SP-A is necessary for increased clearance of alveolar DPPC with hyperventilation or secretagogues

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SP-A is necessary for increased clearance of alveolar DPPC with hyperventilation or secretagogues. Am J Physiol Lung Cell Mol Physiol 284: L759–L765, 2003. First published January 10, 2003; 10.1152/ajplung.00200.2002.—The role of surfactant protein-A (SP-A) in pulmonary uptake and metabolism of [3H]dipalmitoylphosphatidylcholine ([3H]DPPC) was studied in SP-A gene-targeted mice (SP-A−/−). Unilamellar liposomes were instilled into the trachea of anesthetized mice. Uptake was measured as dpm in lungs plus liver and kidney for in vivo experiments and in lungs and perfusates for isolated lung experiments. [3H]DPPC uptake increased with CO2-induced hyperventilation in wild-type mice (SP-A+/+) but was unchanged in SP-A−/−. Secretagogue treatment approximately doubled the uptake of [3H]DPPC in isolated lungs from SP-A+/+ but had no effect in SP-A−/−. Lungs degraded 23 ± 12% of internalized [3H]DPPC in SP-A+/+ and 36 ± 6% in SP-A−/−; degradation increased with 8-bromoadenosine 3’,5’-cyclic monophosphate in SP-A+/+ but was unchanged in SP-A−/−. Activity of lysosomal-type phospholipase A2 (PLA2) was significantly greater in lungs from SP-A−/− compared with SP-A+/+. Thus SP-A is necessary for lungs to respond to hyperventilation or secretagogues with increased DPPC uptake and also modulates the PLA2-mediated degradation of internalized DPPC.

PULMONARY SURFACTANT is a highly conserved, heterogeneous mixture of lipids and proteins that is essential for normal lung function. Because either an excess or deficit of surfactant is deleterious, surfactant concentration is stringently regulated by pathways that involve synthesis, storage, secretion, and reuptake of surfactant lipid and protein components (9). A part of the internalized surfactant phospholipid is routed to lamellar bodies for resecretion, whereas the rest is degraded by lysosomes (9). To maintain a normal extracellular pool, the rate of surfactant clearance over the long term must equal the rate of secretion. Thus increased secretion due to hyperventilation or to administration of a secretagogue leads to increased clearance (11, 13, 26, 27).

Clearance of L-α-dipalmitoylphosphatidylcholine (DPPC) in the intact lung is primarily by type II alveolar epithelial cells with a contribution by alveolar macrophages (8, 9, 29, 34). The use of inhibitors has suggested that endocytosis of DPPC by type II cells occurs by both clathrin-dependent and clathrin-independent pathways (2, 25). However, the precise ligand for uptake via the clathrin-dependent pathway is not known. Because surfactant protein A (SP-A) binding to type II cells is clustered in coated pits (30) and some in vitro studies have demonstrated a SP-A-mediated increase in DPPC uptake (1, 33), it has been proposed that the clathrin-dependent process occurs via receptor-mediated uptake of a DPPC/SP-A complex (9, 32). However, comparison of wild-type (SP-A+/+) and SP-A gene-targeted (SP-A−/−) mice showed similar rates of surfactant lipid clearance (19) and similar alveolar phospholipid pool sizes (20, 22). Because regulation of extracellular surfactant pool size is crucial to survival, redundancy and excess capacity in the clearance pathway might be expected. Thus we postulated that SP-A-deficient mice might have normal basal uptake but might show abnormalities in clearance when the rate of surfactant turnover is increased during physiological stresses. To test this hypothesis, we studied DPPC clearance in intact mice with CO2-stimulated hyperventilation and in isolated mouse lungs perfused with secretagogues. We utilized a newly developed SP-A gene-targeted mouse as a model of SP-A deficiency.

MATERIALS AND METHODS

Authentic lipids were obtained from Avanti (Birmingham, AL). Radiochemicals were from New England Nuclear (Boston, MA). 8-Bromoadenosine 3’,5’-cyclic monophosphate (8-Br-cAMP), 12-O-tetradecanoylphorbol-13-acetate (TPA), bovine serum albumin (BSA), and fatty acid-free BSA were from Sigma (St. Louis, MO). 1-Hexadecyl-3-trifluoroethylglycerol-sn-2-phosphomethanol (MJ33), a
specific inhibitor of lysosomal-type phospholipase A\(_2\) activity in lungs (10), was a kind gift from Dr. Mahendra Jain, University of Delaware.

*Generation of SP-A knockout mice.* The SP-A gene was targeted in F1 BL/6/129Sv embryonic stem cells by standard homologous recombination (17). Briefly, the replacement-type targeting vector replaced exons 2, 3, and 4, including the translation start site for murine SP-A, and short segments of flanking intronic sequence. Targeted clones were injected into day 2.5 postcoital eight-cell to morula-stage CD1 zygotes and transferred to pseudopregnant B6D2 females. Chimeric offspring were bred with albino CD1 female mice, and their pups were screened by PCR and Southern analysis for germ-offspring were bred with albino CD1 female mice, and their pups were screened by PCR and Southern analysis for germ-line transmission of the mutant allele. Heterozygous BL6/129Sv-CD1 mice were backcrossed onto a C57BL6 background. Mice used for the initial part of this study were the 6th backcross generation bred at University of California San Francisco. Subsequent experiments used mice from the 10th backcross generation that were the progeny of a breeding pair confirmed the absence of the normal SP-A gene. Southern analysis was used to confirm the absence of the mutant allele in the lung and of SP-A protein in the lung lavage (Western blot).

*Preparation of liposomes.* Stock solutions of DPPC, egg phosphatidylcholine (PC), egg phosphatidyl glycerol, and cholesterol were mixed in a molar ratio of 10:5:2:3 with trace amounts of [choline-methyl-\(^{3}H\)]DPPC (\(^{3}H\)DPPC) to prepare liposomes (10). The phospholipid mixture was evaporated to dryness under \(N_2\), resuspended in phosphate-buffered saline (pH 7.4), and mixed vigorously. The suspension was subjected to three cycles of freezing under liquid nitrogen followed by immediate thawing at 50\(^{\circ}\)C. The phospholipid suspension was extruded under pressure through a 100-pore-size filter. Liposomes were stored at 4\(^{\circ}\)C and used within 1 wk of preparation.

*In vivo uptake of DPPC by mice and effect of hyperventilation.* Mice were anesthetized with intraperitoneal xylazine (5.2 mg/kg) plus ketamine (40 mg/kg), and liposomes (10 nmol of DPPC in 20 \(\mu\)l of saline) were instilled intratracheally with a Hamilton syringe. The instilled dose was corrected for dpm remaining in the syringe plus dpm accumulated by the trachea and major bronchi measured at the end of the experiment. This correction represented <10% of instilled dpm. After recovery from anesthesia (within 20 min), mice were put into an acrylic chamber that was continuously flushed with 5% \(CO_2\) in air. Mice treated similarly, but allowed to breathe room air, were used as controls. After 3 h, mice were anesthetized with pentobarbital sodium (50 mg/kg body wt), and liver, kidney, and lungs were removed. Lungs were lavaged five times with 1-ml aliquots of saline, and lavage was collected. The trachea and major bronchi were separated from the lung parenchyma. The organs were then homogenized and counted for radioactivity. Uptake in tissues was calculated as the sum of dpm in lung, liver, and kidneys, and data were expressed as tissue dpm/lavage dpm.

*In additional mice, ventilation parameters during air or CO\(_2\)-breathing were measured using whole-body plethysmography (Buxco Electronics, Sharon, CT) (16). Briefly, each mouse was placed in the chamber, and the chamber pressure-time wave was measured continuously via a transducer connected to a computerized data-acquisition system. After baseline measurements for 30 min to ensure a steady state, frequency of breathing, tidal volume, and minute volume were recorded at 7.5-min intervals for 90 min during exposure either to air or 5% CO\(_2\). 

*Isolated lung perfusion.* Mice were anesthetized with intraperitoneal pentobarbital sodium (50 mg/kg body wt). \(^{3}H\)DPPC-labeled liposomes (10 nmol of DPPC in 20 \(\mu\)l of saline) were instilled into the lungs through a cannula placed at the level of the tracheal carina. The mice were then continuously ventilated, the thorax was incised, and lungs were cleared of blood by perfusion through the pulmonary artery with buffer (Kreb-sRinger bicarbonate, pH 7.4, with 3% BSA). The total isolation procedure required ~5 min. Isolated lungs were perfused in a closed-circuit recirculating perfusion apparatus similar to that described previously for rat lungs (15). Perfusion buffer was the same as lung clearing buffer plus 10 mM glucose added. In some experiments, either 8-Br-cAMP (0.1 mM) or TPA (30 ng/ml) was added to the perfusate at the start of perfusion. Perfusate was gassed constantly with 5% \(CO_2\) in air. Lungs were ventilated at 60 cycles/min (0.3 liter/minute), and \(2\text{ cm}_2\text{O}_2\) end-expiratory pressure. There was no significant change in the ventilation and perfusion pressures or overt evidence of lung edema during the experiments.

*DPPC uptake.* At the end of the ~5-min period required for lung isolation (baseline) or the 2-h experimental perfusion, lungs were lavaged five times with 1-ml aliquots of ice-cold saline. Lung tissue was then homogenized in saline on ice with a Polytron homogenizer followed by a motorized mortar and pestle. Lung homogenate and perfusate aliquots were counted for dpm to calculate lung uptake of \(^{3}H\)DPPC (dpm in the lung plus perfusate/total dpm instilled) (13). To calculate alveolar phospholipid pool size, we measured lipid phosphorous in the organic extract of lung lavage after cells were removed by centrifugation at 300 g for 10 min (10).

*DPPC metabolism.* Lipid and aqueous fractions from the lung homogenate were extracted by the Bligh and Dyer procedure (3). Phospholipids in the organic phase were fractionated by thin-layer chromatography on silica gel plates with chloroform-methanol-ammonia-water (65:35:2.5:2.5, vol/vol) as the solvent system (10), and bands of interest were scraped and counted for dpm. The disaturated phosphatidylcholine (DSPC) fraction was separated from total PC on a neutral alumina column after osmication of lipids (23). Unsaturated PC, representing PC in which fatty acyl components have one or more double bonds, was calculated as dpm in total PC minus dpm in DSPC. This fraction represents resynthesized PC, since all dpm were initially in DSPC (i.e., DPPC). Degradation of internalized DPPC was calculated from the sum of dpm in lung fractions (lysoPC, aqueous, and unsaturated PC) plus dpm in perfusate. This estimate does not include counts from DPPC metabolites that have been reincorporated into DSPC.

Lysosomal-type PLA\(_2\) activity of lung homogenates was measured in Ca\(^{2+}\)-free, acidic (pH 4.0) buffer and also in pH 8.5 buffer in the presence of 10 mM Ca\(^{2+}\) (14). Substrate for both assays was liposomes with \(^{3}H\)DPPC labeled in sn-2 palmitate as described previously (14).

*Statistical analysis.* All results are expressed as means \pm SE. Multiple group comparisons were done by one-way analysis of variance with Bonferroni's correction or with the Kruskal-Wallis method. Comparison of groups vs. a control was done using Dunn's method or Tukey's test using SigmaStat software version 2.0 (Jandel, San Rafael, CA). \(P < 0.05\) was considered statistically significant.
RESULTS

**DPPC uptake in vivo.** We evaluated the alveolar uptake of DPPC and the effect of hyperventilation in SP-A +/+ and SP-A −/− mice. Recovery of total radioactivity at 3 h after instillation of 3H-labeled liposomes was measured as the sum of dpm in lung lavage fluid, lung tissue, liver, and kidney. Recovery for both SP-A +/+ and SP-A −/− mice varied from 68 to 98% of instilled dpm under control and CO2 exposure conditions, indicating that a variable fraction of dpm ended up in other organs. There was no difference in recoveries between control and CO2-stimulated conditions, although recoveries in both conditions were slightly greater for SP-A −/− compared with SP-A +/+ mice (Table 1). In control SP-A +/+ mice, 55% of instilled dpm were recovered in the lung lavage, 23% in the lung tissue, and 13% in liver and kidney; the distribution of dpm in control SP-A −/− mice was similar.

Compared with room air breathing, SP-A +/+ mice exposed to CO2 showed decreased recovery of dpm in the lavage and increased dpm in lung plus peripheral organs (Table 1), resulting in a significant increase in the tissue to lavage ratio (Fig. 1). The ratio of recovered dpm in SP-A +/+ increased from 0.68 under control conditions to 1.2 with hyperventilation (P < 0.05). These results indicate a hyperventilation-induced increase in uptake of DPPC. The ratio of recovered dpm under control conditions was similar for SP-A −/−, but there was no significant change in the uptake or the tissue to lavage ratio with hyperventilation in SP-A −/− mice (Table 1 and Fig. 1).

The ventilatory response to CO2 was documented by plethysmography. There was no change in respiratory frequency with CO2 but a significant increase in tidal volume, resulting in a 50% increase in minute ventilation. The ventilatory responses to CO2 were similar in SP-A +/+ and SP-A −/− mice, showing that differences in ventilation were not responsible for the differences in DPPC uptake (Table 2).

Dpm recovery in the liver and kidney of mice presumably represents delivery of 3H-labeled products of DPPC degradation to peripheral organs through the circulation. To evaluate this assumption, we added 3 mol% MJ33, an inhibitor of lysosomal-type PLA2 activity, to the instilled liposomes (10). Hyperventilating SP-A +/+ mice instilled with MJ33 showed significantly less liver and kidney dpm than their corresponding controls (control mice: liver, 13%; kidneys, 5%; MJ33-instilled mice: liver, 4%; kidney, 1%) consistent with inhibition of 3H,DPPC degradation.

**DPPC uptake in isolated perfused lungs.** 3H,DPPC present in isolated lungs from SP-A +/+ mice at the start of perfusion (5 min after instillation of 3H,DPPC-labeled liposomes) was 4.4 ± 0.1% of instilled dpm (n = 7) and was similar in SP-A −/− mice (4.3 ± 0.09%, n = 3). This baseline uptake, shown previously to represent primarily the rapid exchange of DPPC with nonlavageable endogenous pools (11), was subtracted from lung tissue values at subsequent time points. Baseline uptake is the same for control and secretagogue-stimulated lungs, since it is measured before the start of secretagogue infusion. Lung uptake of 3H,DPPC during the subsequent 2 h of perfusion in the absence of secretagogues was 5.1 ± 0.4% of instilled and was comparable for SP-A +/+ and SP-A −/− mice (Fig. 2). However, the lungs from SP-A −/− mice responded differently from wild-type mice to secretagogues. With SP-A +/+ mice, pulmonary uptake of 3H,DPPC was significantly stimulated by 2.2-fold by treatment with secretagogues (8-Br-cAMP, TPA), whereas secretagogues had no effect on uptake by lungs from SP-A −/− mice (Fig. 2).

**Phospholipid pool size.** Phospholipid pool size was measured in the alveolar lavage fluid of control mice and after 3 h of exposure to 5% CO2 and also in the isolated lung after 2 h of perfusion. For intact mice, the measurement represents the endogenous pool in the absence of added lipid, whereas measurements in the

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**Table 1. Total recoveries of instilled [3H]DPPC from lungs**

<table>
<thead>
<tr>
<th>Total Recovery</th>
<th>Lavage</th>
<th>Tissue</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>5% CO2</td>
</tr>
<tr>
<td>SP-A +/+</td>
<td>61.4 ± 4.5*</td>
<td>61.2 ± 4.5*</td>
</tr>
<tr>
<td>SP-A −/−</td>
<td>74.2 ± 3.8*</td>
<td>70.3 ± 3.9*</td>
</tr>
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</table>

Values are means ± SE for n = 8–14. *P < 0.05 for comparison of surfactant protein-A (SP-A) +/+ and SP-A −/−.
perfused lung were made at 2 h after instillation of liposomes. The amount of phospholipid instilled in liposomes represented ~5% of the endogenous pool. The pool size was not different between SP-A+/+ and SP-A−/− mice and did not change significantly following treatment with CO2 exposure or with secretagogues (Table 3).

**DISCUSSION**

Surfactant phospholipid secretion responds to a wide variety of mediators, including β-adrenergic agonists such as 8-Br-cAMP and protein kinase C agonists such as phorbol esters (4, 24, 31). Studies with these mediators in intact lungs or in alveolar type II cells in culture have shown that they also promote cellular uptake of phospholipid, thus giving rise to the concept that surfactant secretion and reuptake are linked (7, 9, 13). In support of this hypothesis, hyperventilation induced by exposure to CO2 or by exercise stimulated both surfactant secretion as well as reuptake (26, 27). Studies with isolated type II cells in culture suggested that SP-A plays a role in surfactant homeostasis, since the addition of SP-A to liposomes augmented cellular uptake of DPPC (1, 33). Furthermore, SP-A was shown to inhibit the lysosomal-type phospholipase A2, the enzyme responsible for degradation of internalized DPPC (14). However, SP-A gene-targeted mice had no significant derangement of DPPC homeostasis under

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**Table 2. Effect of 5% CO2 on lung ventilation**

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>5% CO2</th>
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<tbody>
<tr>
<td><strong>SP-A+/+</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Frequency of respiration, breaths/min</td>
<td>343 ± 35</td>
<td>350 ± 7</td>
</tr>
<tr>
<td>Tidal volume, ml</td>
<td>0.31 ± 0.05</td>
<td>0.48 ± 0.06*</td>
</tr>
<tr>
<td>Minute ventilation, ml/min</td>
<td>110 ± 23</td>
<td>165 ± 21*</td>
</tr>
<tr>
<td><strong>SP-A−/−</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Frequency of respiration, breaths/min</td>
<td>332 ± 25</td>
<td>333 ± 22</td>
</tr>
<tr>
<td>Tidal volume, ml</td>
<td>0.37 ± 0.01</td>
<td>0.53 ± 0.02*</td>
</tr>
<tr>
<td>Minute ventilation, ml/min</td>
<td>119 ± 6.0</td>
<td>173 ± 7.0*</td>
</tr>
</tbody>
</table>

Values are means ± SE for n = 3 mice under each condition. Ventilation parameters were measured in mice under control conditions (room air breathing) followed by exposure to 5% CO2. Each individual measurement represents the mean of measurements made at 7.5-min intervals over a 90-min period. None of the differences between SP-A+/+ and SP-A−/− are statistically significant (P > 0.05). *P < 0.05 vs. corresponding control.
basal physiological conditions (18, 19). These results do not exclude the possibility that the role of SP-A might be critical when the rate of pulmonary surfactant turn-over is increased. The objective of the present study was to use genetically transformed SP-A−/− mice to evaluate the role of SP-A in DPPC uptake and metabolism under resting and stimulated conditions.

Hyperventilation with CO₂ was chosen as a model for stimulation of DPPC uptake in intact mice. Hyperventilation significantly enhanced the uptake of intra-tracheally administered [³H]DPPC by the lungs of SP-A+/+ mice as demonstrated by decreased dpm remaining in the lung lavage and increased dpm in the lung tissue plus distal organs (liver and kidney). Using a PLA₂ inhibitor, we demonstrated that liver and kidney dpm arise from degradation of [³H]DPPC in the lung and are presumably transported to the distal organs via the blood stream. Although SP-A+/+ and SP-A−/− mice exposed to CO₂ had a similar degree of hyperventilation, only the wild-type mice showed increased DPPC uptake. These results suggest that SP-A plays a crucial role in the increased DPPC uptake associated with the physiological stress of hyperventilation.

We extended these investigations to in vitro conditions to more closely regulate physiological parameters. The isolated perfused lung model has been used extensively to investigate the synthesis and secretion of alveolar phospholipids as well as to study clearance of phospholipid from the alveoli (4, 10–13). Similar to previous results with rat lungs (11, 13), isolated perfused lungs of SP-A+/+ mice exhibited increased uptake of DPPC in response to secretagogues (cAMP, TPA). On the other hand, SP-A−/− mice showed no change in DPPC uptake in the presence of secretagogues, confirming the differences observed between wild-type and gene-targeted mice with in vivo experiments.

Endocytosed DPPC may be either directly recycled or degraded with subsequent reutilization of degradation products (9). Perfused lungs of SP-A−/− mice showed a significantly higher level of degradation of internalized DPPC under basal conditions compared with wild-type controls. These results suggest that SP-A regulates metabolism of internalized DPPC and are consistent with our previous studies of isolated granular pneumocytes (14). These cells showed a lower rate of degradation of PC in natural surfactant compared with liposomes alone and a decrease in the degradation of liposomal DPPC with added SP-A. This effect can be explained by in vitro experiments showing that SP-A inhibits lysosomal-type PLA₂ activity, the enzyme responsible for the initial step in degradation of DPPC (14). Activity of lysosomal-type PLA₂ was significantly higher in lungs of SP-A−/− mice compared with the wild type, compatible with a loss of PLA₂ inhibition. As an alternative mechanism, SP-A could direct intracellular trafficking of the phospholipid-SP-A complex toward a nondegrading compartment in type II cells (33), thereby promoting recycling of surfactant components. Thus the absence of endogenous SP-A could result in failure of targeting and consequent increased rate of DPPC degradation.

### Table 3. Pool size of alveolar total phospholipid in SP-A+/+ and SP-A−/− mice

<table>
<thead>
<tr>
<th>Lung Condition</th>
<th>Intact Mice</th>
<th>Perfused Lung</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>244 ± 9.9</td>
<td>255 ± 3.0</td>
</tr>
<tr>
<td>5% CO₂</td>
<td>237 ± 13.0</td>
<td>251 ± 8.4</td>
</tr>
</tbody>
</table>

Values are means ± SE for intact mice (n = 5) and for control perfused lung and 8-bromoadenosine 3’,5’-cyclic monophosphate (8-Br-cAMP) (n = 3) and means ± range for 12-O-tetradecanoylphorbol-13-acetate (TPA) (n = 2). Phospholipid content was measured in the lavage fluid obtained after 3 h of CO₂ exposure in intact mice or after 2 h of isolated lung perfusion. For isolated lungs experiments, lungs were instilled with liposomes containing 10 nmol of DPPC (equivalent to 12.75 µg total phospholipid) and then were isolated and perfused under control conditions (no additions) or in the presence of either 0.1 mM 8-Br-cAMP or 30 ng/ml of TPA. None of the differences between SP-A+/+ and SP-A−/− are statistically significant (P > 0.05). of SP-A and DPPC clearance

### Table 4. Recovery of dpm in lung fractions after isolated lung perfusion

<table>
<thead>
<tr>
<th>Lung Fraction</th>
<th>SP-A+/+</th>
<th>SP-A−/−</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>% of recovered dpm</td>
<td>% of recovered dpm</td>
</tr>
<tr>
<td>Control</td>
<td>77.5 ± 1.6</td>
<td>70.3 ± 1.0</td>
</tr>
<tr>
<td>8-Br-cAMP</td>
<td>58.4 ± 0.6*</td>
<td>71.0 ± 1.2</td>
</tr>
<tr>
<td>Unsaturated PC</td>
<td>12.6 ± 1.2</td>
<td>19.7 ± 1.9</td>
</tr>
<tr>
<td>LysoPC</td>
<td>1.0 ± 0.04</td>
<td>3.8 ± 0.3</td>
</tr>
<tr>
<td>Aqueous fraction</td>
<td>9.2 ± 0.3</td>
<td>19.8 ± 0.5*</td>
</tr>
<tr>
<td>Total degradation</td>
<td>22.8 ± 1.2</td>
<td>36.5 ± 0.5</td>
</tr>
</tbody>
</table>

Values are means ± SE for n = 5. Lungs were perfused for 2 h after liposome instillation and then extracted, separated into lipid classes, and analyzed for dpm. Aqueous fraction is aqueous soluble dpm in lungs plus perfusate. Total degradation is the sum of unsaturated PC, lysoPC, and aqueous fractions. All of the values for SP-A−/− are statistically different (P < 0.05) compared to the corresponding values for SP-A+/+. *All values are significantly different from the corresponding controls.

### Table 5. Phospholipase A₂ activities of mouse lung homogenate

<table>
<thead>
<tr>
<th>Conditions of Assay</th>
<th>SP-A+/+</th>
<th>SP-A−/−</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH 4, no Ca²⁺</td>
<td>8.8 ± 0.4</td>
<td>13.2 ± 0.2*</td>
</tr>
<tr>
<td>+M33</td>
<td>1.7 ± 0.1</td>
<td>2.3 ± 0.3</td>
</tr>
<tr>
<td>pH 9.5, plus Ca²⁺</td>
<td>9.0 ± 0.1</td>
<td>8.9 ± 0.3</td>
</tr>
<tr>
<td>+M33</td>
<td>8.8 ± 0.1</td>
<td>9.1 ± 0.2</td>
</tr>
</tbody>
</table>

Values are means ± SE for n = 4. PLA₂ activity was measured in the lung homogenate using two different assays with [³H]DPPC in liposomes as substrate. 1-Hexadecyl-3-trifluoroethylglycero-en-2-phosphomethanol (M33) when present was added to liposomes at 3 mol%. *P < 0.05 vs. corresponding value for SP-A+/+.
The present results indicate the presence of two different mechanisms for DPPC uptake by lung cells. The first is an SP-A-independent mechanism that has sufficient activity to support basal levels of phospholipid recycling. The second is an SP-A-dependent mechanism that is required to maintain normal DPPC recycling rates under conditions of increased surfactant turnover. On the basis of previous results, these studies presumably reflect uptake by type II cells, since they are responsible for the major fraction of DPPC clearance (8, 9, 29, 34). However, alveolar macrophages or other cells may also participate. The SP-A-dependent pathway most likely involves uptake of an SP-A/DPPC complex. We have shown previously that SP-A dependent uptake of DPPC in the type II cell occurs via a cell membrane SP-A receptor that can be recruited to the cell surface by secretagogues (6). Because SP-A binding has been localized to coated pits in type II cells (30), uptake of the DPPC/SP-A complex likely occurs via clathrin-mediated endocytosis, whereas SP-A-independent uptake may occur by another pathway, possibly involving cell membrane retrieval (2, 25).

The physiological implication of a failure to increase clearance with hyperventilation or secretagogues would be a transient elevation of alveolar phospholipid, assuming that secretary response to those stimuli remains intact. A previous study of hyperventilation in SP-A−/− mice evaluated the DPPC pool size after 1 h of exercise by running or swimming (18). In that study, alveolar DPPC content after running was greater in SP-A−/− mice than for SP-A+/+ mice, although there was no difference following swimming exercise (18). DPPC pool size also was similar in SP-A−/+ and SP-A+/− mice with hyperventilation or following treatment with 8-Br-cAMP or TPA in the present experiments. Thus the regulation of alveolar DPPC pool size appears to involve additional levels of control.

In conclusion, we have shown an increased lung uptake of [3H]DPPC in wild-type mice on exposure to a physical (hyperventilation) or chemical (secretagogue) stimulus. Increased [3H]DPPC uptake did not occur under those conditions in mice with genetic deficiency of SP-A. We propose that SP-A is necessary to maintain normal rates of DPPC clearance from the alveolar space under conditions of increased surfactant turnover.

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