Pulmonary Surfactant Secretion Is Regulated by the Physical State of Extracellular Phosphatidylcholine*

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*This work was supported by National Institutes of Health Grants HL 29891, AG04418, and AA03527 and was performed in the Lord and Taylor Laboratory for Lung Biochemistry and the Anna Perahia Adatto Clinical Research Center. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Pulmonary alveolar type II cells synthesize, secrete, and recycle the components of pulmonary surfactant. In this report we present evidence that dipalmitoylphosphatidylcholine is a potent inhibitor of surfactant lipid secretion by type II cells. Monoenoic and dienoic phosphatidylcholines with fatty acids of 16 or 18 carbons are ineffective as inhibitors of surfactant lipid secretion. In contrast, disaturated phosphatidylcholines, with either symmetric or asymmetric pairs of fatty acids of 14, 16, or 18 carbons, exhibit inhibition of surfactant secretion that correlates extremely well with the phase transition temperature (Tc) of the phospholipid. The inhibitory activity of dipalmitoylphosphatidylcholine is not dependent upon lipid stereochemistry, with either symmetric or asymmetric pairs of phosphatidylcholine. Other lipid components include unsaturated phosphatidylcholines characterized (18). The major component is dipalmitoylphosphatidylcholine (DPPC), which comprises about 50% of the lipid. Other lipid components include unsaturated phosphatidylcholines (~20%) phosphatidylglycerol (~10%), and minor amounts of cholesterol and other lipids. Dipalmitoylphosphatidylcholine plays a critical role in the function of surfactant since this lipid alone can account for the surface tension lowering properties that prevent alveolar collapse and are essential for normal gas exchange to occur in the lung.

The alveolar type II cell of the lung produces pulmonary surfactant, a complex mixture of lipids and proteins that is secreted into the alveolar space and ultimately functions to reduce surface tension at the air-liquid interface (1). Several of the protein components that are secreted by type II cells have been identified and these are denoted SP (for surfactant protein) (2-4), SP-B (5-7), SP-C (8-10), and SP-D (11, 12). SP-B and SP-C are extremely hydrophobic proteins that facilitate lipid adsorption from the alveolar hypophase to the air water interface (5-10). SP-A also facilitates lipid adsorption (7) and along with SP-B functions in phospholipid reorganization in the alveolar hypophase from a lamellar form to a tubular myelin form (13). In addition, SP-A functions as a potent inhibitor of surfactant secretion in vitro (14-17) and has been postulated to be a regulator of surfactant homeostasis in vivo. No specific functions have yet been described for SP-D.

A central question of surfactant biology is how is the secretion of the lipids and proteins regulated? Previous efforts in this laboratory have focused on the ability of SP-A to regulate surfactant secretion in vitro (16-17). In this report the focus is the lipid components of surfactant. The purpose of this study was to address several basic questions about phospholipid interactions with type II cells. The questions addressed included the following: 1) Does phosphatidylcholine alter surfactant secretion? 2) Is there specificity to the phosphatidylcholine-mediated regulation of secretion? 3) What is the alveolar type II cells synthesize, secrete, and recycle the components of pulmonary surfactant. In this report we present evidence that dipalmitoylphosphatidylcholine is a potent inhibitor of surfactant lipid secretion by type II cells. Monoenoic and dienoic phosphatidylcholines with fatty acids of 16 or 18 carbons are ineffective as inhibitors of surfactant lipid secretion. In contrast, disaturated phosphatidylcholines, with either symmetric or asymmetric pairs of fatty acids of 14, 16, or 18 carbons, exhibit inhibition of surfactant secretion that correlates extremely well with the phase transition temperature (Tc) of the phospholipid. The inhibitory activity of dipalmitoylphosphatidylcholine is not dependent upon lipid stereochemistry, with either symmetric or asymmetric pairs of phosphatidylcholine. Other lipid components include unsaturated phosphatidylcholines characterized (18). The major component is dipalmitoylphosphatidylcholine (DPPC), which comprises about 50% of the lipid. Other lipid components include unsaturated phosphatidylcholines (~20%) phosphatidylglycerol (~10%), and minor amounts of cholesterol and other lipids. Dipalmitoylphosphatidylcholine plays a critical role in the function of surfactant since this lipid alone can account for the surface tension lowering properties that prevent alveolar collapse and are essential for normal gas exchange to occur in the lung (19, 20).

The phospholipid components, which comprise approximately 90% of the mass of surfactant, have been well characterized (18). The major component is dipalmitoylphosphatidylcholine (DPPC), which comprises about 50% of the lipid. Other lipid components include unsaturated phosphatidylcholines (~20%) phosphatidylglycerol (~10%), and minor amounts of cholesterol and other lipids. Dipalmitoylphosphatidylcholine plays a critical role in the function of surfactant since this lipid alone can account for the surface tension lowering properties that prevent alveolar collapse and are essential for normal gas exchange to occur in the lung (19, 20).

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the physical basis by which phosphatidylcholine regulates surfactant secretion? The results provide strong evidence that disaturated phosphatidylcholines can play a crucial regulatory role in surfactant homeostasis.

MATERIALS AND METHODS

Chemicals—Simple salts, buffers, and organic solvents were purchased from Sigma, Mallinckrodt, and Fisher. The [H]choline was obtained from Du Pont-New England Nuclear. Phospholipids were obtained from Avanti Polar Lipids and Sigma.

Cells—Alveolar type II cells were isolated from adult male Sprague-Dawley rats by tissue dissociation with collagenase and purification on metrizamide density gradients (21). The cells were maintained in Dulbecco’s modified Eagle’s medium (DMEM) supplemented to 10% fetal bovine serum, 100 units/ml penicillin, 50 μg/ml streptomycin, and 10 μg/ml gentamicin.

Secretion of Surfactant Lipid by Alveolar Type II Cells—Freshly isolated type II cells were incubated in the medium described above, supplemented to 0.5 μCi/ml [H]choline at 37°C for 20–22 h. Non-adherent cells and unincorporated radiolabel were removed by vigorous washing with 10 ml of DMEM containing 10 mM Hepes (pH 7.4) and 1 mg/ml bovine serum albumin. The washed cells were incubated in the same medium at 37°C for 15 min in a CO₂-free incubator. The cells were next replenished with 1.6 ml of DMEM containing 10 mM Hepes, pH 7.4, and the additions of secretagogues and liposomes. The cells were incubated for a period of 3 h during which secretion of [H]phosphatidylcholine occurred. At the end of this period, the cultures were placed on ice and the media and cells were harvested and processed for lipid extraction (21). Prior to lipid extraction, the media were centrifuged at 300 × g to sediment any detached cells. Total lipids were extracted by the method of Bligh and Dyer (22), and radioactivity was measured by liquid scintillation spectrometry. The results of secretion experiments are expressed as percent secretion (radioactive lipid in medium/radioactive lipid in cells × 100%) or as percent of maximal secretion obtained with 10 μM ATP. Lactate dehydrogenase activity in the medium was used to measure nonspecific cell lysis (21) and never exceeded 3% of the value found in intact cells in any of the experiments shown.

In one set of experiments, secretion was conducted with the type II cell monolayer in an inverted configuration to circumvent any possible effects of lipid sedimentation from the media onto the cell monolayer. To accomplish this arrangement, type II cells that had been processed for a normal secretion experiment were overlaid with the dish and the dish inverted, and then secretion was allowed to proceed as described above.

Secretion of Norepinephrine by Adrenal Chromaffin Cells—The ability of phospholipids to regulate exocytosis of cells other than type II cells was examined using primary cultures of bovine adrenal chromaffin cells which were prepared using the method of Waymire et al. (23). The chromaffin cells were maintained in monolayer culture on collagen coated dishes and used within 2 weeks of isolation for secretion experiments as previously described (24, 25). Washed monolayers were incubated in Heps-buffered saline (HBS) (150 mM NaCl, 10 mM Heps, pH 7.4, 5.5 mM d-glucose, 5 mM KCl, 1 mM MgSO₄, 1 mM CaCl₂) supplemented to 2 μCi/2 ml dish of [H]norepinephrine for 60 min. Prelabeled cells were washed 3 times with HBS (5 ml, 5 min ea.) at this point. 5 ml of HBS (with or without DPPC) was put in each dish. Three 0.5-ml samples were taken at 2-min intervals to establish basal release. After removing the third sample, 35 μl of HBS or nicotine stock solution was added (final concentration was 100 μM). 0.5-ml samples were then taken at 2, 5, and 10 min following treatment. Following the final sample, each monolayer was treated with 2 ml of 0.1% NaCl and an aliquot was taken for determination of cellular radioactivity. Results are expressed as percent of total cellular [H]norepinephrine secreted. The secretion of [H]norepinephrine was examined in two separate experiments with duplicate dishes of cells used for each condition.

Liposomes—The liposomes were routinely prepared by removing organic solvents under a stream of N₂ and resuspending the lipid in Ca²⁺- and Mg²⁺-free phosphate-buffered saline to a concentration of 1 mg/ml. The resuspended lipid was next sonicated for 10 min using a Bransonic device at a 50% duty cycle and a setting of 7. This sonication period was more than sufficient to yield a translucent solution for all lipids used. Unsaturated lipids were always sonicated in an ice water bath. The saturated lipids were cooled by room temperature water, which allows the transient warming of distearoyl and dipalmitoylphosphatidylcholine to exceed their phase transition temperatures during sonication. With this procedure the solution warmed to 65°C by the end of the sonication period for all preparations of saturated lipids. Furthermore, in control experiments DPPC and distearoyl PC were individually sonicated in vessels jacketed by 65°C recirculating baths, and these liposomes were found to have properties identical to those prepared by the preceding method. Following sonication, the liposomes were centrifuged at 500 × g × 10 min to remove titanium particles, and the supernatants were used for final preparations in most experiments. In preliminary experiments we determined that there were no significant differences between these liposomes and those further processed by centrifugation at 100,000 × g × 1 h to yield homogeneous populations of small unilamellar vesicles. When experiments used liposomes at final concentrations greater than 100 μg/ml the liposome stock of 1 mg/ml was dialyzed overnight against DMEM prior to use.

RESULTS

Dipalmitoylphosphatidylcholine Inhibits Surfactant Secretion from Alveolar Type II Cells—The initial experiments in this study were conducted to examine if phospholipid was capable of inhibiting [H]phosphatidylcholine secretion from primary cultures of alveolar type II cells. Dipalmitoylphosphatidylcholine (DPPC) and dioleoylphosphatidylcholine (DOPC) were tested for their effects upon the ATP induced secretion of surfactant from type II cells, and the results are shown in Fig. 1A. The data presented in this figure provide clear evidence that DPPC, the major component of pulmonary surfactant, is capable of inhibiting surfactant secretion from alveolar type II cells in a concentration-dependent manner. In contrast, the dioleic lipid, DOPC, is ineffective as an inhibitor. The inhibitory action of DPPC is readily observed with agonist-stimulated cells but does not appear to signifi-

FIG. 1. Dipalmitoylphosphatidylcholine inhibits secretagogue-stimulated surfactant lipid secretion from alveolar type II cells. Primary cultures of type II cells were labeled overnight with [H]choline 0.5 μCi/ml in Dulbecco’s modified Eagle’s medium. The washed monolayers were then incubated either with or without agonists and liposomes for 3 h at 37°C. Phospholipids were recovered from the cells and media by lipid extraction. A, the concentration-dependent effects of dipalmitoylphosphatidylcholine and dioleoylphosphatidylcholine upon [H]phosphatidylcholine secretion from type II cells. Lipid and 10 μM ATP were added to the cells as indicated in the figure. B, the effects of DPPC upon [H]phosphatidylcholine secretion from type II cells stimulated to secrete with different agonists. Lipid and agonists were added at the concentrations shown in the figure. Values shown are the mean ± S.E. for four experiments. Significance: *, p < 0.05; **, p < 0.01.
cantly affect the basal level of secretion observed for unstimulated cells.

The specificity of the inhibitory activity of DPPC was examined with respect to the agonist used for inducing secretion and the results are presented in Fig. 1B. In type II cells ATP has been shown to mobilize Ca\(^{2+}\) via inositol trisphosphate and also act via protein kinase C (26). TPA acts via protein kinase C (27), terbutaline acts via cyclic AMP dependent protein kinase (21, 28), and ionomycin acts by increasing cytosolic Ca\(^{2+}\) (29, 30). The data presented in Fig. 1B demonstrate that dipalmitoylphosphatidylcholine is capable of blocking the secretion induced by each of these agonists. For the remaining experiments in this study, ATP was used as the agonist for secretion because it is the least hydrophobic agonist and the most unlikely to have significant affinity for liposomes.

In the context of these studies, we also sought to rule out several trivial explanations for the results. These possibilities included: 1) DPPC might enhance uptake of secreted \([^{3}H]PC\) into type II cells, 2) DPPC liposomes might settle out of solution and nonspecifically interfere with phospholipid secretion, and 3) during the incubation period, added DPPC liposomes might be rapidly taken up into the surfactant pool in type II cells and be secreted instead of \([^{3}H]PC\) in response to secretagogues. To test the first possibility, type II cells were incubated at 37°C for 20-22 h with or without \([^{3}H]\)choline. Type II cells labeled with \([^{3}H]\)choline were stimulated with ATP (10 \(\mu M\)) for 3 h, and then the secreted \([^{3}H]PC\) was harvested and transferred to unlabeled type II cells in the presence or absence of DPPC liposomes (100 \(\mu g/ml\)). Cells were further incubated at 37°C for 3 h, and then media and cells were separately processed for lipid extraction. Specific cell-associated \([^{3}H]PC\) was only 4% of total transferred \([^{3}H]PC\) in either the presence or absence of DPPC. Furthermore, the presence of DPPC liposomes in the media did not alter the recovery of \([^{3}H]PC\) that was secreted from type II cells. In addition, DPPC was added to \([^{3}H]\)choline-labeled type II cells 1 or 2 h after addition of ATP, but DPPC did not change the accumulated radioactivity of the medium from type II cells stimulated with ATP for 1 or 2 h (data not shown). The results rule out the possibility that DPPC is causing rapid uptake of newly secreted \([^{3}H]PC\).

To determine if precipitation of DPPC liposomes onto the cell layer was inhibiting secretion, the type II cells were incubated in a dish filled to the brim with media that was covered, sealed, and inverted. In this configuration any DPPC that settled out of solution would be unable to interact with the cell layer. DPPC also inhibited \([^{3}H]PC\) secretion from these cells (percent secretion: basal, 1.62 ± 0.19; ATP, 6.97 ± 0.74%; ATP + 100 \(\mu g/ml\) DPPC, 2.20 ± 0.31%; means ± S.E., \(n = 3, p < 0.01\) for ATP versus ATP + DPPC). These results indicate that DPPC liposomes do not act by physically accumulating at the cell surface and mechanically interfering with \([^{3}H]PC\) secretion from type II cells.

To determine if DPPC was displacing the labeled cellular PC pool, \([^{3}H]PPC\) liposomes (0.25 \(\mu Ci, 100 \mu g/ml\)) were prepared and added to type II cells in the presence of ATP. Cells were incubated at 37°C for 3 h, and then cell-associated \([^{3}H]PPC\) was measured. In addition, a subset of these cells was washed and further incubated at 37°C for 3 h with ATP (10 \(\mu M\)) and unlabeled DPPC (100 \(\mu g/ml\)) to measure lipid secretion. After the first 3-h incubation, cell-associated \([^{3}H]PPC\) was 2.7 ± 0.8 \(\mu g/\)monolayer (mean ± S.E., \(n = 3\)). As cell-derived PC is 50 \(\mu g/\)monolayer, only 5.4% of the cellular PC pool was taken up from exogenous DPPC. Moreover, \([^{3}H]PPC\) taken up into type II cells did not appear in the medium during the second 3-h incubation, suggesting that added DPPC is not secreted in lieu of cell-derived PC. This latter result demonstrates that exogenous DP CPC does not displace the intracellular PC pool destined for secretion. Collectively, the above results provide strong evidence that DPPC is acting as a specific inhibitor of surfactant lipid secretion by alveolar type II cells in vitro.

The Regulatory Effect of DPPC upon Exocytosis Is Specific for Alveolar Type II Cells—The finding that DPPC inhibits the secretion of surfactant raises the question of whether this property is restricted to alveolar type II cells or applies to other secretory cells. This problem was addressed by examining the effects of DPPC upon \([^{3}H]\)norepinephrine secretion by primary cultures of adrenal chromaffin cells. The results of these experiments are shown in Fig. 2. Treatment of cultures of chromaffin cells with DPPC was without effect upon nicotine-induced secretion of \([^{3}H]\)norepinephrine from these cells. This finding demonstrates that the interaction of DPPC with alveolar type II cells is specific and probably involves a unique system present in the lung cells for interacting with extracellular lipid.

Structural Specificity of Lipid-mediated Inhibition of Surfactant Secretion—The specificity of the phospholipid-dependent inhibition of surfactant secretion was next investigated. Dipalmitoylphosphatidylcholine molecules with the polar moiety in the sn-3 position \((L)\) DPPC were compared with those having the polar moiety in the sn-1 position \((D)\) DPPC). The results of this experiment are shown in Fig. 3. Both stereoisomers were equally effective as inhibitors of ATP-induced surfactant secretion from isolated type II cells. These findings provide clear evidence that the antagonist action of phosphatidylcholine occurs via a mechanism that is independent of lipid stereochemistry.

To further evaluate the components of lipid structure that affect surfactant secretion, the action of dipalmitoylphosphatidyl(N,N-monomethyl)ethanolamine, dipalmitoylphosphatidyl(N,N-dimethyl)ethanolamine, and dipalmitoylphosphatidylcholine upon this process was compared. The results of these experiments are presented in Fig. 4 and demonstrate that the potency of inhibition among these structurally related lipids varies with the extent of methyl group substitution of the ethanolamine moiety (choline is more inhibitory than dimethylethanolamine which is more inhibitory than monomethylethanolamine). This finding suggests that part of the

![Fig. 2. DPPC does not inhibit \([^{3}H]\)norepinephrine secretion from bovine adrenal medullary chromaffin cells. The chromaffin cells were prelabeled with \([^{3}H]\)norepinephrine (2 \(\mu Ci/2 ml\)) for 60 min and washed as described under "Materials and Methods." Basal and 100 \(\mu M\) nicotine-induced secretion of \([^{3}H]\)norepinephrine in the presence and absence of 200 \(\mu g/ml\) DPPC was measured. Values shown are the average of duplicate determinations in two independent experiments.](image-url)
A phosphatidylcholine is not stereospecific. The secretion experiments were conducted at 37 °C for 3 h after the addition of agonists were added. Where indicated ATP was added to a final concentration of 10 μM. d- and l-dipalmitoylphosphatidylcholine were added to a final concentration of 100 μg/ml. The secretion experiments were conducted at 37 °C for 3 h after the addition of agonists and antagonists. Values are the mean ± S.E. for three experiments. The difference between the inhibition caused by either isomer is not significant, whereas the difference between each isomer and agonist alone is significant with a p < 0.01.

specificty for inhibition is attributable to the choline moiety of phospholipid.

Inhibition of Surfactant Secretion by Phosphatidylcholine Occurs in a Phase Transition Temperature-dependent Manner—The next series of experiments were designed to investigate the acyl chain specificity of the phosphatidylcholine-mediated inhibition of surfactant secretion. To examine this aspect of lipid-mediated inhibition, 15 different species of saturated and unsaturated phosphatidylcholines (all 100 μg/ml) were prepared and tested for their inhibitory activities upon [3H]PC secretion from type II cells at 37 °C (Fig. 5). Three unsaturated phosphatidylcholines did not inhibit [3H]PC secretion (Fig. 5A). In contrast, disaturated phosphatidylcholines exhibited various degrees of inhibition (Figs. 5B and 5C). This result indicates that the inhibitory action of DPPC is unlikely to be attributable solely to the fact that it contains saturated fatty acids. Phospholipids exhibit discrete gel to liquid crystalline phase transition temperatures (Tc) (31) and we analyzed the inhibitory activity of the lipids as a function of Tc (Fig. 2, B and C). The analysis reveals that the inhibitory activity of disaturated phosphatidylcholines increases with increasing Tc. The correlation was significant (Spearman correlation: r = -0.914, p < 0.001; Kendall correlation: r = 0.763, p < 0.001) and, when the lipids were divided into two groups (Fig. 2, B and C) according to Tc with 37 °C (incubation temperature) as a border, the averaged values of percent ATP-stimulated secretion of disaturated phosphatidylcholines in each group showed a significant difference (B: 82.6 ± 4.9% (n = 5); C: 30.2 ± 7.4% (n = 7), means ± S.E., p < 0.001). These results clearly indicate that disaturated PCs inhibit [3H]PC secretion in a Tc-dependent manner. As disaturated PCs are in the gel state at temperatures below Tc, these results suggested that type II cells can selectively recognize this physical state of disaturated phosphatidylcholines and regulate phospholipid secretion. To further test this hypothesis, we sought to determine if the inhibitory activity of phospholipids that are below their Tc at 37 °C was diminished with increased incubation temperature. The optimal range of experimental temperatures for measuring secretion was from 30 to 42 °C (Fig. 6A). At temperatures below 30 °C, type II cells showed a poor secretory response to ATP and at temperatures above 42 °C cell damage was induced. Therefore, DPPC and 1-myristoyl(C14:0)-2-palmitoyl(C16:0) PC, which have phase transition temperatures in the range from 30 to 42 °C, were chosen for the experiment. As demonstrated in Fig. 6B, DPPC does not inhibit [3H]PC secretion at 42 °C (above Tc), although it inhibits [3H]PC secretion at temperatures below Tc. 1-Myristoyl(C14:0)-2-palmitoyl(C16:0) PC shows a similar pattern to DPPC with respect to its phase transition temperature. Dimyristoyl PC and distearoyl PC were also included in the experiment as negative and positive controls for inhibition of secretion. Predictably, dimyristoyl PC (Te = 23.5 °C) did not inhibit secretion in the range from 30 °C to 37 °C and distearoyl PC (Te = 55.0 °C) inhibited secretion in the range from 37 °C to 42 °C. This result clearly indicates that disaturated PCs inhibit [3H]PC secretion in a Tc-dependent manner, and suggests that type II cells recognize the physical state of disaturated PCs.

We next sought to make a distinction between fluid and nonfluid phospholipid environments as mediators of the inhibitory effect of a given phospholipid. In these experiments DPPC was added to type II cells in either the same liposome
expressed as percent secretion (means ± S.E., n = 4 experiments). Significant values: **, p < 0.01, when compared to +DPPC; §, p < 0.05, when compared to +DPPC+DOPC.

as DOPC or in a separate population of liposomes from DOPC. The liposomes composed of both phospholipids form a monotropic system with a broad Tc for DPPC and an enthalpic maximum centered around 30 °C (32). The results of this experiment are presented in Fig. 7 and demonstrate that, when the 41 °C phase transition of DPPC is ablated by the admixture of DOPC to the liposome, the inhibitory activity of DPPC upon ATP-stimulated surfactant secretion is lost. In contrast, the addition of separate DOPC liposomes to DPPC liposomes does not significantly alter the inhibitory effect of DPPC. These results provide additional evidence that the physical state of phosphatidylcholine can function to regulate surfactant secretion. In addition to these experiments, we conducted secretion studies with lipids derived from pulmonary surfactant. Liposomes prepared from total surfactant lipids are randomly mixed and should give results similar to those obtained with mixtures of DOPC and DPPC. Results from these studies reveal that random mixtures of surfactant lipids fail to significantly inhibit secretion of surfactant from type II cells (basal secretion = 2.1%, ATP stimulated secretion = 10.5% and ATP stimulated secretion + 200 μg/ml surfactant liposomes = 9.0%, n = 2 with duplicate dishes for each experiment).

**DISCUSSION**

The results presented in this report provide clear evidence that phosphatidylcholine can regulate surfactant secretion in vitro. The inhibitory activity occurs with saturated phosphatidylcholines at temperatures below their gel-liquid crystal phase transition. At 37 °C dipalmitoylphosphatidylcholine is a potent inhibitor of surfactant secretion. At concentrations of 100 μg/ml, DPPC can reduce agonist induced secretion at 37 °C by 70–90%. The inhibitory activity of DPPC is observed with protein kinase A-, protein kinase C-, and Ca2+-dependent pathways for inducing secretion. This finding suggests that DPPC acts at a relatively late stage in the signaling cascade for exocytosis. Thus DPPC may inhibit secretion at a similar or identical step to that for the major surfactant protein, SP-A, which can also inhibit induced secretion at a step distal to a Ca2+ transient. These results support the concept that the major protein and lipid components of surfactant are capable of feedback control of their own secretion.

The inhibition of surfactant lipid secretion mediated by DPPC is specific and does not appear to be attributable to either facilitating rapid uptake of newly secreted surfactant or nonspecific precipitation onto the cell surface or displacement of the [3H]phosphatidylcholine pool destined for secretion.

Three lines of evidence support the hypothesis that the physical state of phosphatidylcholine is the important factor governing inhibition of surfactant lipid secretion. First, the inhibitory activity observed at 37 °C for different phosphati-
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dylcholines closely follows the phase transition temperatures of these lipids such that lipids below their Tc are inhibitory whereas those above their Tc are either not inhibitory or only modestly so. Second, the inhibitory activity of a given phospholipid can be changed by simply adjusting the temperature of the experiment as shown in Fig. 6. Thus 1-myristoyl-2-palmitoylphosphatidylcholine inhibits agonist-induced lipid secretion by 77-86% at temperatures below Tc (35.5 °C) and by 26-40% at temperatures above Tc. The results are more striking for the physiologically relevant dipalmitoylphosphatidylcholine with 90-100% inhibition of stimulated secretion at temperatures below Tc (41 °C) and 20% inhibition at temperatures above Tc. For both of these lipids, (as well as others) it is also expected that the formation of liposomes by sonication will modestly reduce Tc by approximately 2-5 °C and significantly broaden the temperature interval for the transition (33), thus making the temperatures farthest from the ideal Tc the most reliable for evaluating the general phenomenon. Third, the reduction of Tc of DPPC by the addition of DOPC to the liposome significantly reduces the inhibitory activity of the lipid upon stimulated secretion. In addition, for DPPC the inhibition is not stereospecific, since the same results are obtained when the phosphocholine moiety is located on either the sn-1 or the sn-3 carbon of the glycerol backbone. This latter observation indicates that some parameter other than specific molecular stereochemistry is being recognized by the type II cell.

A number of factors make it highly probable that the action of DPPC upon type II cells is physiologically relevant. The levels of DPPC used in these experiments are consistent with levels present in the alveolus (1). Lavage of rat lungs yields approximately 2 mg of phospholipid, of which DPPC is approximately 50% of the total. Although the volume of the extracellular alveolar aqueous compartment is unknown, it is almost certainly less than 100 µl. This means that the concentration of DPPC in the alveolus is nominally 10 mg/ml. Wright and Clements (1) have estimated that approximately 50-65% of the alveolar DPPC is present at the air-liquid interface. Thus, the surface layer might be expected to contain 500 µg of DPPC. Using physical measurements, Hildebrand (34) has estimated that the monolayer of lipid at the air-liquid interface is at least 90% DPPC at reduced alveolar volume. Additional physical measurements suggest that the compressed lipid film is highly viscous and probably solid and that these solid domains do not fully intermix with unsaturated lipids upon expansion of the surface film but remain as discrete domains (summarized in Ref. 19). If 5% of the surface layer were to re-enter the hypophase as pure DPPC, its concentration would be minimally 250 µg/ml, a level that readily regulates exocytosis by isolated type II cells. The principal elements of this model for regulation are shown in Fig. 8. It is important to note that newly secreted DPPC in the hypophase will not be inhibitory because it is a mixture of saturated and unsaturated lipid. In contrast, the specific accretion of DPPC at the air-liquid interface (which is supported by physical studies) (19, 20, 34) yields a population of lipid with regulatory potential based upon the results obtained in this report. The regulation that we propose, however, can only occur if a portion of the monolayer enters the hypophase without mixing with the unsaturated lipid also present in the hypophase. This latter assumption seems plausible owing to the poor mixing properties of gel phase and fluid phase lipid and the specificity of other molecules such as SP-A for binding to DPPC.

The mechanism by which type II cells detect the physical state of phosphatidylcholine is completely unknown and represents a new dimension in the field of exocytosis and signal transduction. It is difficult to envision a receptor that is capable of directly sampling the physical state of exogenous lipid. However, surfactant protein A is known to have a high affinity for both saturated phosphatidylcholine and a cell surface receptor, and it is possible that a receptor-SP-A-phospholipid ternary complex may form on the cell surface. Although SP-A was not added during any of the secretion experiments described in this report, we cannot rule out that low levels of this protein may be bound to the surface of the cells. Alternatively DPPC may selectively fuse with the plasma membrane of type II cells and induce structural changes that inhibit exocytosis. This latter mechanism might also utilize SP-A, since this protein has been shown to promote lipid uptake by an undefined mechanism. Another possibility is that DPPC in the gel state may fuse directly with the plasma membrane of type II cells without interacting with any of the surfactant proteins. In order for this latter process to be specific, there would still need to be a macromolecule in the plasma membrane that recognized gel phase phospholipid and promoted its fusion into the plasma membrane.

In summary, DPPC inhibits the HJPC secretion from alveolar type II cells. The inhibition was dose-dependent, cell type-specific, and showed no secretagogue specificity. The inhibitory activity of DPPC was lost at temperatures above the phase transition and other disaturated PCs showed phase transition temperature-dependent inhibition of surfactant secretion. Addition of unsaturated PC to liposomes containing DPPC ablated the inhibitory activity of DPPC. From these results we conclude that type II cells can recognize the physical state of surfactant phospholipids and regulate surfactant secretion accordingly. These results suggest that alveolar type II cells possess a novel mechanism for sensing the quantity and composition of lipid in the alveolar hypophase, and this may be an indirect method of detecting the composition of the film present at the air/water interface within the lung.

Acknowledgments—We thank Cathy Queen and Peggy Hammond for excellent secretarial assistance.

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