activation of amiloride-sensitive Na$^+$ reabsorption
2. activation of an apical Cl- secretion

3.   activation of an apical K^+ secretion

Therefore, the inhibitory effect observed in the absence of drugs is an overlay of these three conductances (Fig. 7). Activation of Na^+ reabsorption as well as an increase of Cl^- secretion will produce an increase of the I_{SC}. In contrast to this, activation of apical K^+ channels will cause a decrease of the I_{SC}. Since the net pressure effect is an inhibition of the I_{SC}, the major response observed by the application of hydrostatic pressure is due to an activation of apical K^+ channels. Accordingly, the inhibitory effect of hydrostatic pressure should be prevented (or reversed → activation of the I_{SC}) following pre-incubation with K^+ channel inhibitors. And indeed this is exactly what we observed (Bogdan et al., 2008). Further, it has been found that the pressure-induced effect was largely prevented by glibenclamide (Bogdan et al., 2008), a compound that is a high affinity inhibitor of ATP-sensitive K^+ channels (K_{ATP}) (Nichols, 2006). Among other activating mechanisms, the activity of K_{ATP} channels is dependent on intracellular cyclic nucleotide levels and ATP in particular (Nichols, 2006). Interestingly, increased extracellular ATP concentrations were observed in response to the application of hydrostatic pressure and this represents a likely mechanism to explain our observations.

K_{ATP} channels are octameric complexes consisting of four pore forming Kir (inward rectifying K^+ channels) subunits and four associated SUR (sulfonylurea receptor) subunits (Nichols, 2006). In particular Kir channels are supposed to be involved in controlling the
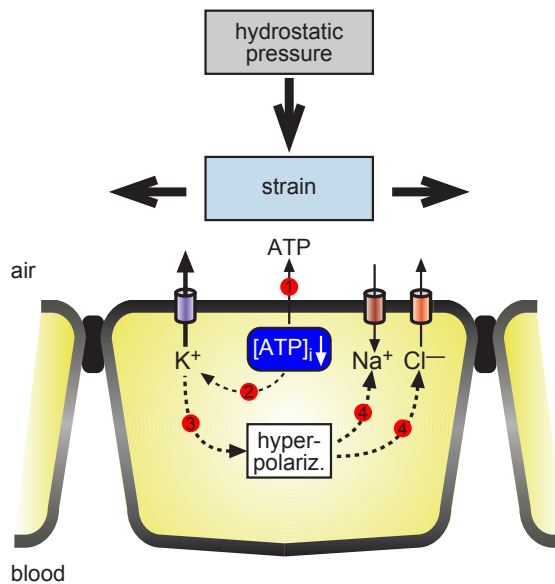

Fig. 7. Scheme illustrating the putative mechanism how hydrostatic pressure acts on epithelial ion transport processes in *Xenopus* lung epithelium (modified from Bogdan et al., 2008). The entire process could be initiated by the release of ATP via a yet unknown mechanism (1). The decrease of intracellular ATP levels ($[ATP]_i$) is likely to activate K_{ATP} channels (2). This will then cause the cell membrane potential to hyperpolarize (3) and subsequently facilitates the uptake of Na^+ via apical Na^+ channels (4) as well as the secretion of Cl^- via apical Cl^- channels.

membrane potential (Nichols, 2006) and this in turn enables the possibility to influence the driving forces for other conductances such as Na$^+$ and Cl$^-$. From this point of view it might be suggested that strain is primarily transduced in activation of K$_{ATP}$ channels and that this in addition influences secondarily Na$^+$ and Cl$^-$ transport as we observe by the application of hydrostatic pressure on *Xenopus* lung epithelia. Interestingly, increased ATP levels are also observed in rats ventilated with injurious ventilation parameters. In those experiments the increase of extracellular ATP concentrations was not reasoned by cell damage or cell lysis (Rich et al., 2003). These observations together with our findings indicate that the release of ATP (by a yet unidentified mechanism) might play a key role concerning the activation of ion channels in response to mechanical forces.

Last but not least it might be highlighted that the effect that was observed in response to the application of hydrostatic pressure (inhibition of the I$_{SC}$) represents a mechanism that impairs ion reabsorption from the alveolar airspace. This will result in a decrease osmotic gradient across the epithelial layer and will subsequently cause a reduction of fluid reabsorption from the airspace. Thus, this is a likely mechanism that impairs the resolution of pulmonary edema or maybe represents a mechanism that – among other incidents – initiates the formation of pulmonary edema in response to strain as induced by artificial ventilation.

5. Conclusions

The fact that mechanical forces directly affect pulmonary epithelial ion transport is important for future therapeutic options. On the one hand it further confirms the observations that modified ventilation strategies with low pressures and reduced volumes are beneficial for the outcome of patients with respiratory failure that are admitted to artificial ventilation. Therefore, the development of new ventilation strategies should be considered with the background that a minimum of mechanical stress should be used because this decreases interference with ion transport processes and this will preserve the ability of the epithelium for effective ion and water reabsorption.

On the other hand, it offers new therapeutic targets since we have evidence that K$^+$ channels and K$_{ATP}$ channels in particular play a major role in the response observed by increased hydrostatic pressure. In our experiments, the inhibition of these channels has been identified to largely abolish the mechanically induced activation of K$^+$ channels and this would be beneficial to prevent the reduced ion transport absorption as that correlates with a decreased water reabsorption from the airspace. Another possibility in order to prevent the effects of strain on ion channels might be represented by the possibility to target the release of ATP, although the mechanisms of ATP release are still under debated.

6. Acknowledgements

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