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Review

Regulatory mechanisms of surfactant secretion

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Abstract

Surfactant secretion is a critical regulated process in the metabolism of pulmonary surfactant. Presumably, because this process is vital to the survival of the organism, there are several independent pathways for stimulating secretion which work through different cell surface receptors and signaling mechanisms. In addition, there is apparent homeostatic regulation in that two components of surfactant, namely SP-A and dipalmitoylphosphatidylcholine, can inhibit secretion. Although secretion of surfactant has been studied for over two decades, there remains some important issues to be resolved. In vivo secretion can be stimulated by hyperventilation or even a single large breath. However, we do not know the biochemical mechanism for this physiologically important form of stimulation. In vitro, we know many of the proximal events in signaling, but we do not know how the lamellar bodies move within a cell or the docking mechanism at the plasma membrane. Many investigators have demonstrated that SP-A will inhibit secretion in vitro, but the mechanism is not known. Finally, there is a route of secretion of SP-A independent of lamellar bodies, but we do not know details of this pathway nor its regulation. © 1998 Elsevier Science B.V. All rights reserved.

Keywords: Dipalmitoylphosphatidylcholine; Alveolar type II cell; Surfactant protein; SP-A; SP-B; SP-C; SP-D; Exocytosis

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1. Introduction

Secretion of pulmonary surfactant is a rate-limiting step in delivery of surfactant to the air–liquid interface to lower surface tension in the lung. Secretion is a critical regulated event in the dynamic metabolism of surfactant. The secretion and re-utilization cycle of surface active material involves a number of complex regulated processes that include: (1) synthesis; (2) intracellular transport; (3) sorting and packaging in the lamellar bodies; (4) movement of lamellar bodies to the apical plasma membrane of the type II cell; (5) exocytosis; (6) absorption to the air–liquid interface; (7) physical separation of some of the surfactant components during compression at the air–liquid interface; (8) uptake of extracellular surfactant; (9) intracellular processing of recycled surfactant components from alveolar fluid (which likely differs from the intracellular processing of newly synthesized material); and finally (10) secretion of recycled material. This chapter will focus on the stimuli for secretion, intracellular movement of the lamellar body to the apical surface of the membrane, and exocytosis, e.g. steps 4 and 5. Readers are referred to other reviews on secretion of surface active material for a different perspective and coverage of additional topics, such as regulation of secretion at the time of the first breath in the infant [1–4]. This review is an update of a previously published review on the secretion of surface active material [5].

2. Assumptions and a proposed model for secretion

Secretion of the phospholipids of surface active material is solely by alveolar type II cells and this intracellular surfactant is stored in lamellar bodies, unique organelles of type II cells. However, secretion and trafficking of the proteins of surfactant are more complex. SP-A may be secreted by routes independent of lamellar bodies in type II cells and is also synthesized by other cell types. SP-A, SP-B, and SP-D are produced by both type II cells and nonciliated bronchiolar (Clara) cells. This is especially true for rodent bronchiolar cells. Human small airways contain little intracellular immunostaining for SP-A or SP-D. SP-D is not processed with the phospholipids of surfactant and is not found in lamellar bodies nor in tubular myelin. Hence, for this review, SP-D is not considered to be part of the surfactant system. For the discussion that follows, we will address issues of lamellar body secretion.

The phospholipid components of surfactant are found within lamellar bodies in type II cells. This conclusion is based on chemical analysis of isolated lamellar bodies, their ultrastructural appearance, and results of pulse chase metabolic labeling studies. The phospholipid bilayers within lamellar bodies have been deemed to be free of protein that spans the bilayer on the basis of freeze fracture analysis [6]. Within lamellar bodies, there is an electron dense rim of granular material that is thought to contain protein. Isolated lamellar bodies have been reported to contain SP-A, SP-B, SP-C, and certain lysosomal enzymes [7,8]. However, Power et al. isolated lamellar bodies and a vesicular fraction from rat lung and found SP-A in the vesicular fraction but not in lamellar bodies [9]. Oosterlaken-Dijksterhuis et al. analyzed isolated rat lamellar bodies for their content of the surfactant proteins [10]. SP-B and SP-C were highly concentrated in lamellar bodies. SP-B and SP-C constituted 28% and 22% of total lamellar body protein, whereas SP-A accounted for only 1% of lamellar body protein. Froh et al. also documented a low amount of SP-A in lamellar bodies and demonstrated that exogenous SP-A was necessary for structural transformation to tubular myelin and rapid surface film formation [11]. Similar analysis of purified extracellular surfactant indicated a much different relative abundance of the surfactant proteins. SP-A accounts for 50%, SP-B for 8%, and SP-C for 4% of the protein in purified surfactant [10]. The molar stoichiometry would make SP-C and SP-B the most abundant surfactant proteins in lamellar bodies. One interpretation of these data is that SP-A is derived in part from sources other than lamellar bodies, within type II cells or Clara cell granules. These observations are compatible with the suggestion that SP-A can be secreted by type II cells independent of lamellar body secretion [12–14]. An alternative interpreta-

tion of the compositional analysis is that the turnover of SP-A in extracellular surfactant is much slower than that of SP-B and SP-C. Our data with type II cells in vitro indicate that the SP-A found in lamellar bodies is derived in large part from extracellular pools (K. Osani et al., Trafficking of newly synthesized surfactant protein A in isolated not alveolar type II cells. Am. G. Respir. Cell Mol. Biol., in press). In this review, the pathway and regulation of SP-A secretion which is independent of lamellar body secretion will not be discussed in more detail because there is little known about this pathway. However, it should be noted that Froh et al. have estimated that this pathway accounts for most of SP-A secretion [12].

A proposed model for exocytosis of lamellar bodies is shown in Fig. 1. This model is designed to conceptualize sites for physiologic and pharmacologic control of lamellar body secretion. Extracellular mediators combine with their cognate cell surface receptors designated R in Fig. 1. The physiologically dominant in vivo agonist and receptor has not been established. As will be discussed below, there are many different agonists that can stimulate secretion. However, the model can be conceptualized from analyses of secretion stimulated by ATP and use the $P2Y_2$ purinoceptor as a model of a seven membrane spanning G-protein coupled receptor [15,16]. The receptor–ligand complex then associates with the heterotrimeric G-proteins (guanine nucleotide binding proteins) to activate phospholipase C which cleaves phosphatidylinositol bisphosphate to form diacylglycerol and IP_3. Diacylglycerol will activate protein kinase C and facilitate the translocation of the soluble form of protein kinase C to the plasma membrane. IP_3 diffuses to intracellular membranes, predominantly the endoplasmic reticulum, to release membrane associated calcium. There is also opening of calcium channels to allow access of extracellular calcium. The result is a transient rise in intracellular calcium. It should be noted that there are also calcium-independent pathways for surfactant secretion that for simplicity are not included in this model, and these include the cyclic AMP-dependent pathway

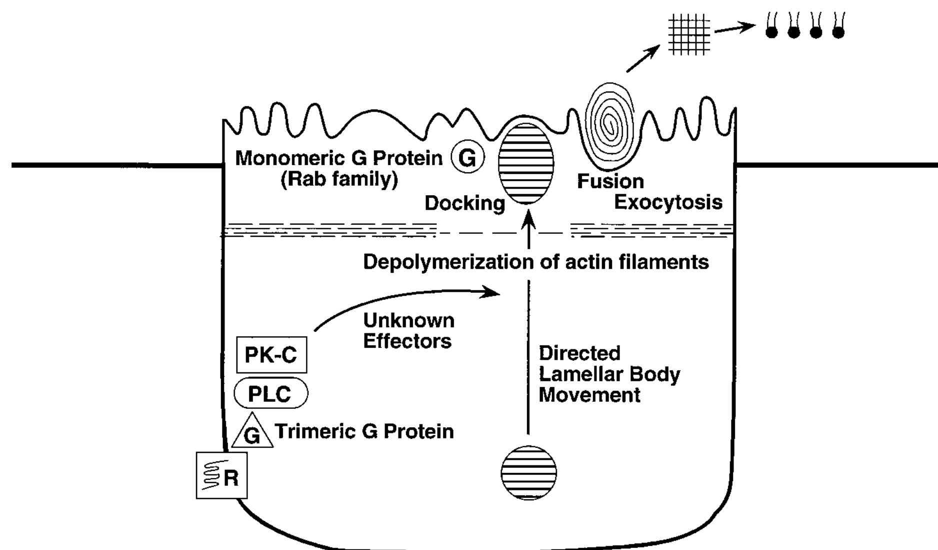

Fig. 1. Proposed general mechanism for exocytosis. As stated in the text, the identity of the dominant receptor R in vivo is not known. The membrane-associated events are well defined in other secretory cell types and in preliminary studies in type II cells. An agonist combines with the receptor to activate phospholipase C (PLC) via heterotrimeric G-proteins and subsequently protein kinase C (PK-C). Other intracellular signals, such as Ca^{2+} and cyclic AMP are generated, but are not shown in this diagram. The major gaps in our knowledge are the subsequent steps. The unknown effectors are presumably cytosolic protein kinases and phosphatases with subsequent protein phosphorylations and dephosphorylations. Presumably these signals activate contractile proteins to move lamellar bodies up to the apical surface and clear the subcortical cytoskeletal network to allow docking with the plasma membrane. Lamellar bodies then fuse with the apical plasma membrane, an effect which is postulated to be regulated by low molecular weight monomeric G-proteins such as Rab3A [25,26]. Exocytosis occurs and the lamellar body transforms into tubular myelin before adsorption to the air–liquid interface. The transformation of lamellar phospholipid into tubular myelin requires SP-B and SP-A.

[17,18]. The specific substrates for protein kinases that participate in exocytosis are not known. Edwards et al. and Takahashi and Voelker independently sought to determine if the MAP (mitogen activated protein kinase) pathways were involved in inhibition of secretion mediated by SP-A in vitro ([19] and unpublished observations). Both groups demonstrated the existence of these kinase pathways in type II cells and activation by secretagogues. However, they were unable to demonstrate any relationship to the inhibition of secretion by SP-A. MAP kinase was activated by TPA, ATP, and ionomycin, and this activation was not inhibited by addition of SP-A. Intracellular effectors presumably calcium, cyclic AMP, and phosphorylated proteins diffuse to activate the lamellar body translocation process. Although the proximal membrane events for intracellular signalling are established, the more distal events leading to translocation of lamellar bodies are not known. A series of coordinated events are needed to move the lamellar bodies intracellularly. Lamellar bodies move vectorially to the apical plasma membrane, fuse with the plasma membrane and are ultimately secreted by exocytosis. The intracellular movement of lamellar bodies presumably involves actin [20] and microtubules for the dynamic movement, intermediate filaments as the guides [21], and specific attachment proteins. There needs to be tethering of the contractile proteins to the plasma membrane and to the cytoplasmic surface of the lamellar body. Annexins, calcium-dependent phospholipid binding proteins, are likely to be important for the terminal events in exocytosis and could dock the lamellar bodies at the plasma membrane prior to membrane fusion and secretion [22,23]. In order to transport the lamellar body to the apical membrane, the subapical or cortical cytoskeletal network of actin filaments must be depolymerized [24]. Once the lamellar body has arrived at the apical membrane, it must fuse with the plasma membrane before exocytosis. The fusion is thought to involve low molecular weight monomeric GTP binding proteins of the Rab family, such as Rab3A [25,26].

3. Regulation of secretion in the intact lung

In order to determine physiologic regulation of secretion and pharmacologic stimulation of surfactant secretion in patients with respiratory insufficiency, we need to understand secretion in the intact lung. However, control of secretion in the intact lung remains largely undefined. For studies of secretion, most investigators have used type II cells in primary culture, which has limitations for extrapolation to events in vivo. Secretion in vivo may involve paracrine and physical forces that are not readily studied with isolated type II cells.

There are two different means of stimulating secretion in the intact lung. One is by hyperventilation, for which agonists (or antagonists) have not been defined, and the other is by agonists that directly stimulate type II cells. The calculated rate of secretion of surface active material in vivo is about 10% of the stored intracellular pool per hour [27,28]. However, the measurements have only quantitated the turnover of the phospholipid components of surfactant. If there is a lamellar body-independent pathway for secretion of SP-A, such a pathway is probably also under pharmacologic control and may have a different turnover rate. Nicholas and colleagues stimulated secretion in rats through hyperventilation caused by swimming or by breathing a gas mixture of 10% oxygen, 5% carbon dioxide, and 85% nitrogen [29–32]. With swimming rats, secretion appears to be maximally stimulated within a few minutes, and this stimulation is not inhibited by propranolol, atropine, cyproheptadine, or indomethacin. These studies were performed before the identification of the purinergic receptor ($P2Y_2$). It is possible that ATP could be the effector molecule, and inhibition of the purinergic receptor could block this effect. The rate of secretion within the first 10 minutes was calculated to increase by 14-fold over the basal rate. In the isolated perfused lung, basal secretion can also be increased by hyperventilation. Several groups have documented that large inflations can increase the amount of phospholipid recovered in lavage [30,33–36]. Interestingly, a single deep breath also causes secretion [37]. Pharmacologic attempts to block increased secretion due to hyperventilation have not been uniformly successful [34,38]. A likely mechanism for the increased secretion due to large tidal volumes is physical stretch which generates a transient increase in cytosolic calcium [39]. Alternative mechanisms include release of mediators such as

arachidonate metabolites from other lung cells which in turn stimulate secretion, a reduction in intracellular pCO_2 which produces intracellular alkalosis, and membrane depolarization due to an opening of ion channels by stretch. Membrane depolarization produced by high extracellular potassium moderately stimulates secretion [40]. The possibility of intracellular alkalosis as a potential mechanism was investigated by Chander in the isolated perfused lung [41]. In addition, Gerboth et al. demonstrated that intracellular alkalinization increases intracellular calcium levels in type II cells [42]. Hence, hyperventilation could stimulate secretion by a calcium-dependent process.

An important clinical issue is whether exogenous surfactant used for replacement therapy alters the secretory rate in vivo. Exogenous surfactant is being used in the treatment of respiratory distress syndrome of the newborn and has been used successfully in the treatment of adult respiratory distress syndrome. Exogenous surfactant could affect endogenous synthesis or secretion. However, data available to date do not indicate a major decrease in the secretory rate nor a feedback inhibition of phospholipid or protein components of surface active material [28,43–45]. In this regard, the in vivo data are different from the in vitro data where both SP-A and dipalmitoylphosphatidylcholine have been shown to inhibit secretion.

Pharmacologic agonists can also be administered to the intact lung to stimulate surfactant secretion. A variety of agonists stimulate secretion in the isolated perfused lung, and these include isoproterenol, terbutaline, 8-bromoadenosine 3′,5′-cyclic monophosphate, TPA and ATP [41,46]. However, the magnitude of secretion in the isolated perfused lung is only about 2% per hour which is low in comparison to turnover data in the intact animal. Maximal secretion is likely to require both physical forces and mediators as occurs with exercise. Since the effects of agonists are modest compared to hyperventilation, it appears that regulation in vivo may be controlled more by physical stretch than by circulating or paracrine mediators.

The role of inhibitors of secretion can be studied in vivo and in the isolated perfused lung. As shown in Fig. 1, there must be some movement of lamellar bodies within the cytoplasm to the apical membrane, and such movement would likely involve contractile proteins, e.g. tubulin and actin. Studies in the intact lung with normal cell shape and cytoarchitecture show that both inhibitors of microtubule assembly (colchicine and vinblastine) and actin polymerization (cytochalasin D) inhibit secretion [20,21]. However, the results of these inhibitors in isolated type II cells are inconsistent [47–49]. The major differences between the studies with the intact lung and with isolated type II cells are that in vitro the cells are flattened, cell polarity is altered, and the cytoskeleton is more disorganized. Thus, the studies with the intact lung are probably more relevant.

4. Regulation of secretion in type II cells in vitro

The development of methods to purify alveolar type II cells has provided essential tools for analyzing factors that stimulate surfactant secretion. This system affords the opportunity of investigating the role of specific second messengers in the signal transduction cascade which cannot be readily studied in the intact lung because of cellular heterogeneity. However, the magnitude of secretion in vitro is less than in vivo. As stated above, basal secretion with normal respiratory movement is about 10% per hour in vivo. Basal secretion in vitro in the absence of exogenous agonists and physical stretch is about 1% per hour. In the presence of maximal stimulation by agonists in vitro, but in the absence of stretch, the secretory rate can reach about 8% per hour. Studies of secretion with isolated type II cells have shown that there are multiple stimulatory agonists (TPA, diacylglycerol, ATP, β-adrenergic agonists, calcium ionophores, arachidonate metabolites, etc.) as well as inhibitors (SP-A, dipalmitoylphosphatidylcholine, lectins). Implications of the numerous potential agonists and antagonists include that control of secretion may be tightly regulated in the intact lung, that numerous backup systems are available should one ever fail, and that secretion may be very difficult to stimulate or inhibit by a single agonist or antagonist in vivo.

Among the most challenging studies on secretion in vitro are those on the role of stretch. Although many investigators considered stretch to be important in vivo, Wirtz and Dobbs were the first to document the role of stretch on secretion in vitro [39].

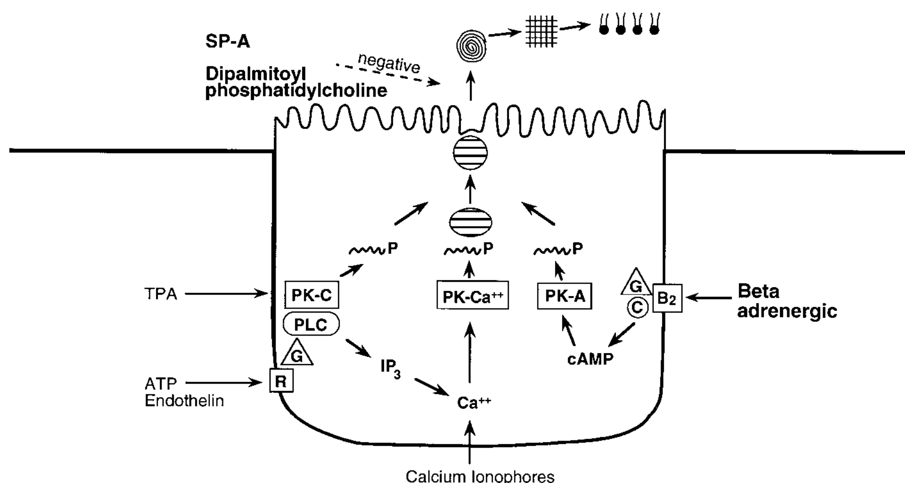

Fig. 2. Regulation of surfactant secretion in vitro. In this diagram, three groups of agonists that result in activation of three different families of protein kinase are shown. Secretion can be stimulated by at least three different pathways and several different receptors may be involved in signaling through the protein kinase C pathway. In addition, the interaction of second messengers is more complex than indicated. For example, agents which increase cyclic AMP can also transiently increase cytosolic calcium. The inhibitors of secretion are depicted to act from the apical surface and the agonists that stimulate secretion are diagrammed to act from the basolateral surface. The mechanisms whereby SP-A and dipalmitoylphosphatidylcholine inhibit secretion is not known. However, for SP-A, the events appear to be distal in that the generation of intracellular mediators, such as cytosolic calcium and cyclic AMP, are not affected.

Type II cells were cultured on elastic silicone membranes coated with fibronectin. A single stretch was applied by hydrostatic pressure and secretion was measured over a 1 h incubation. Because of technical difficulties, rhythmic stretch was not performed. The magnitude of secretion was similar to that obtained with a combination of agonists (ATP, TPA, and terbutaline). Stretch was associated with a transient increase in cytosolic calcium, but no change in cyclic AMP. The stimulation of secretion by stretch was inhibited by SP-A, but not by cyclooxygenase or lipoxygenase inhibitors.

A variety of agonists stimulate secretion in vitro, as diagrammed in Fig. 2. In general, the effect of different types of agonists are additive in the presence of maximal concentrations [50,51]. This implies that there are several independent enzymatic cascades that ultimately lead to secretion. Agonists can be grouped into those that produce a relatively large amount of secretion (4-fold over basal secretion) e.g. TPA and ATP, and those agonists which produced a lower level of secretion (2-fold over baseline), e.g. β-adrenergic agonists, cholera toxin, and calcium ionophores. The implication is that agonists which utilize protein kinase C (ATP and TPA) are more effective than those that work through other protein kinases. In rat type II cells, there is about 6-fold more protein kinase C activity than cyclic AMP-dependent protein kinase [51]. When type II cells are damaged, the β-adrenergic response is lost before the TPA response.

Tetradecanoyl phorbol acetate (TPA) was the first agonist reported to stimulate surfactant secretion in vitro [48]. TPA was especially useful for defining what was secreted, the time course and magnitude of secretion in vitro, and the energy dependence of secretion [52]. TPA activates protein kinase C directly and does not increase cyclic AMP or cytosolic calcium [17,53]. A limitation of studies with TPA is that the physiologic extracellular agonist for activation of protein kinase C in vivo has not been determined. However, TPA will continue to be useful for defining the regulatory role and protein substrates for phosphorylation by protein kinase C.

A variety of β-adrenergic agonists can also stimulate secretion in vitro [46,49,54]. The order of po-

tency is isoproterenol > epinephrine > norepinephrine which is characteristic of the β_2-subtype receptor. β-Receptors are regulated on type II cells, and expression can be stimulated by dexamethasone [55,56]. Addition of β-adrenergic agonists produces a prompt rise in intracellular cyclic AMP [46,49,53]. In addition, forskolin, cholera toxin, and 8-bromoadenosine 3′,5′-cyclic monophosphate which bypass the β-receptor also stimulate secretion. The next step in this pathway is activation of a cyclic AMP-dependent protein kinase.

Purinergic compounds also stimulate secretion [57–60]. The rank order of potency is ATP > ADP > adenosine = AMP which is characteristic of a $P2Y_2$ purinergic receptor. The most potent agonist, ATP, binds this $P2Y_2$ purinergic receptor which is coupled to a heterotrimeric G-protein that upon binding GTP activates phospholipase C to form IP_3 and diacylglycerol [18,60–62]. A $P2Y_2$ purinergic receptor has been cloned from a rat type II cell cDNA library [15]. Because an inhibitor of calcium mobilization prevented the rise of intracellular calcium but failed to block secretion, the increase in intracellular calcium is not thought to be absolutely required for secretion due to ATP [18]. ATP also increases cyclic AMP in type II cells [60]. In addition to the P_2 receptor there is a A_2 subtype of the P_1 purinergic receptor which is activated by AMP and adenosine [63,64]. The physiologic role of ATP, the dominant purinergic agonist, on secretion in vivo is unknown. The effect of ATP in vitro is maximal at 10 μM, intracellular concentration is 4–5 mM, and the calculated concentration in alveolar fluid is 1 mM [65]. This calculation is based on the amount of ATP recovered in lavage and an assumption of 0.1 ml of alveolar fluid in the adult rat lung. Hence, if the purinergic receptor is located on the apical surface of the type II cell, it should be maximally stimulated in vivo by the ATP in alveolar fluid. However, if the purinergic receptor is only on the basolateral side, the receptor may not be maximally stimulated at rest since interstitial fluid should have a lower concentration of ATP. One of the attractions to the importance of the purinergic receptor is that the intracellular concentration of ATP is much higher than that required for maximal stimulation, and ATP, a relatively small molecule, could theoretically be released by stretch. The released ATP would activate surface receptors and stimulate secretion. Hence, a local release of ATP could be part of the signal generation for the effect of stretch. Although this is pure speculation, it could also provide a catabolic and regulatory role for apical membrane alkaline phosphatase.

Calcium ionophores, A23187 or ionomycin, increase cytosolic calcium primarily by an increased uptake of extracellular calcium [17,66]. The stimulation of secretion is thought to be via calcium-dependent protein kinases. Agents other than ionophores that are associated with an increase in cytosolic calcium are stretch, ATP, terbutaline, forskolin, and 8-bromo-cAMP [17,39]. However, as stated above, a rise in cytosolic calcium may, in some circumstances, be independent of the effect on secretion. In addition, lowering intracellular calcium with excess quin 2 has not provided consistent results. Sano et al. chelated cytosolic calcium with excess quin 2 and lowered intracellular calcium from 143 to 31 nM in the absence of extracellular calcium [17]. Lowering cytosolic calcium and preventing a rise in cytosolic calcium only modestly inhibited the secretory response to terbutaline and 3-isobutyl-1-methylxanthine or TPA. Independently, Pian et al. performed similar experiments and inhibited all forms of stimulated secretion when intracellular calcium was maintained below 65 nM [66].

Lipid mediators, i.e. arachidonate, PGE_2, and LTE_4 have been reported to stimulate secretion of phosphatidylcholine by isolated type II cells [67,68]. However, the effect of LTE_4 is not reproducible and the modest stimulatory effects can be duplicated by other unsaturated fatty acids [69]. In addition, cyclooxygenase inhibitors do not block the effect of arachidonate. Type II cells produce large amounts of PGE_2 so in concept PGE_2 could be an autocrine stimulant of secretion [70]. Additional agents that stimulate secretion have been reported, and these include vasopressin [71], endothelin [72], and serum lipoproteins [73,74].

There are several classes of molecules that act as inhibitors of surfactant secretion in vitro. The first are defined components of surfactant that could serve as autocrine physiologic regulators. A second group are the lectins concanavalin A and wheat germ agglutinin which can be used as pharmacologic inhibitors [75]. A final group are metabolic inhibitors

that have been used to demonstrate the energy dependence of secretion and the importance of specific contractile proteins.

There is biologic plausibility for some type of feedback inhibition of secretion by surfactant itself or components of surfactant. Surfactant remains close to the apical surface of type II cells, and there is physical separation of components of surfactant at the air–liquid interface. When surface area is reduced and low surface tension is produced, high film pressure is achieved and components of surfactant are squeezed from the monolayer. Hildebran et al. calculated that the final surface film of surfactant when it was compressed to a low surface tension was composed of at least 90% dipalmitoylphosphatidylcholine [76]. The implication is that the apoproteins, especially SP-A, and the unsaturated phospholipids are physically separated from dipalmitoylphosphatidylcholine in surface film by the surface area compression during normal ventilation. However, nearly 100% of the SP-A in alveolar fluid is presumably bound to phospholipid.

Dobbs et al. and Rice et al. independently discovered that delipidated purified SP-A inhibited secretion in vitro [77,78]. SP-A inhibits all forms of agonist induced secretion and is therefore thought to act distal to the generation of second messengers. For example, SP-A inhibits secretion stimulated by stretch, but does not block the transient increase in cytosolic calcium [39]. There is a very strong correlation between binding of SP-A to type II cells and inhibition of secretion by SP-A (see Table 1). Max-

Table 1
Effect of structural mutations of rat SP-A upon receptor binding and inhibition of surfactant lipid secretion from alveolar type II cells

SP-A variant	Type II cell receptor binding[a]	Inhibition of secretion[b]
Wild-type		
Rat SP-A	Strong	Specific
Recombinant rat SP-A [96]	Moderate	Specific[a]
Oligosaccharide mutations [96]		
N1T	Moderate	Specific
N1T, N187S	Moderate	Specific
N187S	Moderate	Specific
Covalent crosslinking mutations [97]		
C6S	Weak	Very weak, specific
Collagen region deletion [97]		
ΔG8-P80	Absent	Non-specific
CRD mutations [98]		
E195Q, R197D	Absent	Very weak, non-specific
R197A	Moderate	Specific
R197H	Weak	Specific
R197K	Weak	Specific
R197D	Very weak	Weak, specific
R197N	Very weak	Non-specific
Ca^{2+} ligating mutations [99]		
E195A	Absent	Very weak, specific
E202A	Absent	Weak, specific
N214A	Absent	Weak, specific
D215A	Absent	Very weak, specific

[a]The recombinant rat SP-A in these studies was produced in Sf9 or T.*ni* cells using baculovirus vectors. All recombinant proteins in this table have reduced hydroxyproline content in the collagen domain of the molecule. The wild-type recombinant protein has reduced activity relative to the native rat protein purified from lung lavage. In general, the recombinant SP-A has an IC_{50} for competition for receptor binding that is 2–5-fold higher than that for native rat SP-A and this difference is the basis for the strong and moderate designations. The recombinant protein has an IC_{50} for inhibition of secretion that is 2–3-fold higher than that for rat SP-A.
[b]Surfactant lipid secretion can be inhibited by SP-A and a variety of lectins including concanavalin A. The monosaccharide, α-methylmannoside, reverses concanavlin A inhibition of secretion, but not SP-A-mediated inhibition. The reversibility of inhibition by α-methylmannoside is used to discriminate between a non-specific lectin effect and a specific SP-A-mediated effect [79].

imal inhibition of secretion and saturation of high affinity binding of SP-A occurs at a concentration of 1–5 μg/ml [79–82]. Chemical modifications of SP-A that block the inhibition of secretion also block the ability of SP-A to bind to type II cells, and these include heat denaturation, alkylation, reduction, reductive methylation, and treatment with cyclohexanedione, a reagent used to reversibly alter arginine residues [78–80,82]. Removal of the oligosaccharide component of SP-A with endoglycosidase F has no effect on the biologic activity [80]. Predictably, several monoclonal antibodies that block the inhibition of secretion also block binding of SP-A to type II cells [80]. One monoclonal has been identified that fails to block SP-A-mediated inhibition of secretion and only partially block receptor binding. A C-terminal peptide of SP-A beginning at Val^{117} or Gly^{118} was found not to affect secretion [78]. However, other evidence suggests that the oligomeric structure of SP-A is required for binding and for inhibition of secretion. Thus, failure of a peptide to inhibit secretion may in itself be insufficient evidence to conclude that the peptide does not contain the cell recognition site. The biologic effect of SP-A on secretion is largely attributable to the carbohydrate recognition domain (CRD) of SP-A [79,83]. Mannose and α-methylmannoside do not prevent receptor binding and inhibition of secretion by SP-A, suggesting that the CRD recognizes complex oligosaccharides perhaps in combination with peptide determinants. Concanavalin A does not block SP-A receptor binding indicating that this lectin can inhibit secretion via saccharide ligands that are different from those recognized by SP-A. The role of major structural determinants in SP-A upon type II cell receptor binding and the inhibition of secretion are outlined in Tables 1 and 2. The structural variants of the protein were created by site directed mutagenesis. The results shown in Table 1 demonstrate that the oligosaccharides of SP-A do not play an important role in either receptor binding or the regulation of secretion. Domains of SP-A that play important roles in covalent (Cys^6) or non-covalent (collagen region) oligomerization are not required for carbohydrate binding, but are necessary for high affinity receptor binding and effective inhibition of surfactant lipid secretion. A double mutation within the CRD (E195Q and R197D) eliminates receptor binding and specific inhibition of secretion. However, individual mutations to position 197 give a range of activities. The R197A mutation is silent, indicating that a positive charge at this position is not required for activity. The 197H and 197K mutations cause a significant decrease in receptor binding, but still give effective inhibition of secretion. Introduction of negative charge with the R197D mutation significantly reduced both receptor binding and inhibition of secretion. The R197N substitution gave the weakest receptor binding and non-

Table 2
Chimeric protein binding to type II cell receptors and inhibition of secretion

SP-A variant	Type II cell receptor binding[a]	Inhibition of secretion[b]
SP-A:MBP-A chimeras [84]		
SP-A(1–218):MBP-A(210–221)	Moderate	Specific
SP-A(1–203):MBP-A(195–221)	Moderate	Specific
SP-A(1–194):MBP-A(185–221)	Moderate	Specific
MBP-A	Absent	Absent
SP-A:SP-D chimeras [85]		
SP-A(1–194):SP-D(321–355)	Absent	Absent
SP-A(1–173):SP-D(300–355)	Absent	Absent
SP-A(1–134):SP-D(261–355	Absent	Absent
SP-D	Absent	Absent

[a]The chimeric rat SP-A molecules used in these studies were produced in Sf9 or T.*ni* cells using baculovirus vectors. All recombinant proteins in this table have reduced hydroxyproline content in the collagen domain of the molecule. The wild-type recombinant protein has reduced activity relative to the native rat protein purified from lung lavage.

[b]Surfactant lipid secretion can be inhibited by SP-A and a variety of lectins including concanavalin A. The monosaccharide, α-methylmannoside, reverses concanavlin A inhibition of secretion, but not SP-A-mediated inhibition. The reversibility of inhibition by α-methylmannoside is used to discriminate between a non-specific lectin effect and a specific SP-A-mediated effect [79].

specific inhibition of secretion. Thus, the substitutions of position 197 that preserve activity are either of minimal side chain length and uncharged or have some positive character. Mutations within the CRD that eliminate putative Ca^{2+} binding residues at positions 195, 202, 214 or 215 completely eliminate receptor binding and almost all inhibition of secretion.

Another approach to examining the function of elements of the CRD is to create chimeras with the closely related proteins MBP-A and SP-D (see Table 2). Interestingly, chimeras of SP-A and mannose binding protein A (MBP-A) that substituted portions of the CRD of SP-A with portions of MBP-A maintained the ability to inhibit secretion and bind to type II cells [84] (see Table 2). The result with SP-A/MBP-A chimeras was surprising. It was anticipated that substitution of SP-A with portions of the other collectins such as MBP-A or SP-D would abrogate the ability of the chimeras to retain the SP-A functions. Substitution of portions of the CRD of SP-A with comparable portions of SP-D did result in a protein that did not bind to type II cells or inhibit secretion [85]. Additional studies are required to define the epitope on SP-A for binding to type II cells and these future studies would be greatly facilitated by determination of the crystal structure of SP-A.

The effect of purified, delipidated SP-A on secretion is at least partially inhibited in the presence of phospholipid. Dobbs et al. found inhibition of secretion by purified surfactant with an IC_{50} at a concentration of 8 μg of protein per ml. Addition of phospholipid vesicles to SP-A preparations attenuated the protein's inhibitory effect upon secretion [78]. Since SP-A binds phospholipid avidly in the presence of calcium, there is likely to be very little free SP-A in alveolar fluid. The SP-A present in alveolar fluid is almost entirely lipid associated. Secretion in vitro is inhibited by 0.1 μg/ml of purified SP-A in the absence of phospholipid [80] and by about 160 μg/ml of isolated whole surfactant [77]. The calculated concentration of total SP-A in alveolar fluid ranges from 300 to 1800 μg/ml [83].

Although highly specific calcium-dependent binding of SP-A and evidence of internalization of SP-A has been demonstrated [81,82,86], the cell surface receptor on type II cells has not been unambiguously identified. Type II cells have a single class of high affinity receptors for SP-A. There are several candidate receptors reported, but additional studies are required to identify the SP-A receptor which is responsible for inhibition of secretion by SP-A [87–90]. This is an area of research that is progressing rapidly and should be resolved soon. Assuming the molecular mass of SP-A to be 650 kDa, and the maximal amount of SP-A bound is 360 ng/10^6 cells, this calculates to be 3.5×10^5 oligomers of SP-A per cell [81]. SP-A binding is saturable with an apparent dissociation constant (K_d) of 1 μg/ml [81]. Both kinetic and morphologic evidence shows time and temperature-dependent internalization. However, the endosomal compartment which is the initial location of internalized SP-A has not been rigorously defined. The internalized SP-A is not degraded in 20 h [81].

Suwabe et al. demonstrated that saturated phosphatidylcholines below their phase transition temperatures, e.g. dipalmitoylphosphatidylcholine at 37°C, also inhibit secretion in vitro [91]. The biologic implication of this finding is a hypothesis that type II cells sense the physical state of extracellular phospholipid by some currently unknown means. The initial finding was that unilamellar liposomes of dipalmitoylphosphatidylcholine, but not dioleoylphosphatidylcholine inhibit secretion in vitro. A broad range of naturally occurring and chemically synthesized phosphatidylcholines were evaluated, and those with transition temperatures above 37°C were inhibitory at 37°C, whereas those with transition temperatures below 37°C were not. If the temperature of secretion was increased to 42°C or lowered to 32°C, the results were predictable based on the phase transitions of the phosphatidylcholine liposomes. The implication is that if liposomes or vesicles of nearly pure dipalmitoylphosphatidylcholine are formed in vivo, these could serve as autocrine inhibitory regulators of secretion in vivo. As mentioned above, Hildebran et al. have estimated the chemical composition of the compressed surfactant monolayer at times of low surface tension, e.g. high film pressure, to be composed of nearly pure dipalmitoylphosphatidylcholine [76]. Hence, liposomes of nearly pure dipalmitoylphosphatidylcholine could exist in the alveolar subphase in vivo and may constitute an important form of feedback regulation of surfactant

secretion in the alveolus. The role of exogenous surfactants used in replacement therapy have not been studied in similar in vitro studies but would be unlikely to inhibit secretion as liposomes of mixed phosphatidylcholine species.

5. Other secretory proteins

Alveolar type II cells also secrete a variety of other proteins that are likely to be independent of lamellar body secretion. The major one is surfactant protein D which is a calcium-dependent lectin and part of the host-defense system. SP-D binds to a variety of microorganisms including viruses, bacteria, *Mycobacteria*, fungi and *Pneumocystis carinii* [83]. Readers are referred to the article by Reid in this series for a comprehensive review on SP-D. Type II cells also secrete complement components, lysozyme, plasminogen activator, basement membrane components, and hydrogen ions. In addition, type II cells secrete a variety of mediators, cytokines and growth factors including arachidonate metabolites, IL-8, IL-6, GM-CSF, TGF-β and potential autocrine growth factors (TGF-α and heparin binding EGF). Hence, although this review focused on surfactant, type II cells are potentially involved in a variety of inflammatory and reparative processes in the lung. The secretion of GM-CSF may, in addition, play a regulatory role on surfactant catabolism [92].

6. Problems and future directions

Although a great deal has been learned about agonists that stimulate secretion and the second messengers that these agonists generate, there is still much that is not known. We do not know the linkage between the early signals of protein kinase activation and the later steps of intracellular movement of lamellar bodies to the apical surface and exocytosis. Although it appears that the mature processed forms of the hydrophobic proteins SP-B and SP-C are contained within lamellar bodies, the intracellular trafficking of SP-A and its pathway for secretion is controversial. The pathway and regulation of SP-A secretion that is independent of lamellar body secretion needs to be defined more precisely. It is clear that SP-A is synthesized by bronchiolar cells as well as by type II cells in rodent lungs. Is this SP-A in bronchiolar cells regulated differently from the SP-A in type II cells? In human airway submucosal glands only the SP-A 2 gene is expressed [93]. The role of SP-A in regulation of surfactant secretion in vivo also requires further study. SP-A inhibits secretion in vitro and increases uptake of liposomal lipid. However, there is little alteration in surfactant homeostasis in mice with homozygous null alleles for SP-A [94,95]. It is difficult to resolve the in vitro findings of inhibition of secretion and uptake of liposomal lipid by SP-A with the apparent normal function of the surfactant system in mice that do not express this protein. Like many important biologic systems, there may be redundancy, such that in the normal state, there are no physiologic abnormalities and that the deficiencies only become manifest under conditions of stress, such as oxygen toxicity, acute lung injury, etc. The crystal structure of SP-A needs to be resolved so that more precise structure/functional point mutations can be constructed to define the binding of SP-A to receptors on type II cells and to account for the ability of SP-A to inhibit secretion. Finally, the cDNA for the high affinity receptor for SP-A on type II cells needs to be cloned and characterized in detail.

The regulation of secretion in vivo is also incompletely understood. The relative role of physical signals, such as stretch and biochemical mediators (such as ATP and β-adrenergic agonists) remains to be defined. By extrapolation from studies in vitro, maximal secretion in vivo is likely to require multiple agonists. It is not known if pharmacologic stimuli can augment the increased secretion due to hyperventilation. If hyperventilation produces maximal secretion, administration of pharmacologic stimulants to patients who are already hyperventilating may be ineffective. By analogy to other processes in the lung, the physiologic response is likely to be significantly modified by the local biochemical milieu. The role of various cytokines and the transepithelial potential difference and pH gradient that occur in vivo have not been investigated as modulators of secretion. More research is needed before we can devise a strategy for stimulating secretion in vivo as part of the treatment of patients with functional surfactant deficiencies.

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References

[1] A. Chander, A.B. Fisher, Regulation of lung surfactant secretion, Am. J. Physiol. 258 (1990) L241–L253.

[2] L.A.J.M. Creuwels, L.M.G. van Golde, H.P. Haagsman, The pulmonary surfactant system: biochemical and clinical aspects, Lung 175 (1997) 1–39.

[3] J.R. Wright, L.G. Dobbs, Regulation of pulmonary surfactant secretion and clearance, Annu. Rev. Physiol. 53 (1991) 395–414.

[4] H. Wirtz, M. Schmidt, Ventilation and secretion of pulmonary surfactant, Clin. Invest. 70 (1992) 3–13.

[5] R.J. Mason, Surfactant secretion, in: B. Robertson, L.M.G. Van Golde, J.J. Batenburg (Eds.), Pulmonary Surfactant: From Molecular Biology to Clinical Practice, Elsevier, Amsterdam, 1992, pp. 295–312.

[6] M.C. Williams, Freeze-fracture studies of tubular myelin and lamellar bodies in fetal and adult rat lungs, J. Ultrastruct. Res. 64 (1978) 352–361.

[7] K.F. Gibson, C.C. Widnell, The relationship between lamellar bodies and lysosomes in type II pneumocytes, Am. J. Respir. Cell Mol. Biol. 4 (1991) 504–513.

[8] J.J. Coalson, V.T. Winter, H.M. Martin, R.J. King, Colloidal gold immunoultrastructural localization of rat surfactant, Am. Rev. Respir. Dis. 133 (1986) 230–237.

[9] J.H. Power, H.A. Barr, T.E. Nicholas, Surfactant-associated 15- and 35-kDa proteins are concentrated in different organelles in rat lung tissue, Exp. Lung. Res. 14 (1988) 209–224.

[10] M.A. Oosterlaken-Dijksterhuis, M. van Eijk, B.L. van Buel, L.M. van Golde, H.P. Haagsman, Surfactant protein composition of lamellar bodies isolated from rat lung, Biochem. J. 274 (1991) 115–119.

[11] D. Froh, P.L. Ballard, M.C. Williams, J. Gonzales, J. Goerke, M.W. Odom, L.W. Gonzales, Lamellar bodies of cultured human fetal lung: content of surfactant protein A (SP-A), surface film formation and structural transformation in vitro, Biochim. Biophys. Acta 1052 (1990) 78–79.

[12] D. Froh, L.W. Gonzales, P. Ballard, Secretion of surfactant protein A and phosphatidylcholine from type II cells of human fetal lung, Am. J. Respir. Cell Mol. Biol. 8 (1993) 556–561.

[13] S.A. Rooney, L.I. Gobran, T.M. Umstead, D.S. Phelps, Secretion of surfactant protein A from rat type II pneumocytes, Am. J. Physiol. 265 (1993) L586–L590.

[14] M. Ikegami, A.H. Jobe, Surfactant metabolism, Semin. Perinatol. 4 (1993) 233–240.

[15] W.R. Rice, F.M. Burton, D.T. Fiedeldey, Cloning and expression of the alveolar type II cell P_{2u}-purinergic receptor, Am. J. Respir. Cell Mol. Biol. 12 (1995) 27–32.

[16] L. Gobran, Z. Xu, Z. Lu, S.A. Rooney, P2u purinoceptor stimulation of surfactant secretion coupled to phosphatidylcholine hydrolysis in type II cells, Am. J. Physiol. 267 (1994) L625–L633.

[17] K. Sano, D.R. Voelker, R.J. Mason, Effect of secretagogues on cytoplasmic free calcium in alveolar type II epithelial cells, Am. J. Physiol. 253 (1987) C679–C686.

[18] W.R. Rice, C.C. Dorn, F.M. Singleton, P_2-purinoceptor regulation of surfactant phosphatidylcholine secretion. Relative roles of calcium and protein kinase C, Biochem. J. 266 (1990) 407–413.

[19] Y.S. Edwards, J.H.T. Power, T.E. Nicholas, A.W. Murray, Effect of secretagogues on protein kinase-C (PKC) and mitogen-activated protein kinase (MAPK)-regulated pathways in rat alveolar type II cells, Am. J. Resp. Crit. Care Med. 155 (1997) A211A.

[20] E.C. Tsilibary, M.C. Williams, Actin and secretion of surfactant, J. Histochem. Cytochem. 31 (1983) 1298–1304.

[21] L.S. Brown, S.M. Pasquale, W.J. Longmore, Role of microtubules in surfactant secretion, J. Appl. Physiol. 58 (1985) 1866–1873.

[22] L. Liu, M. Wang, A.B. Fisher, U.-J.P. Zimmerman, Involvement of annexin II in exocytosis of lamellar bodies from alveolar epithelial type II cells, Am. J. Physiol. 270 (1996) L668–L676.

[23] N. Sen, A.R. Spitzer, A. Chander, Calcium-dependence of synexin binding may determine aggregation and fusion of lamellar bodies, Biochem. J. 15 (1997) 103–109.

[24] V. Bhandari, H. Lu, J. Pachter, M.J. Kresch, Actin depolymerization is developmentally regulated in rat type II cells exposed to terbutaline, Pediatr. Res. 41 (1997) 166–171.

[25] Y. Takai, T. Sasaki, H. Shirataki, H. Nakanishi, Rab3A small GTP-binding protein in CA^{2+}-dependent exocytosis, Genes Cells 1 (1996) 615–632.

[26] A. Morgan, Exocytosis, Essays Biochem. 30 (1995) 77–95.

[27] J.R. Wright, J.A. Clements, Metabolism and turnover of lung surfactant, Am. Rev. Respir. Dis. 135 (1987) 426.

[28] J.R. Wright, Clearance and recycling of pulmonary surfactant, Am. J. Physiol. 259 (1990) L1–L12.

[29] J.H.T. Power, M.E. Jones, H.A. Barr, T.E. Nicholas, Analysis of pulmonary phospholipid compartments during prolonged periods of hyperpnea, Exp. Lung Res. 11 (1986) 105–128.

[30] T.E. Nicholas, J.H. Power, H.A. Barr, Surfactant homeostasis in the rat lung during swimming exercise, J. Appl. Physiol. 53 (1982) 314–324.

[31] J.H.T. Power, H.A. Barr, M.E. Jones, T.E. Nicholas, Changes in surfactant pools after a physiological increase in alveolar surfactant, Am. J. Physiol. 63 (1987) 1902–1911.
[32] T.E. Nicholas, H.A. Barr, The release of surfactant in rat lung by brief periods of hyperventilation, Respir. Physiol. 52 (1983) 69–83.
[33] E.E. Faridy, Effect of distension on release of surfactant in excised dogs' lungs, Respir. Physiol. 27 (1976) 99–114.
[34] M.J. Oyarzun, J.A. Clements, Control of lung surfactant by ventilation, adrenergic mediators, and prostaglandins in the rabbit, Am. Rev. Respir. Dis. 117 (1978) 879–891.
[35] M.J. Oyarzun, J.A. Clements, Ventilatory and cholinergic control of pulmonary surfactant in the rabbit, J. Appl. Physiol. 43 (1977) 39–45.
[36] G.D. Massaro, D. Massaro, Morphologic evidence that large inflations of the lung stimulate secretion of surfactant, Am. Rev. Respir. Dis. 127 (1983) 235–236.
[37] T.E. Nicholas, J.H.T. Power, H.A. Barr, The pulmonary consequences of a deep breath, Respir. Physiol. 49 (1982) 315–324.
[38] T.E. Nicholas, H.A. Barr, Control of release of surfactant phospholipids in the isolated perfused rat lung, J. Appl. Physiol. 51 (1981) 90–98.
[39] H.R.W. Wirtz, L.G. Dobbs, Calcium mobilization and exocytosis after one mechanical stretch of lung epithelial cells, Science 250 (1990) 1266–1269.
[40] K. Sano, D.R. Voelker, R.J. Mason, Tetradecanoylphorbol acetate and terbutaline stimulate surfactant secretion in alveolar type II cells without changing the membrane potential, Biochim. Biophys. Acta 902 (1987) 317–326.
[41] A. Chander, Regulation of lung surfactant secretion by intracellular pH, Am. J. Physiol. 257 (1989) L354–L360.
[42] G.D. Gerboth, R.M. Effros, R.J. Roman, E.R. Jacobs, pH-induced calcium transients in type II alveolar epithelial cells, Am. J. Physiol. 264 (1993) L448–L457.
[43] K. Oguchi, M. Ikegami, H. Jacobs, A. Jobe, Clearance of large amounts of natural surfactants and liposomes of dipalmitoylphosphatidylcholine from the lungs of rabbits, Exp. Lung Res. 9 (1985) 221–235.
[44] S.B. Oetomo, J. Lewis, M. Ikegami, A.H. Jobe, Surfactant treatments alter endogenous surfactant metabolism in rabbit lungs, Am. J. Physiol. 68 (1990) 1590–1596.
[45] A. Pettenazzo, M. Ikegami, S. Seidner, A. Jobe, Clearance of surfactant phosphatidylcholine from adult rabbit lungs, J. Appl. Physiol. 64 (1988) 120–127.
[46] L.A. Brown, W.J. Longmore, Adrenergic and cholinergic regulation of lung surfactant secretion in the isolated perfused rat lung and in the alveolar type II cell in culture, J. Biol. Chem. 256 (1981) 66–72.
[47] W.R. Rice, K.C. Osterhoudt, J.A. Whitsett, Effect of cytochalasins on surfactant release from alveolar type II cells, Biochim. Biophys. Acta 805 (1984) 12–18.
[48] L.G. Dobbs, R.J. Mason, Stimulation of secretion of disaturated phosphatidylcholine from isolated alveolar type II cells by 12-O-tetradecanoyl-13-phorbol acetate, Am. Rev. Respir. Dis. 118 (1978) 705–733.
[49] N.R. Mettler, M.E. Gray, S. Schuffman, V.S. LeQuire, β-Adrenergic induced synthesis and secretion of phosphatidylcholine by isolated pulmonary alveolar type II cells, Lab. Invest. 45 (1981) 575–586.
[50] L.G. Dobbs, R.F. Gonzalez, L.A. Marinari, E.J. Mescher, S. Hawgood, The role of calcium in the secretion of surfactant by rat alveolar type II cells, Biochim. Biophys. Acta 877 (1986) 305–313.
[51] K. Sano, D.R. Voelker, R.J. Mason, Involvement of protein kinase C in pulmonary surfactant secretion from alveolar type II cells, J. Biol. Chem. 260 (1985) 12725–12729.
[52] L.G. Dobbs, R.J. Mason, M.C. Williams, B.J. Benson, K. Sueishi, Secretion of surfactant by primary cultures of alveolar type II cells isolated from rats, Biochim. Biophys. Acta 713 (1982) 118–127.
[53] E.J. Mescher, L.G. Dobbs, R.J. Mason, Cholera toxin stimulates secretion of saturated phosphatidylcholine and increases cellular cyclic AMP in isolated rat alveolar type II cells, Exp. Lung Res. 5 (1983) 173–182.
[54] L.G. Dobbs, R.J. Mason, Pulmonary alveolar type II cells isolated from rats. Release of phosphatidylcholine in response to beta-adrenergic stimulation, J. Clin. Invest. 63 (1979) 378–387.
[55] J.C. Mak, M. Nishikawa, E.B. Haddad, O.J. Kwon, S.J. Hirst, C.H. Twort, P.J. Barnes, Localisation and expression of beta-adrenoceptor subtype mRNAs in human lung, Eur. J. Pharmacol. 302 (1996) 215–221.
[56] J.P. Fabisiak, E.S. Vessell, D.E. Rannels, Interactions of beta adrenergic antagonists with isolated rat alveolar type II pneumocytes. I. Analysis, characterization and regulation of specific beta adrenergic receptors, J. Pharmacol. Exp. Ther. 241 (1987) 722–727.
[57] A.M. Gilfillan, S.A. Rooney, Purinoceptor agonists stimulate phosphatidylcholine secretion in primary culture of adult rat type II pneumocytes, Biochim. Biophys. Acta 917 (1987) 18–23.
[58] W.R. Rice, F.M. Singleton, P2-purinoceptors regulate surfactant secretion from rat isolated alveolar type II cells, Br. J. Pharmacol. 89 (1986) 485–491.
[59] W.R. Rice, F.M. Singleton, P2Y-purinoceptor regulation of surfactant secretion from rat isolated alveolar type II cells is associated with mobilization of intracellular calcium, Br. J. Pharmacol. 91 (1987) 833–838.
[60] D. Warburton, S. Buckley, L. Cosico, P1 and P2 purinergic receptor signal transduction in rat type II pneumocytes, J. Appl. Physiol. 66 (1989) 901–905.
[61] D. Tio, A. Tayag, M. Rehn, D. Warburton, Role of inositol triphosphate isomer formation in type II pneumocyte signal transduction, Am. J. Respir. Cell Mol. Biol. 4 (1991) 514–518.
[62] A. Chander, N. Sen, A.-M. Wu, A.R. Spitzer, Protein kinase C in ATP regulation of lung surfactant secretion in type II cells, Am. J. Physiol. 268 (1995) L108–L116.
[63] M. Griese, L.I. Gobran, J.S. Douglas, S.A. Rooney, Adenosine A2-receptor-mediated phosphatidylcholine secretion in type II pneumocytes, Am. J. Physiol. 260 (1991) L52–L60.

[64] L.I. Gobran, S.A. Rooney, Adenosine A_1 receptor-mediated inhibition of surfactant secretion in rat type II pneumocytes, Am. J. Physiol. 258 (1990) L45–L51.

[65] W.F. Rice, M. Burhans, J.R. Wispe, Effect of oxygen exposure on ATP content of rat bronchoalveolar lavage, Pediatr. Res. 25 (1989) 396–398.

[66] M.S. Pian, L.G. Dobbs, N. Duzgunes, Positive correlation between cytosolic free calcium and surfactant secretion in cultured rat alveolar type II cells, Biochim. Biophys. Acta 960 (1988) 43–53.

[67] A.M. Gilfillan, S.A. Rooney, Arachidonic acid metabolites stimulate phosphatidylcholine secretion in primary cultures of type II pneumocytes, Biochim. Biophys. Acta 833 (1985) 336–341.

[68] A.M. Gilfillan, S.A. Rooney, Leukotrienes stimulate phosphatidylcholine secretion in cultured type II pneumocytes, Biochim. Biophys. Acta 876 (1986) 22–27.

[69] R.C. Baybutt, J.E. Smith, M.N. Gillespie, T.G. Newcomb, Y.-Y. Yeh, Arachidonic acid and eicosapentaenoic acid stimulate type II pneumocyte surfactant secretion, Lipids 29 (1994) 535–539.

[70] G.R. Cott, J.Y. Westcott, N.F. Voelkel, Prostaglandin and leukotriene production by alveolar type II cells in vitro, Am. J. Physiol. 258 (1990) L179–L187.

[71] L.A. Brown, L.H. Wood, Stimulation of surfactant secretion by vasopressin in primary cultures of adult rat type II pneumocytes, Biochim. Biophys. Acta 1001 (1989) 76–81.

[72] N. Sen, M.M. Grunstein, A. Chander, Stimulation of lung surfactant secretion by endothelin-1 from rat alveolar type II cells, Am. J. Physiol. 266 (1994) L255–L262.

[73] M.S. Pian, L.G. Dobbs, Lipoprotein-stimulated surfactant secretion in alveolar type II cells: mediation by heterotrimeric G proteins, Am. J. Physiol. 273 (1997) L634–L639.

[74] T.A. Voyno-Yasenetskaya, L.G. Dobbs, S.K. Erickson, R.L. Hamilton, Low density lipoprotein- and high density lipoprotein-mediated signal transduction and exocytosis in alveolar type II cells, Proc. Natl. Acad. Sci. USA 90 (1993) 4256–4260.

[75] W.R. Rice, F.M. Singleton, Regulation of surfactant phospholipid secretion from isolated rat alveolar type II cells by lectins, Biochim. Biophys. Acta 958 (1988) 205–210.

[76] J.N. Hildebran, J. Goerke, J.A. Clements, Pulmonary surface film stability and composition, J. Appl. Physiol. 47 (1979) 604–611.

[77] L.G. Dobbs, J.R. Wright, S. Hawgood, R. Gonzalez, K. Venstrom, J. Nellenbogen, Pulmonary surfactant and its components inhibit secretion of phosphatidylcholine from cultured rat alveolar type II cells, Proc. Natl. Acad. Sci. USA 84 (1987) 1010–1014.

[78] W.R. Rice, G.F. Ross, F.M. Singleton, S. Dingle, J.A. Whitsett, Surfactant-associated protein inhibits phospholipid secretion from type II cells, J. Appl. Physiol. 63 (1987) 692–698.

[79] Y. Kuroki, R.J. Mason, D.R. Voelker, Chemical modification of surfactant protein A alters high affinity binding to rat alveolar type II cells and regulation of phospholipid secretion, J. Biol. Chem. 263 (1988) 17596–17602.

[80] Y. Kuroki, R.J. Mason, D.R. Voelker, Pulmonary surfactant apoprotein A structure and modulation of surfactant secretion by rat alveolar type II cells, J. Biol. Chem. 263 (1988) 3388–3394.

[81] Y. Kuroki, R.J. Mason, D.R. Voelker, Alveolar type II cells express a high-affinity receptor for pulmonary surfactant protein A, Proc. Natl. Acad. Sci. USA 85 (1988) 5566–5570.

[82] J.R. Wright, J.D. Borchelt, S. Hawgood, Lung surfactant apoprotein SP-A (26-36 kDa) binds with high affinity to isolated alveolar type II cells, Proc. Natl. Acad. Sci. USA 86 (1989) 5410–5414.

[83] J.R. Wright, Immunomodulatory functions of surfactant, Physiol. Rev. 77 (1997) 931–962.

[84] T. Honma, Y. Kuroki, W. Tsunezawa, Y. Ogasawara, H. Sohma, D.R. Voelker, T. Akino, The mannose binding protein A region of Glu 185-Ala 221 can functionally replace the surfactant protein A region of Glu 195-Phe 228 without loss of interaction with lipids and alveolar type II cells, Biochemistry 36 (1997) 7176–7184.

[85] H. Sano, Y. Kuroki, T. Honma, Y. Ogaswara, H. Sohma, D.R. Voelker, T. Akino, Analysis of chimeric proteins identifies the regions in the carbohydrate recognition domains of rat lung collectins that are essential for interactions with phospholipids, glycolipids, and alveolar type II cells, J. Biol. Chem. 273 (1998) 4783–4789.

[86] M. Kalina, R. Socher, Internalization of pulmonary surfactant into lamellar bodies of cultured rat pulmonary type II cells, J. Histochem. Cytochem. 38 (1990) 483–492.

[87] Z.C. Chroneos, R. Abdolrasulnia, J.A. Whitsett, W.R. Rice, V.L. Shepherd, Purification of a cell-surface receptor for surfactant protein A, J. Biol. Chem. 271 (1996) 16375–16383.

[88] D.S. Strayer, R. Pinder, A. Chander, Receptor-mediated regulation of pulmonary surfactant secretion, Exp. Cell Res. 226 (1996) 90–97.

[89] R.R. Nepomuceno, A.H. Henschen-Edman, W.H. Burgess, A.J. Tenner, cDNA cloning and primary structure analysis of C1qRp, the human C1q/MBL/SPA receptor that mediates enhanced phagocytosis in vitro, Immunity 6 (1997) 119–129.

[90] H. Wissel, A.C. Looman, I. Fritzsche, B. Rustow, P.A. Stevens, SP-A-binding protein BP55 is involved in surfactant endocytosis by type II pneumocytes, Am. J. Physiol. 271 (1996) L432–L440.

[91] A. Suwabe, R.J. Mason, D.R. Voelker, Temporal segregation of surfactant secretion and lamellar body biogenesis in primary cultures of rat alveolar type II cells, Am. J. Respir. Cell Mol. Biol. 5 (1991) 80–86.

[92] G. Dranoff, A.D. Crawford, M. Sadelain, B. Ream, A. Rashid, R.T. Bronson, G.R. Dickersin, C.J. Bachurski, E.L. Mark, J.A. Whitsett, R.C. Mulligan, Involvement of granulocyte-macrophage colony-stimulating factor in pulmonary homeostasis, Science 264 (1994) 713–716.

[93] H. Saitoh, H. Okayama, T. Fushimi, S. Shimura, K. Shirato, K. Hosoda, Surfactant protein-A secretion and genotype in

human airway submucosal glands, Am. J. Resp. Crit. Care Med. 155 (1997) A210.

[94] T.R. Korfhagen, M.D. Bruno, G.F. Ross, K.M. Huelsman, M. Ikegami, A.H. Jobe, S.E. Wert, B.E. Stripp, R.E. Morris, S.W. Glasser, C.J. Bachurski, H.S. Iwamoto, J.A. Whitsett, Altered surfactant function and structure in SP-A gene target mice, Proc. Natl. Acad. Sci. USA 93 (1996) 9594–9599.

[95] M. Ikegami, T.R. Korfhagen, M.D. Bruno, J.A. Whitsett, A.H. Jobe, Surfactant metabolism in surfactant protein A-deficient mice, Am. J. Physiol. 272 (1997) L479–L485.

[96] F.X. McCormack, H.M. Calvert, P.A. Watson, D.L. Smith, R.J. Mason, D.R. Voelker, The structure and function of surfactant protein A: hydroxyproline and carbohydrate-deficient mutant proteins, J. Biol. Chem. 269 (1994) 5833–5841.

[97] F.X. McCormack, S. Pattanajitvilai, J. Stewart, F. Possmayer, K. Inchley, D.R. Voelker, The cys^{6} intermolecular disulfide bond and the collagen-like region of rat SP-A play critical roles in interactions with alveolar type II cells and surfactant lipids, J. Biol. Chem. 272 (1997) 27971–27979.

[98] F.X. McCormack, Y. Kuroki, J.J. Stewart, R.J. Mason, D.R. Voelker, Surfactant protein A amino acids Glu^{195} and Arg^{197} are essential for receptor binding, phospholipid aggregation, regulation of secretion, and the facilitated uptake of phospholipid by type II cells, J. Biol. Chem. 269 (1994) 29801–29807.

[99] F.X. McCormack, J. Stewart, D.R. Voelker, M. Damodarasamy, Alanine mutagenesis of surfactant protein A reveals that lipid binding and pH-dependent liposome aggregation are mediated by the carbohydrate recognition domain, Biochemistry 36 (1997) 13963–13971.