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Exocytosis of Lung Surfactants

A surfactant, by definition, is any molecule that is attracted to an interfacial region by nature of its structure (Notter 2000). Naturally then, the surface of the interior of the lungs has surfactants, termed lung surfactants, because of its interfacial properties (Notter 2000). On the surface of the lungs, there exists a constant interfacial surface tension between the liquid and gas phases. The lungs are the place where respiration and thus gas exchange occurs. More specifically, gas exchange occurs at the surface of the alveoli in the lungs (Moyes 2008). The alveoli are small hollow sacs that extend from the bronchiole of the lungs and in total there are millions of them within the lungs (Moyes 2008). Due to their size and unique shape, alveoli's primary function is to extend the surface area of the lungs in order to facilitate gas exchange with the external environment (Moyes 2008).

There are two types of cells that make up the alveoli. Type I alveolar cells make up most of the alveoli, have very thin walls, and are responsible for gas exchange (Moyes 2008). Within each alveolus, there exist fewer of the type II alveolar cells. These type II alveolar cells are responsible for secreting the components of the lung surfactant (Moyes 2008). The presence of the surfactant molecules at the interfacial region disrupts the cohesive forces between the water molecules in the alveoli fluid. This reduces surface tension at each alveolus (Moyes 2008). By reducing the surface tension at the alveolus, this increases the compliance of the lung. Lung compliance is a term used to describe how easily the lungs stretch during inhalation (Moyes 2008). Reducing the surface tension thus allows the hollow sac of the alveoli to expand more readily, using less energy to overcome the surface tension barrier (Notter 2000). The magnitude by which surface tension is lowered by the presence of surfactants depends on a variety of factors. One example of an important factor is surfactant concentration; generally, the more surfactant present, the stronger its ability is to lower surface tension and increase lung compliancy (Notter 2000). Some other examples of factors which affect the magnitude by which surface tension can be lowered by surfactants are temperature changes, rate of surface film compression, and the type of surfactant molecules present in the surfactant film (Notter 2000).

Lung surfactant, sometimes referred to as pulmonary surfactant, consists of a mixture of 85-90 percent phospholipids, 6-8 percent proteins, and 4-7 neutral lipids (Notter 2000). Of the phospholipid portion of surfactant, Dipalmitoylphosphatidylcholine (DPPC) is the major contributor and accounts for approximately 85 percent of the total phospholipid material (Notter 2000). The phospholipid portion of surfactant material is secreted via specialized vesicles called lamellar bodies. On the other hand, the protein portion of the surfactant can be secreted independently of these lamellar bodies or in conjunction with lamellar bodies (Mason 1998).

Of the protein portion of the surfactant secretion, protein A is the major contributor and accounts for 50 percent of total protein content. This protein has actually been shown to inhibit secretion of lamellar bodies and thus it serves as a regulatory mechanism for the control of surfactant secretion (Dietl 2000). Smaller protein contributors include protein B, which accounts for 8 percent of surfactant protein, protein C, which accounts for 4 percent, and protein D, which accounts for around 1 percent (Notter 2000). Proteins B and C are small hydrophobic molecules which are localized within lamellar bodies and are thus co-secreted with lamellar bodies'

contents (Deitl 2000). In contrast, proteins A and D are large hydrophilic molecules whose secretion is generally independent of lamellar bodies (Dietl 2000).

The excretion of lung surfactant from type II alveolar cells to the alveolar interfacial region is termed “exocytosis of lung surfactant” and regulation of this process is extremely important for maintaining normal and efficient respiratory function. The overall module that has been proposed for surfactant secretion involves multiple steps, many of which can be regulated individually by a variety of different factors. The first step in surfactant secretion is the synthesis and packaging of the surfactant molecules in the endoplasmic reticulum and the golgi body respectively (Weaver 2002). As discussed previously, most surfactant molecules are packaged into vesicles called lamellar bodies; however in contrast, others are packaged into multivesicular bodies which are thought to eventually fuse with lamellar bodies at some point before surfactant secretion (Dietl, 2000 and Weaver, 2002). Lamellar bodies (LB's) are specialized secretory granules that function in transporting the surfactant molecules from the area of synthesis outwards towards the apical plasma membrane of the cell where the contents will eventually be released via LB fusion (Mason 1998). These lamellar bodies are actually lysosome-related organelles containing lysosomal enzymes and an acidic pH as a result of an H^+-ATP-ase (Weaver 2003). Not only do lamellar bodies transport surfactant, but they also function in storing surfactant until the cell has been adequately stimulated. The second step in surfactant secretion thus is the stimulation of the type II alveolar cells.

Overall there are two main ways in which type II alveolar cells can be stimulated; by hyperventilation and by binding of agonists to receptor proteins (Mason 1998). There are several proposed mechanisms by which stimulation of exocytosis of lung surfactants via hyperventilation can occur. One likely mechanism that has been proposed is that physical stretch of the alveolar type II cells in the lung alveoli during tidal ventilation generates a transient increase in the cytoplasmic calcium concentrations which then stimulates secretion (Mason 1998). Another possible mechanism is that perhaps surrounding lung cells, such as the type I alveolar cells are actually responsible for detecting the mechano-signal generated during tidal ventilation. Once these cells are stimulated by the hyperventilation, they may release a mediator, such as arachidonate metabolites, which can either travel via gap-junctions or diffuse through extracellular fluid. When the mediator binds to a receptor on the type II alveolar cell this then serves as the stimulation for secretion (Mason 1998). Other possible mechanisms include the reduction of P_{C02} during hyperventilation causing production of intracellular alkalosis or physical stretch of cells causing mechano-gated ion channels to open thereby causing membrane depolarization (Mason 1998). It has also been proven that even a deep inhalation can cause increased surfactant secretion via the same mechanisms as hyperventilation (Mason 1998).

Although agonists play an important role in stimulation of type II alveolar cells to release surfactants, it is thought that their effects are modest in comparison to the stimulation produced by hyperventilation (Mason 1998). An agonist is a molecule that binds to a receptor of a cell and triggers a response (Moyes 2008). Often times, agonists are drugs or other chemicals that mimic the shape and function of the true molecule that is responsible for stimulation of that specific receptor (Moyes 2008). In pulmonary type II cells, agonists are classified by two main types; those that cause a large impact on cell (increase of 4x normal secretion level) and those that have minimal impact on a cell (increase of <2x normal secretion level). Examples of agonists that cause a large impact on surfactant secretion rates are TPA, ATP, and other puregenic receptors (Mason, 1998 and Dietl, 2004). Examples of other agonists that cause a change in secretion rates include β_2-mimetic drugs, gastrin releasing peptide, prostaglandin debris, vasopresin,

endothelium-1, cholera toxin, and calcium ioniophase (Mason, 1998 and Dietl, 2000). It has been found that those agonists that have the largest effect on secretion generally act via a mechanism utilizing protein kinase C; however, details of this mechanism, while heavily researched, are still unclear (Mason 1998).

The complete mechanism by which many typical pharmaceutical agonists stimulate exocytosis of lung surfactant is a multi-step pathway. First, the agonist binds to the receptor on the cell surface. Second, this receptor-agonist complex then associates with heterotrimeric G-proteins to activate phospholipase C. Phospholipase C then cleaves phosphatidylinositol bisphosphate to form diacylglycerol and IP_3. Diacyglycerol then activates protein kinase C and meanwhile IP_3 diffuses to the endoplasmic reticulum where it stimulates the release of calcium stores within this organelle. At this same time, channels on the extracellular matrix of the cell are stimulated to open up and more calcium is brought into the cell from the external environment. This series of events ultimately causes intracellular calcium concentrations to rise (Mason 1998).

Much research suggests that this rise in intracellular calcium levels is most likely the defining step in regulated exocytosis of lung surfactant in type II alveolar cells (Dietl 2000). This rise in Ca^{2+} levels is generally a key step no matter what the original mode of stimulation was: mechanosensors during hyperventilation and deep inhalations or chemoreceptors that respond to agonists. While scientists have suggested a few exceptions that involve cAMP acting as the primary second messenger rather than Ca^{2+} , these theories still remain unclear and difficult to prove (Dietl 2000). It is difficult to tell when cAMP acts, whether it is acting independently of Ca^{2+} or whether it acting in conjunction with Ca^{2+} (Dietl 2000)

Once Ca^{2+} levels have increased to a sufficient concentration, the pre-fusion phase of exocytosis begins. The pre-fusion phase of exocytosis is defined by three key steps; the stimulation of vesicles to move, the movement of vesicles through cytoplasm, and the docking of vesicles at docking proteins along the apical plasma membrane (Dietl 2000). When Ca^{2+} levels within the cell rise, this stimulates the LB's located throughout the cytoplasm to move towards the plasma membrane. The effect of Ca^{2+} stimulation on the relocation of LB's from inside of the cell to the plasma membrane of the cell is varied. A variety of factors influence the rate at which these LB's move towards the plasma membrane. For example, the spatial-temporal spreading of the Ca^{2+} signal and the size and location of the vesicle have effects on the rate at which the LB moves (Dietl 2000). The two most defining factors that are responsible for determining the movement of the vesicle are the duration of Ca^{2+} signal and the distance of the vesicle from the plasma membrane (Dietl 2000). Studies have shown that the further away a vesicle is from the Ca^{2+} signal or the shorter the Ca^{2+} signal is, the likelihood that the LB will move towards the plasma membrane and eventually fuse is reduced (Dietl 2000). The reason for this is because most of the barriers that the LB must pass before it reaches the cell membrane are Ca^{2+} - dependent (Dietl, 2000 and Signh 2004).

In order for the LB's to move through the cell and out to the apical plasma membrane a series of interconnected events must occur, of which many are Ca^{2+} concentration dependent. Again, the first step is the stimulation of the vesicles to move from their resting location. This stimulation comes from the increased Ca^{2+} levels that develop within the cell once it has had the proper stimulation (Taran 2003). Then, in order for the vesicles to move through the cytosplasm, rearrangement of the cytoskeleton, that is acting as a barrier surrounding the vesicles, must occur (Taran 2003). This is an extremely important regulatory step in the process of exocytosis of lung surfactants and thus serves as another regulatory mechanism for controlling surfactant secretion.

The cytoskeleton has many important functions in regards to surfactant secretion. It

serves as a barrier to regulate the release of secretory vesicles, a track for motor-mediated translocation of secretory vesicles, and a buffer surrounding the internal storage pool of secretory vesicles within the cell (Signh 2004). When the increased Ca^{2+} levels reach the F-actin strands surrounding the vesicles, the strands begin to decay which allows the vesicles to be released from storage. The exact reason why the F-actin strands begin to dissemble is not completely known. Some studies suggest that when Ca^{2+} levels reach a peak, this triggers exogenous annexin II to bind to specific segments of the F-actin strand, thereby disrupting its structure and causing it to partially disassemble (Signh 2004). Also, some studies have discussed the possibility of the existence of a second unique form of annexin II. This other form of annexin II is thought to act via a cAMP-dependent pathway rather than a Ca^{2+} -dependent pathway. This second form of annexin II is thought to already be bound to F-actin chains when the stimulus signal occurs (Signh 2004). When the alveolar cell is stimulated by some types of agonists, such as the β_2-mimetic drugs, this eventually results in a rise of cAMP and eventual activation of protein kinase A, which then phosphorylates the annexin II that is bound to the cytoskeleton (Signh 2004). Upon phosphorylation, the annexin II dissociates from the cytoskeleton, dismisses its F-actin binding activity, and therefore induces cytoskelton reorganization (Signh 2004).

Research also suggests one other possible mechanism that may explain the reorganization of the cytoskeleton during exocytosis of lung surfactants. This proposed mechanism involves Ca^{2+} -dependent actin-severing proteins. When intracellular Ca^{2+} levels rise within the cell, this activates the actin-severing proteins and as a result they bind to F-actin chains causing disruption of actin cross-linking (Signh 2004). Two examples of actin severing proteins are gelsolin and villin (Signh 2004). Research has proven also that at low Ca^{2+} concentrations, these two proteins actually assist with actin cross-linking thereby further supporting the conclusion that cytoskeltal reorganization during exocytosis of lung surfactants is highly Ca^{2+} dependent (Signh 2004). This research also supports the conclusion that as intracellular Ca^{2+} levels rise, this will eventually result in increased stimulation of surfactant secretion (Signh 2004

Studies suggest that with this reorganization of cytoskeleton, there is a change in shape of the alveolar type II cells (Dudek 2003). This change in shape suggests a contractile mechanism for the movement of LB's in which the LB's are propelled outwards towards the apical plasma membrane (Dietl 2000). This rearrangement of cytoskeleton thus directs the vesicles along certain pathways towards the membrane (Dudek 2001)). Once the vesicles reach and attach to the docking proteins on the apical plasma membrane, the LB fusion stage begins.

The LB itself has a lipid bilayer that surrounds the lumen. When this comes into contact with the phospholipid bilayer of the plasma membrane, the two membranes begin to fuse together (Haller 2001). The first step in LB fusion is the formation of a small fusion pore. This fusion pore is an aqueous channel between the vesicle lumen and the extracellular space (Haller 2000). In the early stages for most fusions, the fusion pore is unstable and thus will generally fluctuate for several rounds between an open or closed state (Haller 2001). However, this is not the case for LB fusion within type II alveolar cells (Dietl 2000). Morphological and functional evidence suggest that LB fusion pores in type II alveolar cells are actually very stable and long lasting structures which allow for the slow and continual release of surfactants (Dietl 2000).

The difference in LB fusion in alveolar type II cells from other types of vesicle fusions is a result of the high lipid content within the LB (Dietl 2000). LB's of the alveolar type II cells carry large amount of phospholipids, such as DPPC, and when the aqueous fusion pore forms, water is not attracted to the hydrophobic contents of the LB (Dietl 2000). In other fusion pores, where the contents are hydrophilic, water rushes into the vesicle causing it to swell and rapidly

expand the size of the fusion pore allowing for a quick release of the its contents (Dietl, 2000 and Haller, 2001). However, due to the hydrophobic nature of the LB contents, it suggests that another mechanism for fusion pore expansion must be directing this process. That mechanism is most likely Ca^{2+} dependent, as are most other regulatory mechanisms controlling the exocytosis of lung surfactants. Studies show that any increase in intracellular [Ca^{2+}] causes acceleration of fusion pore expansion (Dietl 2000). Studies also show though that fusion pore expansion rate could also be a result of mechanical strain (Dietl 2000). It is not clear yet whether the mechanical strain acts directly on the fusion pore via cytoskeletal rearrangement of monomeric actin or if it acts indirectly via a resulting increase in [Ca^{2+}] within the cell (Dietl 2000).

Once the LB fusion pore is formed, it will generally stay open and the LB will maintain its rigid shape until all of the contents have been expelled (Dietl 2000). However, sometimes a transient fusion results where the fusion pore closes up before all of the surfactant has been released. It is unclear whether the transient fusions result in decreased surfactant secretion or whether they result in simply a modification of surfactant secretion (Dietl 2000). It is possible that during this transient fusion, significant amounts of small and unbound LB constituents are released into the alveolar lining fluid (ALF) at this time (Dietl 2000). Also, there is the possibility that transient fusions may result in the backwards uptake and recycling of surfactant components (Dietl 2000).

Another unique event that sometimes occurs after LB fusion with the plasma membrane, is LB-LB fusion termed compound exocytosis (Dietl 2000). In this situation, a LB fuses with another LB and the contents of both vesicles are secreted via a single fusion pore. It is thought that this unique event is perhaps a method for focusing release of high levels of surfactant in a localized area where there are not enough docking proteins to support all of the LB's that have been stimulated to move towards the plasma membrane (Dietl 2000). Also, it has been suggested that compound exocytosis may actually be a mechanism that allows for slow and sustained release of surfactants to a specific area (Dietl 2000).

Over the time span of a few minutes to a half hour, LB's will continue fusing with the plasma membrane even after the [Ca^{2+}] has begin to decline (Dietl 2000). Once the surfactant molecules reach the ALF, they quickly arrange themselves into a variety of structures including lamellar body-like particles (LBP's), tubular myelin (TM), a lipid-protein film, small vesicles(SV's) and others (Dietl, 2000 and Wright, 1991). These structures make up what is called the surfactant film (Wright, 1991). The specific arrangement of the surfactant film in the ALF greatly reduces surface tension in the lungs and helps to prevent alveolar collapse (Dietl 2000). This surface film however is not a stagnant film and its contents are continually being reabsorbed, recycled, degraded, and rearranged as often as with each inhalation (Notter, 2000 and Wright, 1991). For example, it is estimated that the pool of surfactant lipids present within the ALF has a turn-over rate of approximately 5-10 hours (Wright 1991). The surfactant film is constantly changing in order to meet the demands of the organism. For example, during exercise when the alveoli are processing an increased amount of air at an increased rate, the surfactant pool can increase by nearly 60 percent and then return back to normal levels when exercise is completed (Wright 1991).

As there are a variety of factors that stimulate rates of surfactant secretion, there are also as many factors stimulating rates of surfactant clearance. It is the precise balance between these two antagonistic processes that maintains the proper amount and the proper type of surfactant molecules present within the AFL and thus maintains efficient respiratory function.

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