A competitive inhibitor of phospholipase A₂ decreases surfactant phosphatidylcholine degradation by the rat lung

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We have shown previously that radiolabelled phosphatidylcholine (PC) in liposomes or natural surfactant is removed from the alveolar space and metabolically recycled in a process that is stimulated by cyclic AMP (cAMP). In this study, we evaluated the effect of a transition-state phospholipid analogue (MJ33; 1-hexadecyl-3-trifluoroethylglycero-*sn*-2-phosphomethanol) that competitively inhibited acidic phospholipase A₂ (PLA₂) activity (pH 4.0) of lung homogenate by more than 97%, but had no effect on PLA₂ activity at pH 8.5. MJ33 incorporated into unilamellar liposomes (dipalmitoyl PC/egg PC/cholesterol/phosphatidylglycerol, molar proportions 10:5:3:2) or co-sonicated with biosynthesized natural surfactant was instilled into the trachea of the anaesthetized rat; lungs were then removed for 2 h perfusion in the absence or presence of 0.1 mM-8-bromo cAMP. Total uptake for phospholipid was unchanged in the presence of the inhibitor MJ33. Degradation of labelled PC during 2 h perfusion in the absence of MJ33 was approx. 26% of that instilled for choline-labelled liposomal PC, 16% for liposomal PC labelled in the second fatty-acyl position, and 33% for choline-labelled natural surfactant. Degradation of PC was decreased by approx. 25–40% for each substrate in the presence of MJ33. Inhibition of lipid degradation depended on the mole fraction of MJ33 in the liposomes and was maximal at 1 mol%. These studies demonstrate a significant role for acidic Ca²⁺-independent PLA₂ in the degradation of internalized alveolar PC, but further indicate that this enzyme accounts for a minor fraction of total lung PC metabolism.

INTRODUCTION

It is now generally accepted that the major pathway for surfactant removal from the alveolar space is through re-uptake by granular pneumocytes, followed by intracellular processing [1,2]. Both protein and lipid moieties of surfactant are cleared by this mechanism, although specific routes for uptake and intracellular processing may differ for the various components [3]. In the case of the major phospholipid component of surfactant, i.e. phosphatidylcholine (PC), internalized material may be in part targeted to lamellar bodies for re-secretion [4], although this appears to be the minor pathway for dealing with internalized PC in adult lungs [5]. The major fraction of internalized PC is degraded, possibly in the lysosomal compartment, and components utilized in part for resynthesis of phospholipid [6–8].

In our studies with PC radiolabelled in the choline moiety, significant radiolabelled lysoPC was identified as a metabolite in both isolated perfused lung and isolated granular pneumocytes, indicating activity of a phospholipase A pathway (either A₁ or A₂) in the degradation of internalized lung phospholipids [6–8]. The lysoPC that is generated is further degraded to aqueous soluble choline metabolites, including free choline, an essential substrate for PC synthesis [9]. Incubation of type 2 cells with a non-specific phospholipase A₂ (PLA₂) inhibitor (quinacrine, *p*-bromophenacyl bromide) or PLA₂ activator (mellitin) resulted respectively in decreased or increased synthesis of PC [10,11]. Thus phospholipase A activity may have an important role in modulating surfactant degradation and synthesis by the lung epithelium. PLA₂ has a further important role in the remodelling of monoenoic PC to form the disaturated species [12,13].

Previous studies of lung have indicated the presence of several distinct forms of PLA₂. A Ca²⁺-independent enzyme with acidic pH optimum (assayed at pH 4) has been identified in the lamellar

bodies, the organelle for surfactant storage and secretion [14]. A Ca²⁺-dependent enzyme with alkaline pH optimum (assayed at pH 8.5) has been identified in lung homogenates and has been associated with a lung microsomal or mitochondrial fraction [13,15]. Both of these enzymes also have been demonstrated in isolated granular pneumocytes (type 2 lung epithelial cells) [16]. Recently, a Ca²⁺-dependent alkaline-pH-optimum PLA₂ of the secretory class of enzyme has been identified in lung cytosol, indicating the presence of at least three PLA₂ forms in the lung [17].

Previous analysis of the role of phospholipase A in surfactant PC degradation was based on identification of metabolic products [7,8]. In the present study, we have utilized an active-site-directed specific competitive inhibitor of PLA₂ activity in order to explore further the role of this enzyme. The inhibitor, 1-hexadecyl-3-trifluoroethylglycero-*sn*-2-phosphomethanol (called MJ33), is a newly described transition-state analogue that interferes with the catalytic turnover of PLA₂ bound to the lipid interface of isolated vesicles [18]. The results of the studies reported in the present paper provide evidence for activity of this novel inhibitor on the acidic PLA₂ in the intact lung. Further, the use of this inhibitor has indicated the relative role of the two forms of lung PLA₂ in the degradation of internalized surfactant.

MATERIALS AND METHODS

Materials

Authentic lipids were obtained from Avanti (Birmingham, AL, U.S.A.). Radiochemicals were obtained from Amersham (Arlington Heights, IL, U.S.A.) or New England Nuclear (Boston, MA, U.S.A.) and were reported as > 99% purity. MJ33 was synthesized as previously described [18]. Sprague–

Abbreviations used: MJ33, 1-hexadecyl-3-trifluoroethylglycero-*sn*-2-phosphomethanol; PLA₂, phospholipase A₂; PC, phosphatidylcholine; DPPC, dipalmitoyl PC; cAMP, cyclic AMP.

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Dawley male rats weighing approx. 200 g, were obtained from Charles River Breeding Laboratories (Kingston, NY, U.S.A.).

Liposome and surfactant preparation

Unilamellar liposomes were prepared as previously described from a lipid mixture of dipalmitoyl PC (DPPC)/egg PC/cholesterol/phosphatidylglycerol (molar proportions 10:5:3:2) to reflect the approximate composition of surfactant [6]. The lipid mixture was evaporated to dryness and resuspended in 1 ml of saline with or without MJ33. Liposomes were prepared by sonication at 50 °C by using a cup horn sonicator (Heat Systems Ultrasonics, Plainview, NY, U.S.A.) at 70 % of maximum power with 6 × 3 min bursts at 1 min intervals. For most studies, the radiolabel used in liposome preparation was DPPC containing [*methyl*-³H]choline. In some experiments, 1-palmitoyl-2-[9,10-³H]palmitoyl-PC was used as the DPPC label and 1-palmitoyl-2-[1-¹⁴C]oleoyl-PC was substituted for egg PC. Liposomes were stored overnight at 4 °C and then centrifuged at 1000 *g* for 10 min to remove aggregates.

Radiolabelled natural surfactant was prepared as a biosynthesized product by using isolated perfused rat lungs with [³H]choline added to the perfusate as previously described [7]. The biosynthesized surfactant was isolated by lung lavage, followed by density-gradient centrifugation and dialysis. It was stored as a freeze-dried material at -80 °C and reconstituted in saline by vigorous vortex-mixing followed by sonication with the same protocol as for liposomes. Analysis of the biosynthesized surfactant has shown that the radiolabel is more than 98 % in PC, with approx. 70 % in the disaturated fraction [7].

Isolated-lung perfusion

The experimental protocol for the study of uptake and degradation of liposomes or radiolabelled natural surfactant from the alveolar space of the isolated perfused lung has been described previously in detail [8,19,20]. In brief, rats were anaesthetized with pentobarbital (50 mg/kg intraperitoneally) and radiolabelled liposomes (0.67 or 1 μmol of PC) or natural surfactant (1 μmol of PC) was instilled through the trachea at approximately the level of the tracheal carina. The respiratory movements assist in the movement of labelled lipid throughout the tracheobronchial tree [19]. The thorax was incised and the lungs were cleared of blood by perfusion through the pulmonary artery with Krebs bicarbonate buffer using gravity flow of perfusate (approx. 25 cmH₂O). The lungs were removed from the thorax and placed in the organ-perfusion chamber at 37 °C for recirculating perfusion using a peristaltic pump. The perfusate was Krebs bicarbonate buffer containing 10 mM-glucose and 3 % fatty-acid-free BSA. The time from instillation of liposomes to the start of isolated-organ perfusion was approx. 5 min.

Different lung-perfusion experiments were carried out in the presence or absence of 0.1 mM-8-bromo cyclic AMP (cAMP) added to the perfusate. We have previously shown that this agonist significantly stimulates the uptake and degradation of lipids by the perfused lung, but has no effect on PLA₂ activity of the lung homogenate [19].

Uptake and degradation of lipid

At the end of the 2 h perfusion, lungs were lavaged through the tracheal cannula five times with saline (7 ml per lavage). The lung tissue was homogenized in 20 vol. of ice-cold saline with a Polytron (P10 probe) at 50 % of maximum power for 2 × 15 s, followed by two strokes with a motor-driven Teflon pestle in a Potter-Elvehjem vessel. A portion of the homogenate was analysed for total radioactivity (d.p.m.), and uptake of phospholipid was calculated as lavage-resistant radiolabel

retained in the lung. Recovery of radiolabel in the lung perfusate at 2 h was less than 10 % of the total label in homogenate, and this has not been included in the calculation of lipid uptake. We have shown previously that the sum of recoveries of radiolabel at 2 h in the lung lavage fluid, perfusate and lung tissue essentially yields 100 % recovery of instilled radiolabelled lipid [3].

The lung homogenate was extracted by the Bligh & Dyer [21] method to give aqueous and organic fractions. The organic fraction was further analysed by t.l.c. For studies with choline-labelled PC, the t.l.c. system was chloroform/methanol/NH₃/water (26:14:1:1, by vol.) [22]. The bands corresponding to authentic PC and lysoPC were scraped and analysed along with the aqueous fraction for radioactivity (d.p.m.). No significant radioactivity was found in any other fraction. In some experiments, the aqueous fraction was further analysed by t.l.c. for glycerophosphocholine, phosphocholine, CDP-choline and free choline as previously described [6]. For studies of fatty-acid-labelled PC, we analysed both for phospholipid as above and for neutral lipids by a t.l.c. system using hexane/diethyl ether/acetic acid (6:4:1, by vol.) for one half of the plate, followed by the same solvents at 90:10:1 (by vol.) for the second half [23]. Bands corresponding to authentic free fatty acid and diacylglycerol were analysed for radiolabel. Total combined recovery from the phospho- and neutral-lipid fractions exceeded 98 % of radioactivity (d.p.m.) present in the original homogenate.

Phospholipase assay

Phospholipase activity of the lung homogenate was measured in the presence and absence of MJ33 by modification of our previously described method [6,8]. Lungs were cleared of blood and homogenized as above. A sample of homogenate (approx. 0.5 mg of protein/ml) was incubated at pH 4.0 or at pH 8.5 and 37 °C for 60 min. The acidic assay buffer was 40 mM-sodium acetate/5 mM-EDTA. The alkaline assay buffer was 50 mM-Tris/HCl/1 mM-EGTA/10 mM-CaCl₂. The reaction was started by addition of substrate. The substrate was the surfactant-like liposome preparation (with or without MJ33) with [9,10-³H]-palmitate in the 2-position of DPPC. The concentration of DPPC was 2 mM and the specific radioactivity was 2 μCi/μmol. The assay was linear with time (0-60 min) and protein (0.05-1 mg/ml). The reaction was stopped with chloroform/methanol (1:2, v/v) and the lipid fraction was isolated [21]. The phospholipids and neutral lipids were separated by t.l.c. as described above. Total phospholipase activity was calculated from the decrease in radioactivity (d.p.m.) in PC and the precursor specific radioactivity; results are expressed per mg of protein in the assay buffer. Production of metabolic products was calculated from the appearance of radioactivity (d.p.m.) in free fatty acid and diacylglycerol. Protein was measured by Coomassie Blue dye binding (Bio-Rad, Richmond, CA, U.S.A.) by using bovine γ-globulin as the standard [24].

Statistical analysis

Results are expressed as means ± S.E.M. Statistical analysis was carried out by ANOVA followed by Dunnett's *t* test, and the level of statistical significance was taken as *P* < 0.05 [25].

RESULTS

Phospholipase activity

Phospholipase activity was measured in the lung homogenate at pH 4.0 and 8.5. There was a significant 70 % decrease in acidic phospholipase activity in the presence of MJ33 in liposomes, but no effect with the alkaline assay (Table 1). No additional

Table 1. Effect of MJ33 on phospholipase activity in lung homogenate

Lung homogenate (2 mg of protein/ml) was incubated with liposomes at 37 °C for 60 min without (control) or with 3 mol % MJ33 at pH 4.0 or 8.5. The substrate was DPPC labelled in the 2-position (1-palmitoyl-2-[9,10-³H]palmitoyl-glycerophosphocholine) (2 mM; sp. radioactivity 2 µCi/µmol). Phospholipase activity was measured by the decrease in [³H]PC in the incubation vessel. Production of metabolic products was calculated from the recovery of radiolabel. Results are means ± S.E.M. (n = 4): *P < 0.05 versus control.

	Activity (nmol/60 min per mg of protein)			
	pH 4.0		pH 8.5	
	Control	MJ33	Control	MJ33
Total phospholipase activity	24.3 ± 0.8	7.2 ± 0.5*	28.3 ± 1.2	31.2 ± 1.2
Free fatty acid production	17.2 ± 1.2	0.5 ± 0.06*	20.9 ± 0.5	22.9 ± 0.7
Diacylglycerol production	6.4 ± 0.3	6.5 ± 0.5	7.1 ± 0.2	7.1 ± 0.1

inhibition was seen when MJ33 was added to medium (1 mM) in addition to liposomes (results not shown). Analysis of the metabolic products indicated a 97 % decrease in the recovery of label in free fatty acid and no change in the recovery in diacylglycerol for the assay at pH 4.0 (Table 1). At pH 8.5, recovery of both metabolites was unchanged by MJ33. The recovery of label in fatty acid plus diacylglycerol at either pH accounted for the total disappearance of PC from the assay, suggesting that significant labelled lysoPC or phosphatidic acid was not generated. These results indicate nearly complete inhibition of acidic PLA₂ and no effect on alkaline PLA₂ or on phospholipase C activity by MJ33.

Uptake and degradation of liposomes

The initial experiments with the intact isolated lung evaluated liposomal PC with label in the choline moiety of DPPC. Similarly to our previous results [3,19,20], uptake of this substrate by the lung at 2 h (in the presence of 8-bromo cAMP) was approx. 33 % of the radiolabel instilled into the trachea (Table 2). There was no significant effect of MJ33 on uptake (Table 2).

Metabolism of PC was evaluated by measurement of label in degradation products. Total metabolism represents the sum of the aqueous and lysoPC fractions and is expressed as a percentage of the internalized lipid. Metabolism measured at 2 h of perfusion with 8-bromo cAMP is approx. 26 % of internalized radioactivity,

Table 3. Metabolic products of [³H]choline-labelled PC in isolated perfused rat lung

Effect of 3 mol % MJ33 on the recovery of ³H in lysoPC and in aqueous metabolites in homogenates of isolated rat lung after 2 h of perfusion. Lungs were instilled with [methyl-³H]choline-labelled liposomal dipalmitoyl PC or biosynthesized surfactant PC. Values are means ± S.E.M. for the numbers of experiments indicated in Table 2: *P < 0.05 versus minus MJ33.

	Liposomes		Surfactant	
	– MJ33	+ MJ33	– MJ33	+ MJ33
LysoPC	5.7 ± 0.3	3.9 ± 0.3*	5.4 ± 0.2	4.0 ± 0.2*
Aqueous fraction	20.6 ± 0.6	14.3 ± 0.3*	27.6 ± 1.0	20.6 ± 0.7*

similar to our previous reports (Table 2) [8,20]. Metabolism was decreased by approx. 30 % when liposomes contained MJ33. Analysis of the distribution of metabolic products indicated that only about 20 % of recovered radioactivity was in lysoPC, whereas 4 times as much was present in the aqueous fraction; recovery in both compartments was decreased in the presence of MJ33 (Table 3).

The above studies with MJ33 in liposomes were carried out with 3 mol % (w/w) of the inhibitor, equivalent to total instilled MJ33 of 20–30 nmol. The effect of MJ33 concentration on DPPC metabolism was studied by varying the inhibitor concentration from 0.1 to 6 mol %. MJ33 in liposomes at 0.1 mol % showed no inhibition of DPPC degradation, but increasing inhibition was observed at concentrations up to 1 mol % (Fig. 1). No additional effect on DPPC metabolism was observed between 1 and 6 mol %. All further studies were carried out with 3 mol % MJ33 in the liposomes.

The effect of adding free MJ33 to lung perfusate was also evaluated. In these experiments, the liposomes instilled into the trachea did not contain MJ33. With either 0.5 or 5 µmol of MJ33 in 40 ml of perfusate (n = 4 for each), there was less than 14 % inhibition of the metabolism of liposomal PC (results not shown). The results with perfusate MJ33 were not significantly different from controls, suggesting that free MJ33 does not readily cross cell membranes.

The effect of MJ33 on the metabolism of liposomes with radiolabel in the second-position fatty acid of PC was evaluated as a double-label experiment. Liposomes were prepared with ³H-labelled palmitate in the 2-position of DPPC and ¹⁴C-labelled oleate in the 2-position of PC, substituted for egg PC in our standard liposome preparation. Uptake of radiolabelled lipid by

Table 2. Uptake and metabolism of PC by isolated perfused rat lung

Lungs were instilled with 1 µmol of total PC and uptake was measured after 2 h perfusion in the presence of 0.1 mM-8-bromo cAMP. MJ33 when present was 3 mol % (w/w). Mean uptake expressed as percentage of instilled radiolabel is indicated in parentheses. Values are means ± S.E.M. for n = 4 under each condition (n = 8 for surfactant minus MJ33): †P < 0.05 versus minus MJ33.

	Label instilled (nmol of PC)	Uptake		Metabolism (% of lung radioactivity)	
		– MJ33	+ MJ33	– MJ33	+ MJ33
1,2-Dipalmitoyl[<i>choline</i> - ³ H]PC	670	219 ± 8.0 (33)	203 ± 4.7 (30)	26.3 ± 1.0	18.2 ± 0.6†
1-Palmitoyl-2-[9,10- ³ H]palmitoyl-PC*	670	234 ± 1.2 (35)	235 ± 3.5 (35)	16.0 ± 0.5	10.6 ± 0.4†
1-Palmitoyl-2-[1- ¹⁴ C]oleoyl-PC*	330	109 ± 3.3 (33)	113 ± 0.6 (34)	16.7 ± 0.5	10.5 ± 0.2†
[<i>choline</i> - ³ H]Surfactant	1000	568 ± 8.3 (57)	590 ± 8.0 (59)	33.4 ± 0.8	24.5 ± 0.8†

* Double-label experiment.

Fig. 1. Effect of MJ33 concentration on metabolism of [^3H]choline-labelled DPPC in liposomes

MJ33 of various concentration expressed as mol % of the lipid was co-sonicated with lipids for preparation of liposomes as described in the text. Results are expressed as percentages of the radioactivity (d.p.m.) recovered in the lung homogenate after 2 h of perfusion followed by lung lavage. Total metabolism (▽) represents the sum of radioactivity recovered in the aqueous fraction of a Bligh & Dyer [21] extract (○) and in lysoPC (●).

Table 4. Metabolic products of fatty-acid-labelled PC in isolated perfused rat lung

Values are means $\pm$ S.E.M. ($n = 4$): * $P < 0.05$ versus minus MJ33. Abbreviations: DPPC, 1-palmitoyl-2-[9,10- ^3H]palmitoyl-PC; POPC, 1-palmitoyl-2-[1- ^{14}C]oleoyl-PC.

	DPPC (% of homogenate radioactivity)		POPC (% of homogenate radioactivity)	
	– MJ33	+ MJ33	– MJ33	+ MJ33
Free fatty acids	13.7 $\pm$ 0.4	8.0 $\pm$ 0.4*	14.3 $\pm$ 0.4	7.9 $\pm$ 0.2*
LysoPC	0.08 $\pm$ 0.01	0.05 $\pm$ 0.004	0.06 $\pm$ 0.02	0.04 $\pm$ 0.008
Di- and tri- acylglycerols	0.4 $\pm$ 0.05	0.7 $\pm$ 0.03*	0.4 $\pm$ 0.09	0.7 $\pm$ 0.05*
Aqueous fraction	0.3 $\pm$ 0.06	0.1 $\pm$ 0.006	0.3 $\pm$ 0.06	0.2 $\pm$ 0.01

the lung, expressed as a percentage of instilled label, was similar for the DPPC and PC labels and was also similar to results when label was in the choline moiety (Table 2). Approx. 16% of PC was metabolized under control conditions (Table 2). Results were similar for both fatty acid labels, but significantly less than with choline-labelled PC (Table 2). Metabolism of PC was inhibited by approx. 40% in the presence of MJ33 (Table 2).

The distribution of the recovered radioactivity present in metabolites of fatty-acid-labelled PC is shown in Table 4. Under control conditions (without MJ33), more than 90% of metabolite radioactivity was recovered in the free-fatty-acid fraction; relatively little label were recovered in diacylglycerol, triacylglycerol, lysoPC or the aqueous fraction. In the presence of MJ33, there was a significant decrease in recovery of label in free fatty acids (Table 4).

As a further control, lungs were instilled with non-radioactive liposomes with or without MJ33 at 3 mol % and perfused for 2 h. Lungs were then lavaged, homogenized, and assayed *in vitro* for phospholipase activity at pH 4 as described above with 2-[^3H -

palmitoyl]DPPC as substrate. PLA₂ activity based on recovery of fatty acid label was decreased by 57% (mean for $n = 2$) in the lungs that had been instilled with MJ33. The lesser degree of inhibition compared with addition of MJ33 directly to the homogenate can be explained by the limited accessibility of airway-instilled liposomes to cells other than the epithelium.

Uptake and degradation of natural surfactant

The effect of MJ33 on the degradation of biosynthesized radiolabelled natural surfactant was evaluated. Both the uptake of PC in natural surfactant as well as the percentage degradation of internalized lipid under control conditions was significantly greater than with liposomes (Table 2). The presence of MJ33 had no effect on surfactant PC uptake, but inhibited degradation by approx. 25% (Table 2). As with choline-labelled liposomes, the distribution of metabolites was predominantly into the aqueous fraction, with a lesser amount in lysoPC (Table 3). Of the radioactivity in the aqueous fraction, the greatest component (65–70%) was in free choline, in both the presence and the absence of MJ33, with the remainder in phosphocholine, glycerophosphocholine and CDP-choline (results not shown).

The above studies were carried out in the presence of 8-bromo cAMP. When lungs were perfused in its absence, the uptake of [^3H]choline-labelled natural surfactant was significantly less, $46.2 \pm 0.8\%$ of instilled in 2 h ($n = 9$), as previously reported [19]. Recovery of label in the aqueous fraction of the lung extract was $13.2 \pm 0.5\%$ of lung radioactivity ($n = 9$) in the absence of MJ33 and decreased to $10.9 \pm 0.4\%$ ($n = 4$) in its presence. This 17% inhibition of metabolism by MJ33 in the absence of 8-bromo cAMP was statistically significant ($P < 0.05$).

DISCUSSION

The results of the present investigation confirm our previous studies of radiolabelled PC uptake from the alveolar space of the isolated perfused rat lung [3,8,19,20]. PC, either in synthetic liposomes or in natural surfactant, is accumulated by the lung and, at least in part, metabolized. The uptake and metabolism of PC in natural surfactant are greater than for liposomes and are stimulated by the presence of a cAMP analogue. For natural surfactant, uptake of PC, defined as resistance to removal by lung lavage, was nearly 60% of instilled PC at 2 h of perfusion in the presence of 8-bromo cAMP; approximately one-third of the lung-associated label was recovered in metabolic products. This study has not evaluated the uptake or metabolism of the components of surfactant other than PC.

Phospholipases of the A, C, and D classes have been described in lung tissue, and theoretically could each be responsible for breakdown of internalized surfactant PC [13–15,26,27]. The primary catabolic products would be lysoPC plus fatty acid for phospholipase A, phosphocholine plus diacylglycerol for phospholipase C, and choline plus phosphatidic acid for phospholipase D. The present studies with choline-labelled PC in liposomes and in natural surfactant, showing the generation of labelled lysoPC, demonstrate a specific role for PLA₂ in the degradation of internalized alveolar PC. The results also raise the possibility for concurrent activity of other phospholipases (i.e. C or D), based on the generation of labelled choline-containing aqueous metabolites.

Alternatively, the aqueous metabolites could be produced from choline-labelled lysoPC through activity of lysophospholipase and phosphatases which would generate labelled phosphocholine and choline. Evidence against a major role for phospholipases C and D was obtained by studies of PC labelled with fatty acid in the 2 position, since $> 90\%$ of radiolabel recovered in metabolites was found in free fatty acid, compatible

with degradation via PLA₂. PLA₂ activity for PC with palmitate or oleate in the 2 position was similar, showing a lack of substrate specificity for this enzyme. We conclude that the major pathway for degradation of PC internalized from the alveolar space is PLA₂. This does not exclude a role for phospholipase C or D in the breakdown of membrane PC or other endogenous phospholipids, which were not evaluated in the present study. The assays *in vitro* with lung homogenate (Table 1) show significant phospholipase C activity for PC, but activity of phospholipase A₁ or D was not detected.

Further evidence for the role of PLA₂ in the degradation of internalized alveolar PC was obtained through the use of a specific PLA₂ inhibitor, MJ33. The study *in vitro* with lung homogenate showed no effect of MJ33 on phospholipase C activity (Table 1), confirming previous results with lipolytic and interfacial enzymes [18]. The presence of MJ33 resulted in essentially complete inhibition of acidic PLA₂ activity in the lung homogenate, but had no effect on activity of the alkaline enzyme. In the perfused lung, MJ33 at maximal concentration inhibited degradation of phospholipid by approximately one-third. This result suggests that degradation of internalized PC occurs in part by the acidic PLA₂ and presumably in an acidic organelle such as the lysosome or lamellar body [28]. However, the major fraction of PC degradation was not inhibited by MJ33, raising the possibility of degradation via another PLA₂. The localization of this latter activity in the lung cell has not been precisely defined, but it may be associated with mitochondria, microsomes, cytoplasm or other organelles [13,15,17,26].

Our previous studies have demonstrated that the metabolic products of PC catabolism are utilized by the lung for resynthesis of phospholipid [6,8]. The supporting evidence was the appearance of radiolabel in CDP-choline and also in PC with unsaturated fatty acids after incubation with choline-labelled DPPC. There are two likely pathways for PC resynthesis. The first is the re-esterification of lysoPC, and the second is the reutilization of choline through the 'de novo' pathway via CDP-choline [29]. DPPC is the major product of either pathway in the lung [29]. Consequently, the actual rate of PC catabolism could be underestimated in this study, since the metabolic products are reutilized to re-form the original substrate. Similar reutilization may occur with the products generated from fatty-acid-labelled PC, and the apparently lower degradation rate of fatty-acid-labelled PC compared with choline-labelled PC may be related to the more rapid reutilization of the fatty-acyl moiety.

In summary, we have demonstrated that the acidic Ca²⁺-independent PLA₂ of lung plays a major role in the degradation of internalized surfactant PC. MJ33, a competitive inhibitor of acidic PLA₂ activity in the lung, is effective in significantly decreasing the degradation rate of internalized surfactant.

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