GURGLING AND BUBBLING IN THE LUNG

Alveolar fluid reabsorption and high altitude pulmonary edema

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With this article I would like to draw your attention to a possible role of the alveolar epithelium as one factor that might contribute to formation of high altitude pulmonary edema (HAPE). Doing so I am passing over the most commonly used explanations of HAPE which are pulmonary hypertension and increased vascular permeability. Two facts leave the door open for alternate or additional explanations: (i) Pulmonary hypertension is more pronounced in HAPE-susceptibles exposed to hypoxia than in non-susceptibles, yet, not all susceptibles develop HAPE (1). (ii) Very often no history of infections was reported of mountaineers who did develop HAPE during high altitude exposure, nor were there any signs of markers of inflammation like cytokines (1). So, what else is going on? The above mentioned models explain mainly interstitial edema; what about the alveolar side?

Fluid balance in alveoli

Before evaluating other possibilities that might or might not explain HAPE processes involved in the clearance of fluid from the alveolar space have to be considered. Since one does not constantly suffer from alveolar flooding effective mechanisms of fluid removal have to be assumed. This was studied extensively on lungs in situ, on isolated, perfused lung models and on alveolar epithelial cells isolated from lung tissue that were kept in tissue culture (12;18). Beforehand, it has to be pointed out that a significant difference in fluid balance exists between the fetal and the adult lung: Whereas in the fetal lung fluid is actively secreted by distal lung epithelial cells causing the lung to be filled with fluid, the adult lung is “dry” because of fluid reabsorption (14).

In the in situ and isolated lung models from adult animals it was found that instilled fluid is readily reabsorbed. The site of reabsorption appeares to be the alveolar space. Addition of inhibitors indicate the significance of the Na+/K+ pump and of Na+ transporting cation channels in fluid reabsorption (12,14). Reabsorption could be stimulated by a variety of substances that were added either to the instillate or the perfusate. Among the most potent ones were epinephrine and its analogues acting via cyclic AMP (12).

From these studies as well as follow up experiments on isolated lung alveolar epithelial cells a model of fluid reabsorption was derived (12) that is similar to many other reabsorptive epithelia (fig.1): Na+/K+ pumps located in the basolateral plasma membrane at the endothelial-alveolar interface of alveolar epithelial cells pump Na+ out of the cell to keep the intracellular Na+ concentration low. Due to the high extracellular Na+, pumping generated a concentration gradient facing into the cell, which allows Na+ to enter via various pathways located in the plasma membrane of the apical side of the cell, i.e. the side facing toward the alveolar space. Those entry pathways include Na+ permeable ion channels and secondary active systems transporting different substances coupled to the transport of Na+ (12). Any Na+ that

entered the cell will again be pumped out by the Na+/K+ pump. This creates a stream of Na+ as well as an electrical potential (apical side negative) across the epithelium that allows chloride to follow. The reabsorption of NaCl generates the osmotic gradient that pulls water from the alveolar to the interstitial side. The pathways that mediate the movement of Cl- and water are not very well understood.

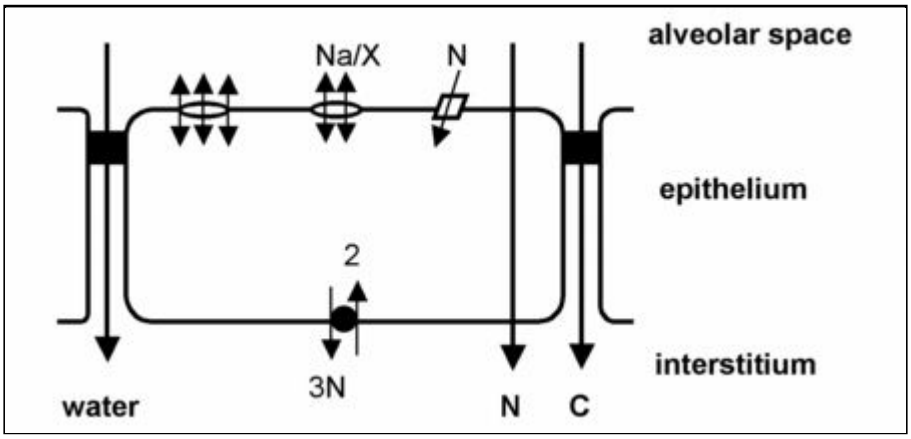

Figure 1. Model of NaCl and water reabsorption across the alveolar epithelium. Na+/K+/2Cl- ... Na+/K+/2Cl- cotransport; Na+/X ... Na+ coupled cotransport; Na+ ... Na+ permeable cation channels; 3Na+/2K+ ... Na+/K+ pump.

It is not clear yet which of the cells of the alveolar barrier mediate transepithelial Na+ and water movements. Alveolar type I cells cover most of the alveolar surface and would therefore be ideal candidates. However, barely any Na+/K+ pumps have been found there. Alveolar type II cells cover only a small portion of the alveolar surface. Their major functions appear to be secretion of surfactant and repair of damaged alveolar epithelium. When isolated for primary culture these cells also perform active Na+ transport from the apical to the basolateral cell surface that can be measured as electric current in Ussing chambers (5). During in vitro maturation alveolar type II cells differentiate to cells similar in structure and function to type I cells but still show active transport (4). In both cell types in culture the reabsorption of Na+ can be inhibited by amiloride, a blocker of epithelial Na+ channels (ENaC) and of non-selective, Na+ and K+ permeable cation channels (11). Of course, transport is also blocked by inhibiting the Na+/K+ pump. Other transporters like Na+/K+/2Cl- cotransport, Na+/H+ exchange, Na+/glucose-, Na+/phosphate- and Na+/amino acid-cotransport seem to play only minor roles although exhibiting quite some activity (11).

A variety of substances was shown to modulate Na+ transport in alveolar epithelial cells. b-adrenergic agonists stimulate Na+ reabsorption by activating apical Na+ entry mediated by the intracellular messenger adenosine 3',5'-cyclic monophosphate (cAMP) (12). Terbutaline effects on isolated lungs as well as isolated alveolar type II cells were blocked by propranolol indicating specific mechanisms (18). Cyclic AMP acts directly by affecting protein kinases or via calcium dependent mechanisms (Cai). An increased Cai was shown to activate cation transport of A549 cells (9). Non-catecholamine-dependent stimulators of alveolar Na+ and water reabsorption include various growth factors like the epidermal growth factor, transforming growth factor and keratinocyte growth factor (6;12), which are thought to be involved in epithelial repair.

Alveolar fluid reabsorption in hypoxia

Recently, several studies documented that hypoxia might impair alveolar fluid transport. Planes et al. (17) reported an inhibition of the Na+/K+ pump in a cell line derived from rat alveolar epithelial cells after SV-40 virus transformation. We found that in primary cultured rat alveolar type II cells and a human lung carcinoma cell line (A549) with functions similar to type II cells hypoxia not only inhibited the Na+/K+ pump, but also Na+ entry via amiloride sensitive pathways and Na+/K+/2Cl- cotransport (10). Planes et al. (16), reported a downregulation of the expression of ENaC and the Na+/K+ pump in primary cultured rat alveolar epithelial cells by hypoxia. Another very interesting result comes from Pitkänen et al. (15), who works on the transition of fetal to adult alveolar type II cells. These authors reported that fetal type II cells kept in tissue culture at about 3% O2 estimated to match the value in the fetal lung exhibit a low capacity for transepithelial Na+ reabsorption, whereas exposure to 21% O2 activates Na+ reabsorption considerably (15). They argue that the increase in PO2 might be significant for drying the lung after birth (15).

The mechanisms that cause the hypoxic inhibition of alveolar Na+ transport are not understood. Based on available results inhibition occurs in an initial phase by inhibition of transport probably by inactivation and/or internalization of transporters, in a second phase by inhibition of protein synthesis (10). Planes et al. (17) reported an increased Ca++-entry in hypoxia and that nifedipine, the drug used successfully to prevent HAPE (2), also prevented inhibition of the Na+/K+ pump. However, in primary cultured rat alveolar type II cells and A549 cells this effect of nifedipine could not be confirmed (10). It is by now not known to what extent mechanisms involved in oxygen sensing by e.g. pulmonary artery smooth muscles (20) or erythropoietin-producing cells (19) also are effective in lung alveolar epithelium.

Significance of Na+ transport for fluid reabsorption and HAPE

Reabsorption of fluid from the alveolar space is of vital significance (3). In the perinatal lung, when the function of the alveolar epithelium switches from secretory to reabsorption, the above described transport systems mediate the removal of fluid contained in the lungs (14). This process is stimulated by increased levels of stress hormones liberated during birth (14). The significance of Na+ reabsorption via ENaC has recently been demonstrated impressively. Knockout mice with a deletion of the gene encoding for the a-subunit of ENaC die within about 40 hours after delivery since reabsorption of alveolar fluid is entirely absent (8). Gene recovery results in heterocygotes with a decreased amount of ENaC protein in alveolar epithelial cells. However, this seems sufficient for adequate fluid reabsorption from the lungs and survival of these mice.

In the adult lung reabsorption has to prevent alveolar flooding by removal of accumulated fluid, which is of importance, since the barrier for gas diffusion has to be kept as thin as possible to allow proper exchange of respiratory gases. The role of transport in clearing alveolar edema particularly of HAPE is unclear. Studies on fluid reabsorption of the intact human have been performed (13) but have not yet been applied to HAPE. It is difficult to consider what might be going on: If alveolar ion transport is inhibited by hypoxia in vivo, a diminished rate of fluid reabsorption has

to be expected, which might cause alveolar edema. This, however, should occur in HAPE-susceptibles and non-susceptibles as well. Alveolar epithelial cells of HAPE-susceptibles might be exposed to an increased degree of hypoxia because of their blunted HVR and widened alveolar-to-arterial oxygen difference(1), which might augment the inhibition of transport (10). On the other hand, increased levels of catecholamines were reported in individuals suffering from HAPE (7). Since catecholamines potently activate fluid reabsorption in the lung, increased levels might to some extent compensate for the decreased basal transport activity. b-adrenergic agents have been shown to be effective in acute pulmonary edema (3). However, it has not yet been demonstrated that these agents can be used to prevent or treat HAPE.

The results on ion transport across the alveolar epithelium indicate clearly the important role in fluid clearance from the alveolar space. However, it is impossible to state the significance of this mechanisms in maintaining the fluid balance in the lung in hypoxia relative to the effects of pulmonary hypertension and to changes in permeability. It can be assumed that the “liquid pump” (3) described above is important as long as the epithelium is intact, but that it looses its significance with increasing leakiness.

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