Spatio-temporal aspects, pathways and actions of Ca^{2+} in surfactant secreting pulmonary alveolar type II pneumocytes

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SUMMARY

The type II cell of the pulmonary alveolus is a polarized epithelial cell that secretes surfactant into the alveolar space by regulated exocytosis of lamellar bodies (LBs). This process consists of multiple sequential steps and is correlated to elevations of the cytoplasmic Ca^{2+} concentration ($[Ca^{2+}]_c$) required for extended periods of secretory activity. Both chemical (purinergic) and mechanical (cell stretch or exposure to an air–liquid interface) stimuli give rise to complex Ca^{2+} signals (such as Ca^{2+} peaks, spikes and plateaus) that differ in shape, origin and spatio-temporal behavior. This review summarizes current knowledge about Ca^{2+} channels, including vesicular P2X4 purinoceptors, in type II cells and associated signaling cascades within the alveolar microenvironment, and relates stimulus-dependent activation of these pathways with distinct stages of surfactant secretion, including pre- and postfusion stages of LB exocytosis.

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1. Introduction

The pulmonary alveolus is the terminal structure of the airways where gas exchange between air and blood takes place. About 480 million alveoli in the human lung establish a surface area of about 130 m^2 [1], providing ample capacity for the diffusional exchange of O_2 and CO_2. The barrier between air and blood is composed of just 2 thin cell layers with a variable thickness averaging 1.3 μm, consisting of either type I or type II epithelial cells (also termed pneumocytes) on the luminal side, and endothelial cells on the blood side [1].

Each alveolus is a small, nearly spherical structure with a diameter of about 200 μm. It is generally believed that the epithelial cells are covered by a thin lining fluid (also termed hypophase). Its average thickness was estimated 0.2 μm by low temperature electron microscopy (EM) [2]. Due to surface tension at a curved air–liquid interface (ALI), alveoli are considered to be intrinsically unstable structures prone to collapse. It is the main purpose of surfactant [3], a complex material consisting primarily of phospholipids and 4 different surfactant proteins (SP-A to SP-D), to reduce this

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surface tension in a surface area-dependent way by forming a dynamic interfacial film at the ALI (recently reviewed in [4]). The continuous presence of surfactant is crucial for lung mechanics and survival, its deficiency causes respiratory failure, most impressively manifested as infant respiratory distress syndrome (IRDS) of newborns with immature lungs [5].

Surfactant is produced by type II pneumocytes and stored in characteristic organelles termed lamellar bodies (LBs). LBs contain surfactant as concentric, extremely compressed stacks of lipid bilayers (=lamellae) visualized by EM [6]. To continuously provide the ALI with surfactant and prevent surfactant deficiency, type II cells must cope with increased demands by regulated exocytosis of LBs. Surfactant turnover was intensively investigated in the 70 s and 80 s using morphological and biochemical techniques including incorporation and release of radioisotopes of choline or other surfactant components. These studies indicated that the major fraction of secreted surfactant is recycled by endocytosis and other mechanisms, only a small portion is continuously lost by mucociliary clearance (reviewed in detail in [7]). The turnover of surfactant is slow (hours)[7], but accelerated under conditions of physical (e.g. swimming) exercise [8]. Importantly, when lung lavages were analyzed for the amount of surfactant phospholipids shortly after a single deep breath, this was found to be increased, consistent with the idea that a sigh or yawn is sufficient to rapidly translocate a certain amount of surfactant into the alveolar space [9,10]. Moreover, ventilation with enhanced tidal volumes decreased the cellular density of LBs, corroborating the idea of regulated exocytosis of LBs from type II cells [11].

2. Stimuli of surfactant secretion in the isolated type II cell

With the development of techniques enabling the isolation of type II cells from rat lungs and preparation of primary cultures [12], agonists and cellular/molecular mechanisms that modulate surfactant secretion on the level of tissue cultures and single cells have been intensively investigated. In summary, a variety of para- or endocrine mediators including nucleotides, catecholamines and eicosanoids, or physico-chemical factors such as cell stretch, CO_2 or pH, may stimulate surfactant secretion through intracellular signaling pathways such as protein kinases A, C and Ca^{2+}/calmodulin-dependent kinase (reviewed in detail in [7,13–19]). SP-A, a surfactant protein which is secreted from type II cells involving routes independent from LB exocytosis, inhibits surfactant secretion probably by a receptor P63/CKAP4-mediated process [20,21]. To this end, the origin and/or (patho)physiological significance of many of these factors is not entirely clear. It appears that surfactant secretion is such a basic prerequisite for survival that a redundancy of mechanisms keeps the supply of surfactant at a sufficient rate. One possible explanation is that mechanosensitive – and hence evolutionary most ancient and conserved – responses sustain vesicle fusion with the plasma membrane by a simple chain of reactions that are largely independent of receptor-mediated trans-membrane signaling (see below). In fact, as noted above, a single deep breath is sufficient to stimulate surfactant secretion in intact lungs, and these findings were corroborated in isolated type II pneumocytes, where a single stretch of cells grown on elastic membranes elicited LB exocytosis [22,23]. Although there is still some ambiguity from morphological data about the extent of lung inflation that finally leads to stretch type II pneumocytes in vivo [24,25], the alveolar volume changes during normal breathing is estimated to cause a 4% linear distension of the basement membrane [25]. About 10% equi-biaxial tensile strain was found to be the threshold mechanical stimulus in isolated type II cells grown on elastic supports that causes an elevation of $[Ca^{2+}]_c$ and surfactant secretion [22,23].

3. The role of Ca^{2+} for surfactant secretion

An elevation of the $[Ca^{2+}]_c$ was early recognized as a potent stimulus of surfactant secretion [26], and this is consistent with the well-known roles of Ca^{2+} in regulated exocytosis in general. Several proteins associated with SNARE-mediated membrane merger and fusion, hallmarks of regulated (Ca^{2+}-induced) exocytosis, have been identified in type II cells so far and were associated with surfactant secretion [27–30]. However, the threshold Ca^{2+} concentration required to induce LB fusion with the plasma membrane was established to be about 320 nmol/l, a value far lower than for SNARE-mediated fusion in neurons [31–33]. Moreover, Ca^{2+}-induced fusion of LBs with the plasma membrane is a function of the integrated $[Ca^{2+}]_c$ value over time (=dose) rather than a function of the $[Ca^{2+}]_c$ peak itself, at least when purinergic stimulation is considered [34]. Patch clamp experiments revealed that type II cells lack a readily releasable pool of LBs, consistent with the morphology of the type II cell showing LBs distributed throughout the cell but not clustered at a distinct zone near the apical membrane [35]. The slow, protracted action of Ca^{2+} to trigger LB fusion in a sequential way suggests a process associated with LB transport to sites of fusion, involving molecular motors and cytoskeletal elements. This notion is supported by many studies demonstrating that disruption of actin or other cytoskeletal elements severely affect surfactant secretion, though in various ways [36–41].

4. Phases, shapes and origin of Ca^{2+} signals in type II cells

Based on the shape of temporal $[Ca^{2+}]_c$ changes in the type II pneumocyte, a Ca^{2+} "peak" and a "plateau" phase can be distinguished, which may be superimposed by Ca^{2+} "spikes". These patterns depend on the amount of Ca^{2+} in the extracellular space and the Ca^{2+} load in intracellular stores [42], both of which can be experimentally modulated (e.g. zero bath Ca^{2+} concentration, thapsigargin). Hence, shapes and stages depend on two mechanisms, Ca^{2+} entry and Ca^{2+} release from stores, respectively.

Plasma membrane receptors coupled to inositide hydrolysis and generation of inositol 1,4,5-trisphosphate (IP$_3$), such as the purinergic P2Y2 receptor expressed in type II cells [43–45], induce a transient Ca^{2+} peak [46]. In the absence of external Ca^{2+}, this short-lasting Ca^{2+} signal (tens of seconds up to minutes, depending on the activity of Ca^{2+} extrusion mechanisms) is followed by a moderate exocytotic response, similar to that induced by flash photolysis of caged Ca^{2+} [31,34]. When Ca^{2+} is present in the extracellular solution, the peak is followed by a sustained elevation of $[Ca^{2+}]_c$, variable in duration as well as amplitude. We denote it as "plateau" in accordance with the generally used nomenclature [47], although it resembles a slow decay of $[Ca^{2+}]_c$ rather than a stable, steady-state plateau [34,48]. The strong dependence of this phase on extracellular Ca^{2+} is generally considered as a proof of Ca^{2+} entry into the cytoplasm. Since Ca^{2+} store depletion by thapsigargin elicits a similar Ca^{2+} plateau, we refer to it as "store-operated Ca^{2+} entry" (SOCE, see also below).

When ATP is used as the stimulating agonist, the plateau is superimposed by "spikes", transient elevations of $[Ca^{2+}]_c$ - characterized by a rapid (ms range) upward deflection followed by a slower decay [49]. Like the plateau these spikes are strictly dependent on the presence of extracellular Ca^{2+}. They are, however, always preceded by single LB fusion events with the plasma membrane. For these reasons, the Ca^{2+} entry mechanism was named "fusion-activated Ca^{2+} entry" (FACE) [49]. In contrast to Ca^{2+} peak and plateau, which are global Ca^{2+} signals, spikes originate from fused LBs and remain localized, that means restricted to cytoplasmic sites around single fused LBs, although they tend to spread through the cytoplasm. This spreading behavior, probably