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Regulation of Lung Surfactant Secretion by Phospholipase A_2

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Abstract Arachidonic acid has been shown to stimulate lung surfactant secretion from alveolar epithelial type II cells. To identify the (phospho)lipases responsible for generating arachidonic acid during lung surfactant secretion, the effects of various (phospho)lipase inhibitors on phosphatidylcholine (PC) secretion from rat alveolar type II cells were investigated. N-(p-amylcinnamoyl)anthranilic acid (ACA), a general inhibitor of phsopholipase A_2 (PLA_2), inhibited ATP-stimulated PC secretion in a dose-dependent manner. ACA also blocked PC secretion from type II cells stimulated by other secretagogues including phorbol 12-myristate 13-acetate, Ca^{2+} ionophore A23187 and terbutaline, indicating that PLA_2 acts at a late step distal to the generation of second messengers. To determine which PLA_2 isoform(s) is involved in lung surfactant secretion, selective inhibitors to different types of PLA_2 were used to inhibit PLA_2 activity in type II cells. The cytosolic PLA_2 ($cPLA_2$) inhibitor, arachidonyl trifluoromethyl ketone, was found to inhibit ATP-stimulated PC secretion, whereas the secretory PLA_2 inhibitors, oleoyloxyethylphosphocholine, aristolochic acid, or *p*-bromophenacyl bromide, and the Ca^{2+}-independent PLA_2 inhibitors, palmitoyl trifluoromethyl ketone, or haloenol lactone suicide substrate, had no effect. In addition to PLA_2, arachidonic acid is released from diacylglycerol (DAG) by DAG and monoacylglycerol lipases. The DAG lipase inhibitor, RHC-80267 also blocked ATP-stimulated PC secretion. The results suggest that both pathways for generating arachidonic acid via $cPLA_2$ and DAG lipase may participate in lung surfactant secretion. J. Cell. Biochem. 72:103–110, 1999.

Key words: exocytosis; arachidonic acid; phospholipase A_2; diacylglycerol lipase; alveolar type II cells

The alveolar epithelium is composed of two phenotypically distinct cell types: squamous type I and cuboidal type II cells. While type I cells are the sites for gas exchange, type II cells produce lung surfactant, a phospholipid-rich lipoprotein complex that lowers surface tension to prevent collapse of the alveoli. Lung surfactant is stored in lamellar bodies, characteristic secretory organelles of type II cells and released via fusion of lamellar bodies with plasma membranes. The secretion of lung surfactant is stimulated by various secretagogues including agonists for β-adrenergic receptors and purinoceptors, calcium ionophores, and protein kinase C activators. Based on second messengers and protein kinases involved, those secretagogues are categorized into three different pathways: cAMP/protein kinase A, diacylglycerol (DAG)/protein kinase C and Ca^{2+}/Ca^{2+}-, and calmodulin-dependent protein kinase [Chander and Fisher, 1990].

Although signal transduction pathways in lung surfactant secretion are relatively well understood the factors involved in membrane fusion are much less known. We have recently shown that annexin II, a member of calcium-dependent phospholipid-binding protein family, promotes in vitro fusion of lamellar bodies with liposomes and restores lung surfactant secretion from permeabilized type II cells [Liu et al., 1995, 1996]. Furthermore, arachidonic acid enhances annexin II-mediated membrane fusion, implying a possible role of arachidonic acid in membrane fusion during lung surfac-

Abbreviations: $sPLA_2$, secretory phospholipase A_2; $cPLA_2$, cytosolic phospholipase A_2; $iPLA_2$, Ca^{2+}-independent phospholipase A_2; ACA, N-(p-amylcinnamoyl)anthranilic acid; $AACOCF_3$, arachidonyl trifluoromethyl ketone; OOPC, oleoyloxyethylphosphocholine; ASA, aristolochic acid; BPB, *p*-bromophenacyl bromide; $PACOCF_3$, palmitoyl trifluoromethyl ketone; HELSS, haloenol lactone suicide substrate; RHC-80267, 1,6-bis (cyclohexyloximinocarbonylamino) hexane; IP_3, *myo*-inositol 1,4,5-trisphosphate; DAG, diacylglycerol; PMA, phorbol 12-myristate 13-acetate.

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tant secretion. Other investigators report that arachidonic acid stimulates surfactant secretion from cultured alveolar type II cells by an undefined mechanism [Gifillan and Rooney, 1985; Baybutt et al., 1994]. In addition, the release of arachidonic acid is increased by incubation of type II cells with lung surfactant secretagogues, Ca^{2+} ionophore and phorbol 12-myristate 13-acetate [Lipchik et al., 1990, Peters-Golden et al., 1992].

There are two major pathways to generate arachidonic acid in cells. One is the hydrolysis of membrane phospholipids by phospholipase A_2 (PLA_2). Another is the liberation of arachidonic acid from DAG by the sequential actions of two lipases: DAG and monoacylglycerol (MAG) lipases [Burgoyne and Morgan, 1990]. Those enzymes have been shown to be involved in exocytosis in various cell types [Frye and Holz, 1983; White et al., 1993; Ramanadham et al., 1993, 1994; Rindlisbacher et al., 1990; Konrad et al., 1994].

Three types of PLA_2s, secretory PLA_2 ($sPLA_2$), cytosolic PLA_2 ($cPLA_2$), and $iPLA_2$ are identified in lung [Lindahl et al., 1989, Nakano and Arita, 1990, Neagos et al., 1993, Peters-Golden and Feyssa, 1994, Pierik et al., 1988]. Phospholipase C and D activities are also detected in type II cells [Griese et al., 1991, Rooney and Gobran, 1993]. However, the specific (phospho)-lipases responsible for the generation of arachidonic acid during lung surfactant secretion are not identified. In this study, the effects of specific inhibitors of various (phospho)lipases on lung surfactant secretion were examined. The results suggest that both $cPLA_2$ and DAG lipase may participate in lung surfactant secretion.

MATERIALS AND METHODS

Materials

N-(p-amylcinnamoyl)anthranilic acid (ACA) was purchased from Biomol Research Laboratories (Plymouth Meeting, PA). Arachidonyl trifluoromethyl ketone ($AACOCF_3$), oleoyloxyethylphosphocholine (OOPC), aristolochic acid (ASA), haloenol lactone suicide substrate (HELSS), palmitoyl trifluoromethyl ketone ($PACOCF_3$), and 1,6-bis(cyclohexyloximinocarbonylamino)hexane (RHC-80267) were from Calbiochem (San Diego, CA). *p*-Bromophenacyl bromide (BPB), calcium ionophore A23187, ATP, phorbol 12-myristate 13-acetate (PMA), and terbutaline were from Sigma (St. Louis, MO). Eagle's Minimal Essential medium (EMEM) was from ICN (Costa Mesta, CA). Fetal calf serum (FCS) was from GIBCO (Grand Island, NY). Elastase was from Worthington Biochemical Co. (Freehold, NJ). [methyl-^{3}H] choline was from NEN (Boston, MA).

Isolation and Culture of Alveolar Epithelial Type II Cells

Type II cells were isolated from the lungs of adult male Sprague-Dawley rats (180–200 g) according to the method of Dobbs et al. [1986]. Rats were anesthetized by intraperitoneal administration of sodium pentobarbital (50 mg/kg body weight). The abdominal cavity was then surgically exposed. Lungs were ventilated and perfused via the pulmonary artery with cold solution II (10 mM Hepes, pH 7.4, 2.6 mM Na_2HPO_4/NaH_2PO_4, 154 mM NaCl, 3.9 mM KCl, 1.7 mM $CaCl_2$, 1.3 mM $MgSO_4$, 10 mM glucose, 100 µg/ml streptomycin, and 60 µg/ml penicillin). After being removed from the thorax, lungs were lavaged six times with cold solution I (Ca^{2+}- and Mg^{2+}-free solution II plus 0.16 mM EGTA) and two times with prewarmed (37°C) solution II. Then, the lungs were instilled intratracheally with 7 ml elastase solution (3 units/ml in solution II) and incubated at 37°C for 12 min, followed by instillation of 6 ml additional elastase solution two times, each incubated for 10 min. At the end of incubation, the lung lobes were minced with a tissue chopper, suspended in 10 ml solution II containing deoxyribonuclease and 3% FCS, and incubated 2 min in a shaking water bath (37°C). The cell suspension was sequentially filtered through nylon gauze (160, 37, and 15 µm mesh size), and centrifuged at 300*g* for 10 min. The cell pellet was suspended in EMEM, plated on a 100 mM bacteriological plastic dish coated with 5 mg rat IgG, and incubated at 37°C for 1 h to remove macrophages. The unattached cells were collected by centrifugation and resuspended in EMEM containing 10% FCS. For secretion studies, isolated type II cells (1×10^6) were plated on 35 mM plastic tissue culture dishes and cultured overnight in 1.5 ml of EMEM containing 10% FCS and 0.6 µCi [methyl-^{3}H] choline at 37°C in 5% CO_2 in humidified air. The purity of overnight-cultured type II cells were greater than 90% as evaluated by staining with phosphine 3R.

Surfactant Secretion Assay

The secretion of lung surfactant was measured by following the release of [^{3}H] phosphatidylcholine (PC) from primary culture of type II cells [Chander and Sen, 1993]. Overnight-cultured and [^{3}H] choline-labeled type II cells were washed six times with EMEM and equilibrated for 30 min in 1 ml of serum-free EMEM in the presence or absence of inhibitors. One set of dishes was removed for analysis of the *time zero* value. Secretagogues were added to the remaining dishes and incubated for another 2 h. Medium and cells were extracted with chloroform-methanol according to the method of Bligh and Dyer [1959]. In order to improve the recovery of labeled lipids, carrier PC (400 µg) was added to each sample during the extraction procedure. The lipid extract was dried in a ventilation hood overnight and counted on a scintillation counter. Since more than 95% of lipid-associated choline radioactivity was recovered in the PC, the extracted lipid [^{3}H] radioactivity was assumed to be all in PC [Chander and Sen, 1993]. Secretion was expressed as (dpm in medium/dpm in medium + dpm in cells) × 100%. All secretion data were corrected by subtracting the *time zero* value (1–2%). Viability of type II cells was evaluated by monitoring the release of lactate dehydrogenase (LDH) from the treated cells. LDH activity was determined by the method of Lee and Lardy [1965]. LDH release was expressed as a percentage of total LDH activity found in the medium.

Statistical Method

Data were expressed as mean ± SE. All secretion studies were carried out in duplicate and the individual results were averaged. Statistical significance was evaluated by one-way analysis of variance (ANOVA), followed by comparison of treated versus control by the Bonferroni procedure using SigmaStat software (Jandel Scientific, San Rafael, CA). The level of significance was taken as $P < 0.05$.

RESULTS

Inhibition of PC Secretion by ACA

The PLA_2 inhibitor, ACA, has been shown to block glucose-stimulated insulin secretion from pancreatic islets [Konrad et al., 1992]. The participation of PLA_2 in lung surfactant secretion was therefore investigated using ACA. The secretion of lung surfactant in primary culture of type II cells was monitored by following the release of [^{3}H] choline-labeled PC. Basal secretion in the absence of any secretagogues was 0.93% during a 2-h incubation. Inclusion of ACA (100 µM) almost completely blocked basal secretion (Table 1). The inhibition was not due to cytotoxic effects, since the rates of LDH release from control and ACA-treated cells were not different (data not shown).

Next, we examined the effects of ACA on secretagogue-stimulated PC secretion. ATP, an agonist of P_{2u} purinoceptor, is known to enhance PC secretion [Chander and Fisher, 1990]. In the presence of 1 mM ATP, PC secretion increased by approximately six-fold over control. ACA (100 µM) inhibited ATP-stimulated secretion by 94% (Table 1). The inhibition was dose-dependent. The concentration required to obtain 50% inhibition (IC_{50}) was ~ 5 µM (Fig. 1).

There are at least three signal transduction pathways in lung surfactant secretion, which involve different second messengers and protein kinases. Therefore, it is of interest to determine whether the ACA inhibition of PC secretion is specific to ATP. PMA stimulates PC secretion by directly activating protein kinase C, whereas terbutaline and Ca^{2+} ionophore A23187 increase PC secretion via β-adrenergic receptor-coupled adenylate cyclase/protein kinase A and Ca^{2+} influx, respectively [Chander and Fisher, 1990]. As shown in Table 1, ACA inhibited all these secretagogue-stimulated PC

TABLE I. Effect of ACA on PC Secretion From Rat Lung Type II Cells[a]

Secretagogues	PC Secretion (%) −ACA	PC Secretion (%) +ACA	Inhibition (%)
None	0.93 ± 0.25	0.03 ± 0.02	97
A23187, 1 µM	3.68 ± 0.68*	0.76 ± 0.54**	79
PMA, 1 µM	6.58 ± 0.27*	0.13 ± 0.08**	98
ATP, 1 mM	5.98 ± 0.72*	0.36 ± 0.20**	94
Terbutaline, 10 µM	1.75 ± 0.37	0.31 ± 0.18**	82

[a][^{3}H] choline-labeled type II cells were stimulated 2 h with 1 µM Ca^{2+} ionophore A23187, 1 µM PMA, 1 mM ATP, or 10 µM terbutaline in the absence or presence of 100 µM ACA. PC secretion was measured and expressed as (dpm in medium/dpm in medium + dpm in cells) × 100%. Data shown are mean ± SE of three independent experiments, each carried out in duplicate using different cell preparations.

*$P < 0.05$ vs. no additions.

**$P < 0.05$ vs. in the absence of inhibitors.

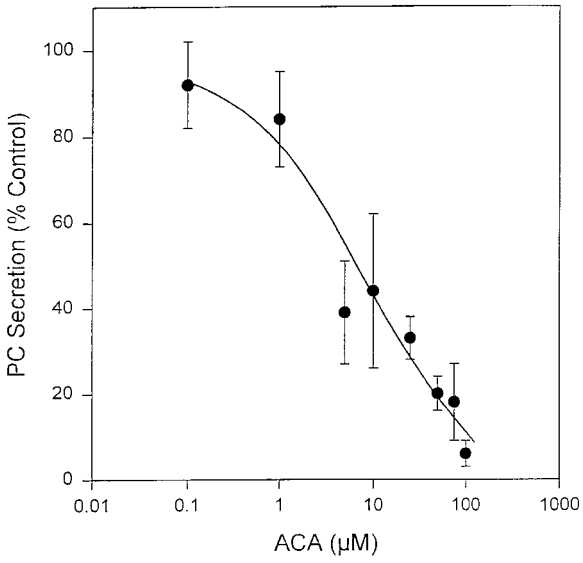

Fig. 1. Dose-dependent inhibition of ATP-stimulated PC secretion by ACA. Type II cells were preincubated 30 min in the presence of varying concentrations of ACA and then stimulated 2 h with 1 mM ATP. PC secretion was measured and expressed as a percentage of the control (in the absence of the inhibitor). Data shown are mean ± SE of three to four experiments, each carried out in duplicate using different cell preparations.

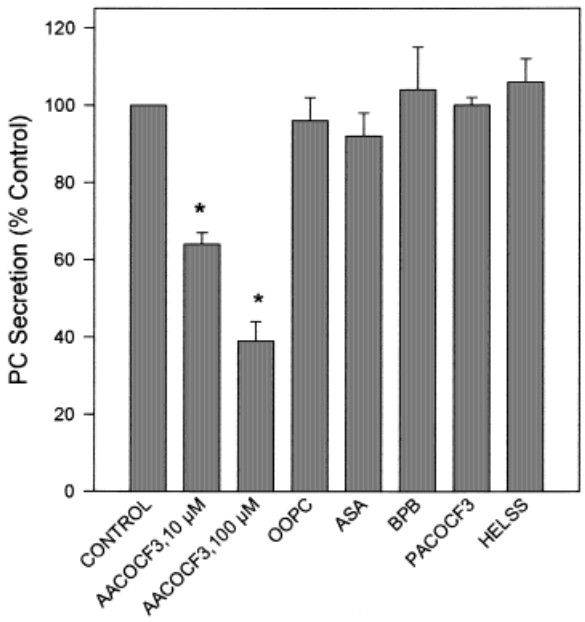

Fig. 2. Effect of selective PLA_2 Inhibitors on ATP-stimulated PC secretion. Type II cells were pretreated 30 min with 10 μM or 100 μM $AACOCF_3$, 10 μM OOPC, 10 μM ASA, 10 μM BPB, 10 μM $PACOCF_3$, or 0.1 μM HELSS and then stimulated 2 h with 1 mM ATP. PC secretion was measured and expressed as a percentage of the control (in the absence of inhibitors). Data shown are mean ± SE of three to five experiments, each carried out in duplicate using different cell preparations. *$P < 0.05$ versus control.

secretions by 79–98%, indicating that PLA_2 may be a downstream component distal to the generation of second messengers, consistent with our previous finding that arachidonic acid increases annexin II-mediated membrane fusion [Liu et al., 1995], a final step of exocytosis.

Inhibition of PC Secretion by Selective Inhibitors of PLA_2

In lung, three types of PLA_2s have been described: $sPLA_2$, $cPLA_2$, and $iPLA_2$ [Lindahl et al., 1989, Nakano and Arita, 1990, Neagos et al., 1993, Peters-Golden and Feyssa, 1994, Pierik et al., 1988]. To identify which PLA_2 isoform(s) is involved in lung surfactant secretion, the effects of selective inhibitors to each type of PLA_2 on PC secretion were examined. Involvement of $cPLA_2$ was evaluated using $AACOCF_3$, a trifluoromethyl ketone analogue of arachidonic acid. $AACOCF_3$ is a cell-permeable and potent slow-binding inhibitor of $cPLA_2$ and is more than 1,000-fold less potent as an inhibitor of $sPLA_2$ [Street et al., 1993, Bartoli et al., 1994, Ackermann et al., 1995]. $AACOCF_3$ at 10 μM and 100 μM significantly inhibited ATP-stimulated PC secretion by 36 and 61%, respectively (Fig. 2).

Inhibition of $sPLA_2$ was accomplished by incubating type II cells with $sPLA_2$ inhibitors. OOPC is a novel site-specific porcine pancreatic PLA_2 inhibitor with IC_{50} of 6.2 μM [Magolda et al., 1985]. ASA, a major chemical component derived from *Aristolochia* plant, inhibits $sPLA_2$ from human synovial fluid, neutrophils, platelets and snake venom [Vishwanath et al., 1988]. BPB modifies a histidine residue in the active site of $sPLA_2$ [Roberts et al., 1977]. The effects of these inhibitors on PC secretion are shown in Figure 2. OOPC, ASA, and BPB show no significant inhibition of ATP-stimulated PC secretion.

Two $iPLA_2$ inhibitors, $PACOCF_3$ and HELSS were employed to investigate the involvement of $iPLA_2$ in lung surfactant secretion. $PACOCF_3$, a trifluoromethyl ketone analogue of palmitic acid, inhibits macrophage $iPLA_2$ with IC_{50} of 3.8 μM [Ackermann et al., 1995]. HELSS, a potent, irreversible, mechanism based inhibitor, inhibits macrophage and myocardial $iPLA_2$ with IC_{50}s of 60 nM and 100 nM, respectively [Ackermann et al., 1995; Hazen et al., 1991].

None of those $iPLA_2$ inhibitors had effect on ATP-stimulated PC secretion (Fig. 2).

Inhibition of PC Secretion by DAG Lipase Inhibitor

The $cPLA_2$ inhibitor only partially blocks PC secretion and none of $sPLA_2$ and $iPLA_2$ inhibitors does, suggesting that another pathway for the generation of arachidonic acid exists. In addition to PLA_2, arachidonic acid can also be produced from DAG by the sequential actions of two enzymes: DAG and MAG lipases. We therefore examined the effects of RHC-80267, a specific inhibitor of DAG lipase, on PC secretion. RHC-80267 displayed a dose dependent inhibition of ATP-stimulated PC secretion (Fig. 3). A 40% inhibition was observed at 50 μM of RHC-80267.

DISCUSSION

By means of specific inhibitors, the (phospho)-lipases involved in lung surfactant secretion from rat alveolar epithelial type II cells were investigated. The present study demonstrates that (i) The general PLA_2 inhibitor, ACA, inhibits various secretagogue-stimulated PC secretions; (ii) The $cPLA_2$ inhibitor, $AACOCF_3$, but not the $sPLA_2$ and $iPLA_2$ inhibitors blocks ATP-stimulated PC secretion; and (iii) The DAG lipase inhibitor, RHC-80267 inhibits ATP-stimulated PC secretion. The results point to roles of $cPLA_2$ and DAG lipase in lung surfactant secretion.

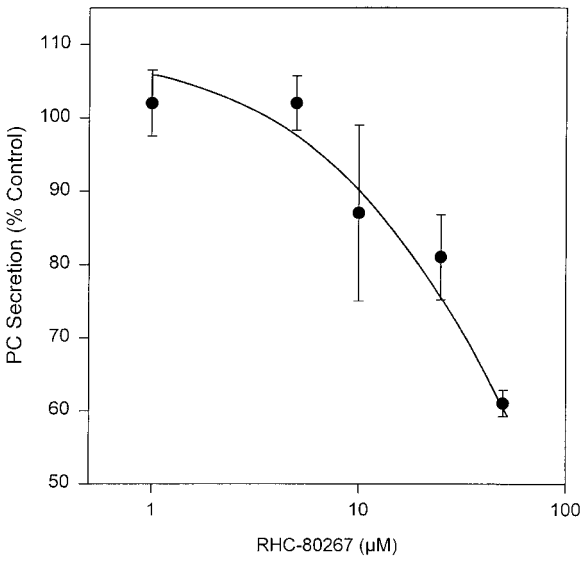

Fig. 3. Effect of DAG lipase inhibitor RHC-80267 on ATP-stimulated PC secretion. Type II cells were preincubated 30 min in the presence of varying concentrations of RHC-80267 and then stimulated 2 h with ATP. PC secretion was measured and expressed as a percentage of the control (in the absence of the inhibitor). Data shown are mean ± SE of three experiments, each carried out in duplicate using different cell preparations.

PLA_2 represents a diverse family of enzymes with heterogeneous biochemical characteristics [Dennis, 1994]. $sPLA_2$ has a low molecular mass (~14 kDa), is sensitive to dithiothreitol and requires mM Ca^{2+} for its activity. These secretory forms of PLA_2 are found in venoms, pancreatic secretions and inflammatory fluids. BPB, an inhibitor of $sPLA_2$, has been shown to block catecholamine secretion from bovine adrenal chromaffin cells [Frye and Holz, 1983] and human esosinophil degranulation [White et al., 1993]. In contrast to those reports, BPB and other $sPLA_2$ inhibitors including OOPC and ASA have no effect on PC secretion from lung type II cells. Although $sPLA_2$ is found in lung [Lindahl et al., 1989; Nakano and Arita, 1990] its existence in alveolar type II cells is controversial. While $sPLA_2$ can not be detected in type II cells by Western blot using antibodies against group I and group II $sPLA_2$s [Neagos et al., 1993] an alkaline Ca^{2+}(mM)-dependent, BPB-sensitive PLA_2 activity is observed in type II cells [Fisher and Dodia, 1996]. In views of low concentration of Ca^{2+} (μM) and high concentration of glutathione (mM), an intracellular role of $sPLA_2$ in type II cells is doubtful. However, the possibility that extracellular $sPLA_2$, secreted either by type II cells or by macrophages, endothelial cells and fibroblast, exerts its role on surfactant secretion can not be excluded, since extracellular mM Ca^{2+} is available. A recent paper reported that PC12 cells release $sPLA_2$ when the cells were stimulated with carbamylcholine or potassium. Furthermore, exogenous $sPLA_2$ increases catecholamine secretion, whereas an antibody against $sPLA_2$ decreases [Matsuzawa et al., 1996].

$iPLA_2$s were isolated from myocardium, macrophage-like cells $P388D_1$ and brain [Ackermann and Dennis, 1995]. Myocardial $iPLA_2$ has a molecular mass of 40 kDa and prefers plasmlogen. Its activity is stimulated by ATP and inhibited by a mechanism-based inhibitor, HELSS. This $iPLA_2$ is also expressed in pancreatic islet β cells and vascular smooth muscle cells. Since HELSS inhibits glucose-induced insulin secretion and a rise of intracellular Ca^{2+} from pancreatic islet β cells it is suggested that secreta-

gogues activate islet $iPLA_2$, release arachidonic acid, facilitate Ca^{2+} entry into β cells, and thus increase insulin secretion [Ramanadham et al., 1993, 1994]. Macrophage $iPLA_2$ has an apparent molecular mass of 80 kDa. Its activity is also stimulated by ATP and inhibited by HELSS and $PACOCF_3$. Previous studies have indicated that macrophage $iPLA_2$ may play roles in the esterification of arachidonic acid into membrane phospholipids and endocytosis [Balsinde et al., 1995; Mayorga et al., 1993]. Although $iPLA_2$ activity is detected in lung [Pierik et al., 1988] whether myocardial and macrophage $iPLA_2$s are present in alveolar type II cells is unknown. The present finding that the inhibitors of $iPLA_2$, $PACOCF_3$, and HELSS have no effect on PC secretion suggests that $iPLA_2$ is not a major player in lung surfactant secretion. Recently, a rat lung 26 kDa acidic Ca^{2+}-independent PLA_2 ($aiPLA_2$) has been isolated and cloned [Kim et al., 1997]. This $aiPLA_2$ shows a maximal activity at acidic pH (pH 4.0), is insensitive to BPB, ATP, and $AACOCF_3$, but is inhibited by a transition state phospholipid analog, MJ33. The major activity of $aiPLA_2$ is found in lamellar body/lysomal compartment. $aiPLA_2$ may be involved in PC remodeling and the degradation of DPPC in granular pneumocytes [Fisher and Dodia, 1996].

$cPLA_2$ is a high molecular mass (~85 kDa), cytosolic and widely distributed enzyme. It requires μm Ca^{2+} for binding to substrates. $cPLA_2$ is resistant to reducing agents, but is inhibited by $AACOCF_3$. Unlike other PLA_2, $cPLA_2$ selectively hydrolyzes arachidonyl phospholipids. Besides, $cPLA_2$ can translocate from cytosol to membrane in a Ca^{2+}-dependent manner and is regulated by protein kinases including protein kinase C and MAP kinase [Channon and Leslie, 1990; Qiu and Leslie, 1994]. These properties make it a good candidate to participate in signal transduction. $cPLA_2$ activity has been characterized in alveolar epithelial type II cells [Neagos et al., 1993]. The present study shows that $AACOCF_3$ inhibits ATP-stimulated PC secretion, suggesting a participation of $cPLA_2$ in lung surfactant secretion.

In type II cells, ATP stimulates P_{2u} puroceptor and increases the hydrolysis of phosphatidylinositol 4,5-bisphosphate to generate inositol trisphosphate (IP_3) and DAG by a G-protein coupled phosphoinositide-specific phospholipase C (PI-PLC) [Griese et al., 1991]. IP_3 leads to the mobilization of intracellular Ca^{2+} and DAG activates protein kinase C. ATP also activates phospholipase D (PLD) in type II cells and results in the formation of phosphatidic acid [Rooney and Gobran, 1993]. The latter is metabolized to DAG via phosphatidic acid phosphatase. While PI-PLC acts at early phase (~10 sec), the activation of PLD occurs at late phase (5–10 min) of stimulation. DAG can be hydrolyzed sequentially by DAG and MAG lipases to produce arachidonic acid. Both lipases translocate from gelatinase containing granules to the plasma membranes of neutrophils in response to stimulation by Ca^{2+} ionophore A23187 [Balsinde et al., 1991]. RHC-80267, a specific inhibitor of DAG lipase, has previously shown to inhibit catechamine release induced by acetylcholine from chromaffin cells [Rindlisbacher et al., 1990] and insulin secretion induced by glucose from pancreatic islets [Konrad et al., 1994]. The present finding that RHC-80267 inhibits ATP-stimulated PC secretion from alveolar type II cells indicates another pathway for the production of arachidonic acid during lung surfactant secretion. However, the relative contributions of two pathways ($cPLA_2$ and DAG lipase) in the secretagogue-induced secretion remain to be determined.

REFERENCES

Ackermann EJ, Dennis EA. 1995. Mammalian calcium-independent phospholipase A_2. Biochim Biophys Acta 1259:125–136.

Ackermann EJ, Conde-Frieboes K, Dennis EA. 1995. Inhibition of macrophage Ca^{2+}-independent phospholipase A_2 by bromoenol lactone and trifluoromethyl ketone. J Biol Chem 270:445–450.

Balsinde J, Bianco ID, Ackermann EJ, Conde-Frieboes K, Dennis EA. 1995. Inhibition of calcium-independent phospholipase A_2 prevents arachidonic acid incorporation and phospholipid remodeling in $P388D_1$ macrophages. Proc Natl Acad Sci USA 92:8527–8531.

Balsinde J, Diez E, Mollinedo F. 1991. Arachidonic acid release from diacylglycerol in human neutrophils: Translocation of diacylglycerol-deacylating enzyme activities from an intracellular pool to plasma membranes upon cell activation. J Biol Chem 266:15638–15643.

Bartoli F, Lin HK, Ghomashchi F, Gelb MH, Jain MK, Apitz-Castro R. 1994. Tight binding inhibitors of 85-kDa phospholipase A_2 but not 14-kDa phospholipase A_2 inhibit release of free arachidonate in thrombin-stimulated human platelets. J Biol Chem 269:15625–15630.

Baybutt RC, Smith JE, Gillespie MN, Newcomb TG, Yeh YY. 1994. Arachidonic acid and eicosapentaenoic acid stimulate type II pneumocyte surfactant secretion. Lipids 29:535–539.

Bligh EG, Dyer WJ. 1959. A rapid method of total lipid extraction and purification. Can J Biochem Physiol 37: 911–917.

Burgoyne R, Morgan A. 1990. The control of free arachidonic acid levels. Trends Biochem Sci 15:365–366.

Chander A, Fisher AB. 1990. Regulation of lung surfactant secretion. Am J Physiol 258:L241–L253.

Chander A, Sen N. 1993. Inhibition of phosphadtidylcholine secretion by stilbene disulfonates in alveolar type II cells. Biochem Pharmacol 45:1905–1912.

Channon JY, Leslie CC. 1990. A calcium-dependent mechanism for associating a soluble arachidonoyl-hydrolyzing phospholipase A_2 with membrane in the macrophage cell line RAW 264.7. J Biol Chem 265:5409–5413.

Dennis EA. 1994. Diversity of group types, regulation, and function of phospholipase A_2. J Biol Chem 269:13057–13060.

Dobbs LG, Gonzalez R, William MC. 1986. An improved method of isolating type II pneumocytes in high yield and purity. Am Rev Respir Dis 134:141–145.

Fisher AB, Dodia C. 1996. Role of phospholipase A_2 enzymes in degradation of dipalmitoylphosphatidylcholine by granular pneumocytes. J Lipid Res 37:1057–1064.

Frye RA, Holz RW. 1983. Phospholipase A_2 inhibitors block catecholamine secretion and calcium uptake in cultured bovine adrenal medullary cells. Mol Pharmacol 23:547–550.

Gifillan AM, Rooney SA. 1985. Arachidonic acid metabolites stimulate phosphatidylcholine secretion in primary cultures of type II cells pneumocytes. Biochim Biophys Acta 833:336–341.

Griese M, Gobran LI, Rooney SA. 1991. ATP-stimulated inositol phospholipid metabolism and surfactant secretion in rat type II pneumocytes. Am J Physiol 258:L586–L593.

Hazen SL, Zupan LA, Weiss RH, Getman DP, Gross RW. 1991. Suicide inhibition of canine myocardial cytosolic calcium-independent phospholipase A_2: Mechanism-based discrimination between calcium-dependent and -independent phospholipase A_2. J Biol Chem 266:7227–7232.

Kim T-S, Sundaresh CS, Feinstein SI, Dodia C, Skach WR, Jain MK, Nagase T, Seki N, Ishikawa K-C, Nomura N, Fisher AB. 1997. Identification of a human cDNA clone for lysosomal type Ca^{2+}-independent phospholipase A_2 and properties of the expressed protein. J Biol Chem 272:2542–2550.

Konrad RJ, Major CD, Wolf BA. 1994. Diacylglycerol hydrolysis to arachidonic acid is necessary for insulin secretion from isolated pancreatic islets: Sequential actions of diacylglycerol and monoacylglycerol lipases. Biochemistry 33:13284–13294.

Konrad RJ, Jolly YC, Major C, Wolf BA. 1992. Inhibition of phospholipase A_2 and insulin secretion in pancreatic islets. Biochim Biophys Acta 1135:215–220.

Lee YP, Lardy HA. 1965. Influence of thyroid hormone on L-α-glycerophosphate dehydrogenase and other dehydrogenases in various organs of rats. J Biol Chem 240:1427–1436.

Lindahl M, von Schenck H, Tagesson C. 1989. Isolation and characterization of phospholipase A_2 from rat lung with affinity chromatography and two-dimensional gel electrophoresis. Biochim Biophys Acta 1005:282–288.

Lipchik RJ, Chauncey JB, Paine R, Simon RH, Peters-Golden M. 1990. Arachidonate metabolism increases as rat alveolar type II cells differentiate in vitro. Am J Physiol 259:L73–L80.

Liu L, Wang M, Fisher AB, Zimmerman U-JP. 1996. Involvement of annexin II in exocytosis of lamellar bodies from alveolar epithelial type II cells. Am J Physiol 270:L668–L676.

Liu L, Fisher AB, Zimmerman U-JP. 1995. Lung annexin II promotes fusion of isolated lamellar bodies with liposomes. Biochim Biophys Acta 1259:166–172.

Magolda RL, Ripka WC, Galbraith W, Johnson PR, Rudnick MS. 1985. Novel synthesis of potent site-specific phospholipase A_2 inhibitors. In: Bailey JM, editor. Prostaglandins leukotriene and lipoxins. New York: Plenum Press, pp 669–676.

Matsuzawa H, Murakami M, Atsumi G, Imai K, Prados P, Inoue K, Kudo I. 1996. Release of secretory phospholipase A_2 from rat neuronal cells and its possible function in the regulation of catecholamine secretion. Biochem J 318:701–709.

Mayorga LS, Colombo MI, Lennartz M, Brown EJ, Rahman KH, Weiss R, Lennon PJ, Stahl PD. 1993. Inhibition of endosome fusion by phospholipase A_2 (PLA_2) inhibitors points to a role for PLA_2 in endocytosis. Proc Natl Acad Sci USA 90:10255–10259.

Nakano T, Arita H. 1990. Enhanced expression of group II phospholipase A_2 gene in the tissues of endotoxin shock rats and its suppression by glucocorticoid. FEBS Lett 273:23–26.

Neagos GR, Feyssa A, Peters-Golden M. 1993. Phospholipase A_2 in alveolar type II epithelial cells: Biochemical and immunologic characterization. Am J Physiol 264: L261–L268.

Peters-Golden M, Feyssa A. 1994. Augmented expression of cytosolic phospholipase A_2 during phenotypic transformation of cultured type II pneumocytes. Am J Physiol 266: C382–C390.

Peters-Golden M, Coburn K, Chauncey JB. 1992. Protein kinase C activation modulates arachidonic acid metabolism in cultured alveolar epithelial cells. Exp Lung Res 18:535–551.

Pierik AJ, Nijssen JG, Aarsman AJ, Van den Bosch H. 1988. Calcium-independent phospholipase A_2 in rat tissue cytosols. Biochim Biophys Acta 962:345–353.

Qiu ZH, Leslie CC. 1994. Protein kinase C-dependent and -independent pathways of mitogen-activated protein kinase activation in macrophages by stimuli that activate phospholipase A_2. J Biol Chem 269:19480–19487.

Ramanadham S, Gross RW, Han X, Turk J. 1993. Inhibition of arachidonate release by secretagogue-stimulated pancreatic islets suppresses both insulin secretion and the rise in β-cell cytsolic calcium ion concentration. Biochemistry 32:337–346.

Ramanadham S, Wolf MJ, Jett PA, Gross RW, Turk J. 1994. Characterization of an ATP-stimulatable Ca^{2+}-independent phospholipase A_2 from clonal insulin-secreting HIT cells and rat pancreatic islets: A possible molecular component of the β-cell fuel sensor. Biochemistry 33:7442–7452.

Rindlisbacher B, Sidle MA, Galatioto LE, Zahler P. 1990. Arachidonic acid liberated by diacylglycerol lipase is essential for the release mechanism in chromaffin cells from bovine adrenal medulla. J Neurochem 54:1247–1252.

Roberts MR, Deems T, Mincey T, Dennis E. 1977. Chemical modification of the histidine residue in phospholipase A_2 Naja naja naja: a case of half-life reactivity. J Biol Chem 252:2405–2411.

Rooney SA, Gobran LI. 1993. Activation of phospholipase D in rat type II pneumocytes by ATP and other surfactant secretagogues. Am J Physiol 264:L133–L140.

Street IP, Lin HK, Laliberte F, Ghomashchi F, Wang Z, Perrier H, Tremblay NM, Huang Z, Weech PK, Gelb MH. 1993. Slow- and tight-binding inhibitors of the 85-kDa human phospholipase A_2. Biochemistry 32:5935–5940.

Vishwanath BS, Fawzy AA, Franson RC. 1988. Edema-inducing activity of phospholipase A_2 purified from human synovial fluid and inhibition by aristolochic acid. Inflammation 12:549–561.

White SR, Strek ME, Kulp GVP, Spaethe SM, Robbi RA, Neeley SP, Leff AR. 1993. Regulation of human eosinophil degranulation and activation by endogenous phospholipase A_2. J Clin Invest 91:2118–2125.