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Pulmonary Consequences of a Deep Breath Revisited

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Key Words

Pulmonary alveoli · Pulmonary surfactants · Lamellar body · Exocytosis · Pulmonary stretch · Mechanotransduction · Membrane fusion

Abstract

About two decades ago, a model was proposed for surfactant release by lung distension. This model implies rapid fusion of lamellar bodies (LBs) with the plasma membrane followed by quick release of surfactant into the alveolus, as reflected by immediate facilitation of lung inflation after a single deep breath. Recent experimental evidence indicates that this two-pool model (intracellular versus alveolar surfactant pool) has to be refined by introducing a third pool, which resides in fused but non-released LBs. Here we discuss the implication of this additional pool for strain-induced surfactant secretion and propose a revised model for the sequence of events following a single deep breath.

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Introduction

From the first breath on, alveolar surface tension is a key determinant of respiration mechanics and is maintained at low values by a sufficient amount of surfactant [1]. Deficiency at birth causes respiratory failure as part of a syndrome elicited by insufficient surfactant biosynthesis (respiratory distress syndrome, RDS) [2]. Later, surfactant dysfunction may accompany or induce various abnormalities of pulmonary function.

Surfactant is produced by and secreted from alveolar type II cells, where it is stored in intracellular vesicles termed lamellar bodies (LBs) [3, 4]. Analysis from alveolar lavage fluid reveals a complex mixture of lipids, mainly dipalmitoylphosphatidylcholine, and specific surfactant proteins (SP-A, SP-B, SP-C and SP-D). Details of the molecular composition of surfactant and its turnover and possible function of surfactant components have been reviewed [5–9]. As for the context of this review, it should be considered that essentially all phospholipids secreted into the alveolus originate from LBs. For that reason, we shall confine our definition of surfactant secretion to the secretion of LBs, and not discuss secretory pathways for other surfactant components, such as SP-A.

The Secretory Path of Surfactant

Following the first morphological description that LBs are secreted by exocytosis [10], this process has been confirmed by many studies with transmission and scanning electron microscopy, and recently also with the patch clamp technique [11]. LBs are large organelles (diameter

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~ 1 μm) densely packed with circular layers of lipid membranes. Upon fusion of the limiting LB membrane with the plasma membrane, electron microscopy (EM) revealed that the surfactant structure may be largely maintained during and after the release from type II cells, resulting in accumulation of spherical particles in the 'hypophase' of the tiny extracellular fluid volume in the alveolar spaces. Alternatively, conversion into a unique lattice-like structure ('tubular myelin', TM) may take place [12]. Following its first description by Policard et al. [13], the mechanisms of formation and possible roles of TM have been extensively studied. Because TM originates from secreted LB material, it is considered a precursor, though not an obligatory one, of the surfactant film at the air-liquid interface. It should be kept in mind, however, that a test of this hypothesis is inherently difficult because TM structure is amenable to high resolution microscopy techniques (EM and AFM) only.

Surfactant Secretion and Its Relation to Lung Distension

The exocytotic process underlying surfactant secretion is a regulated one. Depending on the species [14], it is controlled by a multitude of endogenous or exogenous compounds (neurotransmitters, hormones, auto-/paracrine mediators, toxins and drugs) and physical factors (temperature, pH and mechanical forces), which have been reviewed in detail [9, 15–21]. The need for a regulated versus a constitutive secretion is obvious, as each breathing cycle results in a compression-dependent degree of surfactant 'consumption', comprising everything within the surface film that is either 'squeezed out' and endocytosed by the type II cell (surfactant 'recycling'), or 'lost' into the upper airways. Although, under resting conditions, the loss of alveolar surface activity is quite slow, and the turnover time of alveolar surfactant quite long (several hours, reviewed in [9]), this may considerably change under conditions of enhanced surfactant consumption (e.g. during exercise or in pathological states of enhanced surfactant consumption).

In fact, several studies have confirmed that exercise and increased mechanical ventilation result in an increase of surfactant secretion [22–31]. Nicholas and Barr [29] showed that the tidal volume rather than the frequency accounted for this stimulatory effect and speculated that direct distortion of the type II cell might be a stimulus for secretion. The finding of distension-induced surfactant secretion renewed the interest in the physiological roles of 'yawns' and 'sighs'. Loss of surface activity would result in stiffening of the lung, the development of atelectatic areas and hypoxia [32]. This is thought to trigger a yawning reflex via stimulation of vagal afferent nerves, forcing a single deep breath. The distension of the lungs – and this is likely to include lung distension during a newborn's first breath – would be a stimulus for surfactant secretion and reverse the detrimental effects of surfactant depletion.

Experimental evidence in favor of this hypothesis was first provided by Thet et al. [33], who demonstrated that in the excised rat lung ventilated for 2 h at constant tidal volume, areas of atelectasis could be removed by a single deep breath. Wirtz and Dobbs [34] confirmed many years later that a single distension of type II cells grown on silastic membranes stimulates surfactant secretion. This unambiguously proved that type II cells can be stimulated by physical forces directly, independent of hormones, neurotransmitters or lung tissue interactions. It is difficult to estimate, however, at which volumes of lung inflation the type II cell is actually strained. The relation between alveolar surface and volume is complex and cannot be fitted by a simple geometrical function [35–38]. Nevertheless, morphometric analysis of the epithelial basement membrane in lungs fixed by vascular perfusion is consistent with the idea that a significant distension of the epithelium occurs at least with lung volumes exceeding the tidal volume [39]. Of course, these findings do not rule out an additional mechanism (for example, secretagogue release) for the distension-induced response in the lung, but they indicate that strain of type II cells may by itself be a sufficient secretory stimulus in vivo. It should be noted that most other (secretagogue-induced) types of stimulation are still of unknown physiological importance and will therefore not be considered in this context.

The Model under Scrutiny

In the isolated perfused lung, Nicholas et al. [27] reported an immediate drop of the ventilation pressure needed to maintain a constant tidal volume following a single deep breath. Accordingly, the rate of phospholipid accumulation in alveolar lavages increased rapidly for 2 min and then declined [29]. From these observations, the authors suggested that 'increased tidal volume may directly distort the alveolar type II cell; each cell reacts to its own threshold distortion by releasing a pool of surfactant in an all-or-none fashion' [29]. Their implicit perception was that of 'a certain pool of stored surfactant close to the plasma membrane', assuming a release process similar to

other secretory cell types, where a readily-releasable vesicle pool fuses with the plasma membrane upon stimulation, followed by an instant release of vesicle contents into the extracellular space. Recent evidence indicates that this assumption might be too reductionistic and that the concept of surfactant pools and secretory mechanisms requires rethinking.

The Exocytotic Process Observed with New Fluorescence Techniques

Conventional methods to measure surfactant secretion are based on chemical or scintigraphic analysis of phospholipid accumulation in the extracellular space, either alveolar lavage fluid (lung) or the cell supernatant (type II cell culture). What is actually measured is the transfer of LB contents from a 'fixed' (cell-associated) to a 'removable' (i.e. not cell-associated) compartment, yielding little information about the transfer process itself. Haller et al. [40] have recently developed an approach to solve this problem. By using fluorescent dyes with high affinity for LB contents (FM 1–43), it is not only possible to precisely measure the instant of vesicle fusion, but also to observe the release and further 'fate' (re-shaping) of contents from fused LBs. These and other experiments indicated the following general features of the exocytotic process:

- Although some single LB fusions may occur within periods as short as seconds after stimulation, other LBs fuse with the plasma membrane in a sequential rather than a synchroneous fashion. Thus, the overall fusion activity following stimulation is slow and takes several minutes [40, 41].
- Fused LBs remain in an 'arrested state' for various times, up to hours [42]. This means that surfactant is neither quickly released into the extracellular space nor does it noticeably change its shape. Instead, it remains embraced by the limiting LB membrane of that LB communicating with the extracellular fluid by an exocytotic fusion pore of varying size. These fusion pores are stable structures, expanding slowly and discontinuously [42]. Direct application of mechanical forces with laser tweezers revealed that they are indeed a mechanical barrier for the release of fused LB material [43].
- An elevation of the cytoplasmic Ca^{2+} concentration ($[Ca^{2+}]_c$) is not only a potent stimulus of LB fusion with the plasma membrane in response to various stimuli, but also accelerates the expansion of the fusion pore thereby improving the delivery of LB contents into the alveolar fluid [42].
- Once released into the extracellular space (i.e. the removable fraction), LB contents remain particulate and predominantly form spherical aggregates, which do not disperse in the extracellular fluid for days [44].
- These macromolecular surfactant aggregates instantaneously desintegrate when reaching the air-liquid interface, denoting a flow of material into the interface [44]. This process is dependent on the surface tension and is inhibited at values ≤ 50 mN/m [44].

Effects of Single Cell Strain

The alveolus consists of type I and type II epithelial cells. A clear-cut and general answer to the question which of these two cell types is more subject and sensitive to lung inflation still cannot be given. In an elegant in situ study, Ashino et al. [45] demonstrated that type II cell exocytosis was modulated by $[Ca^{2+}]_c$ waves originating from type I cells and concluded that type II cells may not be the only relevant mechanotransducers. On the other hand, single type II cells studied with a new strain device specifically developed for investigation with high-resolution imaging techniques revealed a very high sensitivity to strain [46]; LB fusion could be triggered when the cell surface area increased by 8% or more. However, this threshold amount of strain varied considerably from cell to cell. A similar value was reported by Wirtz and Dobbs [34], who measured the release of phospholipids into the extracellular space. Considering that increases of epithelial basement membrane surface areas >30% were reported for lung inflations to 100% total capacity [35, 39], these low thresholds for single cells support the notion that physical strain is indeed a direct stimulus for secretion in vivo.

An elevation of $[Ca^{2+}]_c$ is a prerequisite for strain-induced LB fusion with the plasma membrane, and both the $[Ca^{2+}]_c$ elevation and the LB fusion response are graded events with respect to the strain amplitude [46]. Furthermore, extracellular Ca^{2+} is an absolute requirement for this accumulation of Ca^{2+} ions in the cytosol, which suggests that a Ca^{2+} entry pathway may be an important, yet undefined, strain sensor in this cell [46]. In addition to Ca^{2+} entry, it is known that strain elicits Ca^{2+} release from intracellular stores [34], recently identified as a Ca^{2+}-induced Ca^{2+} release mechanism from the endoplasmatic reticulum, operating at higher strain amplitudes [46]. No evidence, however, could be found so far that other second messengers (protein kinase A, C, for example) in addition to Ca^{2+} play a role in strain-induced LB fusion [34, 46]. This is supported by the observation

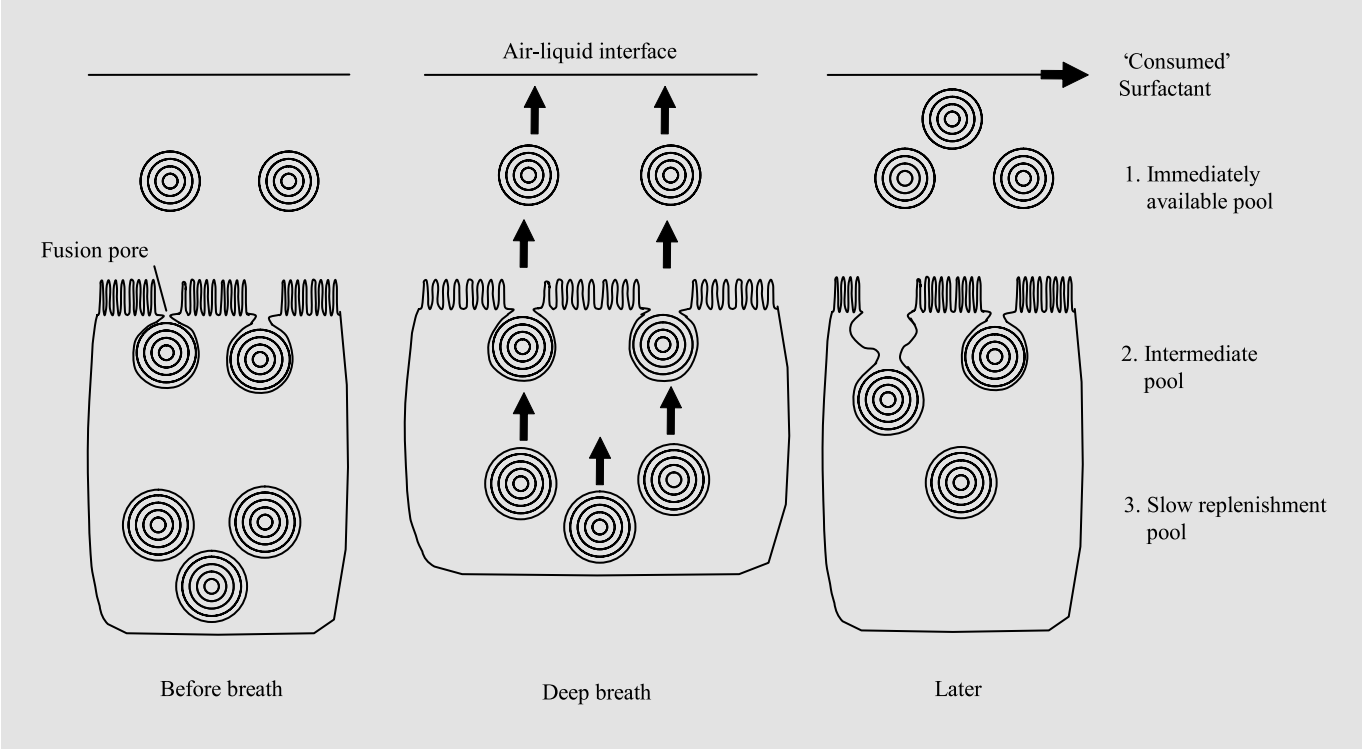

Fig. 1. Schematic illustration of surfactant pools and their secretory pathway following a deep breath. Arrows indicate directions of movement triggered by strain of a type II cell. The illustration neither considers the sequence and time courses of transition between pools (see text) nor does it represent real quantities of the various pools and compartments in relation to each other. The endocytotic pathway is also not considered in this image. See text for further information.

that LB fusion in response to flash photolysis of caged Ca^{2+} [47] (the most selective way to increase $[Ca^{2+}]_c$) is similar, with regard to both magnitude and time course, to a single strain.

The slow fusion response to strain in isolated type II cells is hardly compatible with the almost immediate effect of a deep breath on lung compliance as described by Nicholas et al. [27]. Even if the fusion response is more pronounced and efficient in vivo than in vitro, this does not explain how fused LB material could reach the air-liquid interface so quickly, given the 'arrested state' of fused LBs as described above.

The Revised Model

Based on these new findings, we argue that in addition to the alveolar and the intracellular surfactant pool, a third, yet neglected, pool has to be taken into consideration. The revised three-pool model is schematically shown in figure 1 and includes:

(1) The immediately available surfactant pool. This is the alveolar pool located closest to the site where it is needed (the air-liquid interface). The stability of LB material in an aqueous environment, and recent observations that this material is directly transformed at the air-liquid interface in a surface tension-dependent way, suggest that it is immediately recruitable during inspiration, when the alveolar surface tension increases [44]. Naturally, the fate of released LBs under conditions mimicking the microenvironment of a single alveolus is still elusive, including the roles of intermediate structures like TM or enzymatic processes, which are not considered here.

(2) The intermediate surfactant pool. This is the pool of surfactant stored in, but not released from, LBs fused with the plasma membrane. Singer et al. [43] 'grabbed' fused LB material with laser tweezers and tried to pull this macromolecular aggregate through the fusion pore. These

experiments did not only reveal that the material is coherent in aqueous solutions and exhibits viscoelastic properties, but also that the fusion pore is a mechanical barrier for release. Strain of type II cells apparently widens this barrier, as evidenced by a facilitated release of LB material from fused LBs into the bath solution [43]. This may be mediated by the strain-induced elevation of $[Ca^{2+}]_c$, which is, as noted above, a stimulus for fusion pore expansion [42]. The model implies that fusion pores – and not prefusion events – are the limiting factors for strain-induced surfactant release from alveolar type II cells.

(3) The slow replenishment pool. This pool consists of non-fused, intracellular LBs. It is the pool which reacts mosts slowly to strain and which replenishes the intermediate pool by fusion with the plasma membrane. It should be noted, however, that LBs do not fuse with the plasma membrane exclusively, but also with the limiting membrane of fused LBs, as schematically shown in figure 1 [11, 42]. Evidence for 'transient fusions', i.e. re-closures of fusion pores after fusion, has also been reported [42]. Hence, it is possible that some back-movement from the intermediate pool to the replenishment pool may occur. The physiological importance thereof remains to be determined.

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