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Negative feedback between secretory and cytosolic phospholipase A₂ and their opposing roles in ovalbumin-induced bronchoconstriction in rats

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Negative feedback between secretory and cytosolic phospholipase A₂ and their opposing roles in ovalbumin-induced bronchoconstriction in rats. *Am J Physiol Lung Cell Mol Physiol* 288: L523–L529, 2005. First published November 19, 2004; doi:10.1152/ajplung.00199.2004.—Phospholipase A₂ (PLA₂) hydrolyzes cell membrane phospholipids (PL) to produce arachidonic acid and lyso-PL. The PLA₂ enzymes include the secretory (sPLA₂) and cytosolic (cPLA₂) isoforms, which are assumed to act synergistically in production of eicosanoids that are involved in inflammatory processes. However, growing evidence raises the possibility that in airways and asthma-related inflammatory cells (eosinophils, basophils), the production of the bronchoconstrictor cysteinyl leukotrienes (CysLT) is linked exclusively to sPLA₂, whereas the bronchodilator prostaglandin PGE₂ is produced by cPLA₂. It has been further reported that the capacity of airway epithelial cells to produce CysLT is inversely proportional to PGE₂ production. This seems to suggest that sPLA₂ and cPLA₂ play opposing roles in asthma pathophysiology and the possibility of a negative feedback between the two isoenzymes. To test this hypothesis, we examined the effect of a cell-impermeable extracellular sPLA₂ inhibitor on bronchoconstriction and PLA₂ expression in rats with ovalbumin (OVA)-induced asthma. It was found that OVA-induced bronchoconstriction was associated with elevation of lung sPLA₂ expression and CysLT production, concomitantly with suppression of cPLA₂ expression and PGE₂ production. These were reversed by treatment with the sPLA₂ inhibitor, resulting in amelioration of bronchoconstriction and reduced CysLT production and sPLA₂ expression, concomitantly with enhanced PGE₂ production and cPLA₂ expression. This study demonstrates, for the first time in vivo, a negative feedback between sPLA₂ and cPLA₂ and assigns opposing roles for these enzymes in asthma pathophysiology: sPLA₂ activation induces production of the bronchoconstrictor CysLT and suppresses cPLA₂ expression and the subsequent production of the bronchodilator PGE₂.

asthma; cysteinyl leukotrienes; prostaglandin E₂; secretory phospholipase A₂ inhibitors

PHOSPHOLIPASE A₂ (PLA₂) is a family of enzymes that hydrolyzes cell membrane phospholipids (PL) to produce lysophospholipids (LysoPL) and free fatty acids. The PLA₂ family consists of secreted and intracellular enzymes. The intracellular PLA₂ include the cytosolic (cPLA₂), which is specific for arachidonic acid (AA)-carrying PL, and the calcium-independent PLA₂ (iPLA₂), which has no fatty acid preference. The secretory PLA₂ (sPLA₂) enzymes are secreted by activated

inflammatory and other mammalian cells in inflammatory conditions, such as pancreatitis, respiratory distress, sepsis, asthma, and more (7, 25).

By hydrolyzing cell membrane PL, PLA₂ enzymes initiate the production of numerous lipid mediators of diverse pathological states. When the released fatty acid is AA, it is metabolized into the eicosanoid families, mainly via the lipoxygenase pathways, producing the leukotrienes (LT), and the cyclooxygenase pathways, producing prostaglandins (PG) and thromboxanes. Eicosanoids of the different families are involved in the development of almost any pathological condition, especially those related to inflammatory/allergic processes (6, 41, 42).

LysoPL activate white cells and increase their vascular permeation (31), act as growth factors (especially lysophosphatidic acid), enhance airway smooth muscle (ASM) contractility and cell proliferation (37), and induce eosinophil activation and infiltration (27). Lysophosphatidyl serine, in particular, activates histamine secretion by mast cells (22). Lysophosphatidylcholine is the precursor of platelet-activating factor, a strong proinflammatory mediator (8, 10). Together, PLA₂ enzymes are directly and indirectly involved in diverse pathological processes, mainly those involved in inflammation and allergy.

iPLA₂ is considered a housekeeping enzyme involved in the maintenance of membrane PL composition, although a recent study demonstrated that in some inflammatory states, specifically adjuvant-induced arthritis in rats, this enzyme is the first to produce inflammatory eicosanoids (12). On the other hand, activation of cPLA₂ and sPLA₂ has unequivocally been shown to initiate the production of proinflammatory lipid mediators, eicosanoids in particular (6). The interrelationship between cPLA₂ and sPLA₂ in the induction of inflammatory processes is not unequivocally clear, as disparate results have been reported. For example, cPLA₂ is required for the induction of sPLA₂ expression in fibroblasts and macrophages (21), and cPLA₂ inhibitors block sPLA₂-dependent AA release (36). Conversely, in other cells (e.g., murine mesangial cells, human neutrophils, bone marrow mast cells, and others), sPLA₂ activates cPLA₂ and eicosanoid production (11–13, 17–19, 36). Together, these studies suggest that the relative contribution of the two PLA₂ types to inflammatory conditions is tissue dependent and might differ between tissues and cell types. Yet, it is generally accepted that both cPLA₂ and sPLA₂ take part and

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often act synergistically, in positive feedback mechanism, in inducing inflammatory/allergic processes. However, this generalization seems to be too inclusive, particularly in regard to airway function, in which different eicosanoids appear to play opposing roles: cysteinyl LT (CysLT) are strong bronchoconstrictors and are involved in airway inflammation and remodeling occurring in asthma (17), whereas PGE₂, which is generally considered a strong proinflammatory agent, is a potent bronchodilator and can inhibit ASM proliferation (28, 40). Moreover, it has been shown that intratracheal administration of PGE₂ to rats reduced ovalbumin (OVA)-induced elevation of CysLT level in the bronchoalveolar lavage (BAL) (23). On these grounds, it has been postulated that the lung is a privileged site for the beneficial actions of PGE₂, since in the lung, as opposed to other parts of the body, PGE₂ has a role in limiting the immune-inflammatory responses as well as in tissue repair processes (40). In accordance with that, it was further reported that the capacity of airway epithelial cells to produce CysLT is inversely proportional to PGE₂ production (17). It thus seems that although both eicosanoids are derived from AA subsequent to PLA₂ activation, the production of CysLT and PGE₂ in challenged airways follows different routes. A possible explanation for these disparate routes may be found in reports that in inflammatory cells (basophils, eosinophils), LT are produced from an AA pool linked to sPLA₂, whereas PG are produced from an AA pool provided by cPLA₂ (7, 24). Together, this seems to suggest an inverse relationship between sPLA₂ and cPLA₂ and disparate, possibly opposing, roles in asthma pathophysiology.

To test this hypothesis, in the present study we have examined the effect of a cell-impermeable extracellular inhibitor of sPLA₂ (ExPLI) on bronchoconstriction, concomitantly with PLA₂ expression and eicosanoid production, in rats with OVA-induced asthma. The ExPLIs, designed and synthesized in the laboratory of S. Yedgar (42), have been previously found effective in amelioration of inflammatory processes in cell cultures and in animal models. These include inhibition of lipid membrane hydrolysis by diverse types of sPLA₂ (9), inhibition of group V sPLA₂ activation in LPS-stimulated P388D macrophages (3), endotoxin-induced sepsis in rats, as expressed by reduced mortality rate, blood cytokine level (TNF- α , IL-6), and expression of type IIA sPLA₂ and inducible nitric oxide synthase in liver and kidney of septic rats (4), suppression of sPLA₂ and PGE₂ production by LPS-stimulated cultured rat brain glial cells and experimental allergic encephalomyelitis in rats and mice (29), and trinitrobenzenesulfonic acid-induced colitis in rats, expressed by reduced mortality rate, histology, and blood sPLA₂ activity (20). Using the ExPLIs, in the present study we have found that OVA-induced asthma in rats is associated with elevation of sPLA₂ and suppression of cPLA₂ activity and expression in the lung.

MATERIALS AND METHODS

Animals. Inbred Brown Norway male rats (4 wk old) obtained from Harlan were used in this study. The Hebrew University Animal Welfare Committee approved all protocols.

Induction of asthma. Asthma was induced in rats by sensitization with OVA (Sigma, Rehovot, Israel) according to a previously described protocol (33): on day 0, rats received a single subcutaneous injection of 1 mg of OVA plus aluminum hydroxide (200 mg/ml in 0.9% NaCl; Sigma) and an intraperitoneal injection of 1 ml containing

6×10^9 heat-killed *Bordetella pertussis* bacteria (Pasteur Marieux). Repeated bronchial allergen challenge was performed from day 14 every other day for 1 mo by inhalation of OVA (1 mg/ml in 0.9% normal saline) for 5 min each time in a 20-l box connected to an ultrasonic nebulizer (LS 230 System Villeneuve Sur Lot).

Treatments. Rats were divided into three treatment groups ($n = 10$): 1) no sensitization and no treatment (–/–), used as naïve control; 2) sensitization plus challenge with OVA and no treatment (OVA/OVA), used as positive control; and 3) sensitization plus challenge with OVA and treatment with ExPLI (OVA/ExPLI). The OVA/OVA group received a subcutaneous injection of 1 ml of saline before each challenge.

Treatment with ExPLI. In this study, we employed HyPE, designed and synthesized in the laboratory of S. Yedgar, by linking phosphatidylethanolamine to truncated hyaluronic acid (mol wt ~ 50 kDa) (4, 15). Each rat received a subcutaneous injection of 1 ml of saline containing 15 mg of HyPE (to obtain a concentration of ~ 1 mg/ml of body fluid = 20 μ M) before each challenge.

Assessment of bronchoconstriction. Unrestrained conscious rats were placed in a whole body plethysmograph (Buxco Electronics, Troy, NY) connected to a pneumotach (Type 0000, EMKA Technologies) at one end and to a 10-ml bottle at the other end. The pneumotach was connected to a preamplifier (model MAX2270, Buxco Electronics). Analog signals from the amplifier were converted to a digital signal by an analog-to-digital card (LPM-16; National Instruments, Austin, TX). Bronchoconstriction measures were expressed as the enhanced pause (Penh). $\text{Penh} = (\text{PEF}/\text{PIF}) \times [(T_e - T_r)/T_r]$, where PEF is peak expiratory flow, PIF is peak inspiratory flow, T_e is expiratory time, and T_r is relaxation time = time of the pressure decay to 36% of total box pressure during expiration, according to Ref. 14.

BAL. BAL was performed on day 30. The rats were tracheotomized and cannulated. BAL was performed with 5×10 ml of PBS. BAL fluid was collected into 50-ml sterile tubes, centrifuged (to collect cell-free supernatant), and stored at -80°C .

Lung histological preparation. Lungs were fixed by inflation with paraformaldehyde at a pressure of 20 cmH₂O and embedded in paraffin. Tissue slices were stained with eosin-hematoxylin and periodic acid-Schiff (PAS) for cell identification.

Immunohistochemistry of cPLA₂ in rat lung tissue. Five-micrometer sections were subjected to staining using Histostain-SP kit (Zymed, San Francisco, CA). Polyclonal anti-rabbit cPLA₂ antibody, generously provided by Dr. Lisa Marshall [Glaxo-Smith-Kline (GSK), King of Prussia, PA], was used as first antibody. The antibody complex was visualized by applying aminoethylcarbazol for 3–10 min.

Computerized staining intensity measurement. Staining intensity was quantified by measuring density lamination using the Image Pro Plus V3 computer program (Media Cybernetics, Baltimore, MD). In this method, one measures the light transmittance, which is inverse (reciprocal) to the density of the staining color (i.e., the more cPLA₂ in the tissue, the lesser the light transmittance). Accordingly, cPLA₂ staining was determined by the difference between the maximal light transmittance and that of the measured sample and expressed by total density lamination in lung tissues and point counting in specific cell types.

Protein expression of cPLA₂ and sPLA₂ in lung tissue. Proteins were identified in homogenized lung tissue (100 μ g of protein) using standard Western blot. A specific polyclonal antibody against cPLA₂ (used as first antibody) was provided by Dr. Lisa Marshall and was diluted [Tris buffer saline Tween 20 (TBST) buffer + 0.1% BSA] 1:1,000. Anti-sPLA₂ antibody (Santa Cruz) was diluted 1:500 (vol/vol) in TBST buffer plus 0.1% BSA. The immune reaction was detected by enhanced chemiluminescence.

Determination of cPLA₂ activity. cPLA₂ activity was determined by the hydrolysis of radioactively labeled lipid membranes (liposomes) containing AA-carrying PL, as previously described (20).



Lung tissues were homogenized in a polytron in ice-cold buffer (1:5 wt/vol) containing Tris·HCl (pH 7.6), EDTA (1 mM), EGTA (1 mM), and DTT (5 mM) to abolish sPLA₂ activity. The phospholipid substrate (liposomes) was composed of 1-stearoyl-2-[¹⁴C]-arachidonyl phosphatidylcholine (Amersham), lysophosphatidylcholine, and dioleoyl-phosphatidylcholine (Sigma), suspended in Tris buffer, and supplemented with 1 mg/ml of BSA. The reaction was initiated by the addition of 100 μ l of homogenate and 2 mM CaCl₂ and incubated at 37°C in a shaking water bath for 1 h. Naja Mocambique PLA₂ type 1 (Sigma) was used as positive control. The reaction was stopped by the addition of 1.25 ml of Dole's reagent (isopropyl alcohol/heptane/0.5 M H₂SO₄, 20:5:1, by vol), 1 ml of heptane, and 0.75 ml of water. The mixture was vortexed and centrifuged (1,000 *g* for 10 min). The organic phase (0.8 ml) was added to 20 mg of silica with 1 ml of heptane and was then centrifuged to remove remnants of PL. The supernatant was mixed with scintillation fluid, and ¹⁴C radioactivity was determined in a scintillation counter. PLA₂ activity was defined as the percentage of ¹⁴C-labeled fatty acid in the total radioactivity.

CysLT. CysLT levels were measured in BAL using a kit for direct enzyme immunoassay according to the manufacturer's instructions (Amersham Pharmacia Biotech). The specificity of the kit was 100% for leukotriene C₄, 100% for leukotriene D₄, and 70% for leukotriene E₄. The result range was between 0 and 48 pg.

Determination of PGE₂. PGE₂ levels were determined in BAL fluid by RIA using specific antibody and a radioligand as previously described by Pinto et al. (29). Anti-PGE₂ and standard PGE₂ were purchased from Sigma (St. Louis, MO). [³H]PGE₂ (150–200 Ci/mmol) was purchased from Amersham.

Statistical analysis. All data are expressed as means $\pm$ SE. One-way ANOVA was used to compare treatment groups. Pairwise comparisons were performed by the Tukey-Kramer honestly significant difference test ($P = 0.05$). Where necessary, data were log transformed before analysis to stabilized variances. In all analyses, $P < 0.05$ was considered statistically significant.

RESULTS

Effect of ExPLI on OVA-induced bronchoconstriction. To examine the relationship of respiratory function to the expression and activity of the PLA₂ isoenzymes, we first validated the applicability of the ExPLI (HyPE) as an sPLA₂ inhibitor on respiratory functions of sensitized rats with OVA-induced asthma. As shown in Fig. 1, HyPE effected a dramatic improvement in respiratory functions. Figure 1 shows that the administration of HyPE at a dose corresponding to ~ 10 μ M in body fluid induced an ~ 10 -fold reduction of the OVA-induced bronchoconstriction.

CysLT and PGE₂ in BAL of asthmatic rat. As discussed above, among the lipid mediators involved in asthma pathophysiology, CysLT and PGE₂ are said to play opposing roles. CysLT are considered key mediators of bronchoconstriction, whereas PGE₂ is a bronchodilator. The results of the present study support this notion: Fig. 2 shows that in parallel to the OVA-induced bronchoconstriction, the level of CysLT in BAL was markedly elevated, and treatment with the ExPLI reduced it to that of naïve rats. Contrary to CysLT, the PGE₂ level in the BAL of asthmatic rats was markedly reduced, and this was strongly reversed by treatment with HyPE, bringing it to the level of the normal rats, as shown in Fig. 3.

cPLA₂ and sPLA₂ in lungs of asthmatic rats. As noted in the Introduction, it has been previously suggested that LT are produced from an AA pool provided by sPLA₂, whereas PG are produced from an AA pool provided by cPLA₂ (1, 25). Along this line, we have determined the levels of the PLA₂

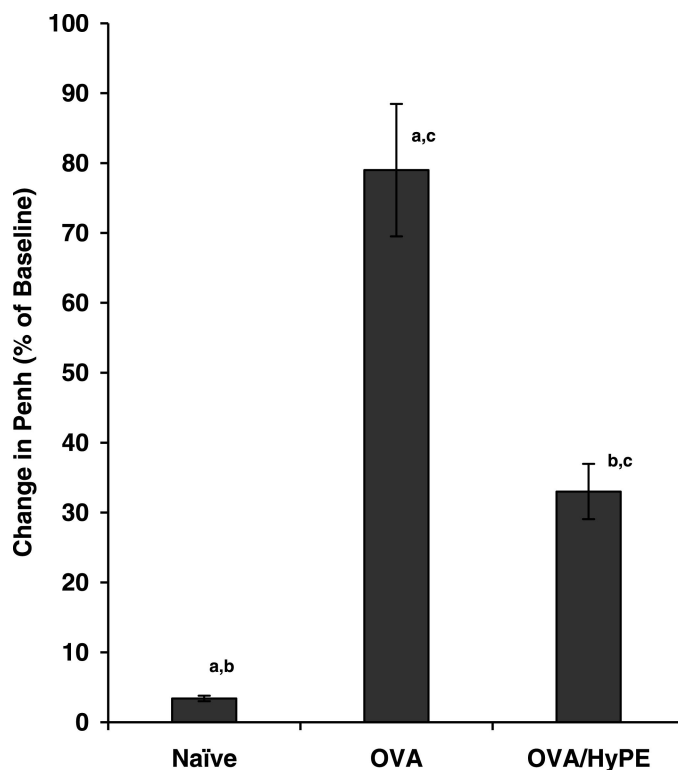


Fig. 1. Effect of phosphatidylethanolamine linked to hyaluronic acid (HyPE), administered subcutaneously, on early asthmatic reaction induced by ovalbumin (OVA) inhalation. Bronchoconstriction was induced in OVA-sensitized rats by inhalation of OVA and expressed by the difference in enhanced pause (Penh) measured before and 5 min after allergen challenge (see MATERIALS AND METHODS for details). Data are means $\pm$ SE for 10 rats. ^a $P < 0.01$; ^b $P < 0.05$.

isoforms in rat lungs following the different treatments, first by immunohistochemistry.

cPLA₂ immunoreactivity was measured by the density of the immunochemical staining. As illustrated in Fig. 4, in general, cPLA₂ was observed in alveolar macrophages, lymphocytes at the site of inflammatory infiltrate (mainly in the untreated asthmatic rats), in ASM cells, and in airway-ciliated epithelial cells. Staining with PAS reagent for mucus-secreting (goblet) cells was negative, implying that goblet cells were not formed, as expected in acute allergic inflammation. It is well known that mucus metaplasia is not significant in acute inflammation and generally occurs in later stages of chronic asthma. In the control and asthmatic rats, the staining in the epithelial cells was mainly in the apical membrane, whereas in the HyPE-treated group, in which the staining was enhanced, cPLA₂ was localized primarily in the cytoplasm of the ASM and epithelial cells.

The corresponding quantitation of cPLA₂ is depicted in Fig. 4D, presenting total staining density, and Fig. 4E, presenting point counting of cPLA₂ in the epithelial cells. The point-counting procedure could not be applied to ASM layer, since it exhibited multiple nuclei, which could not be sufficiently distinguished from the cytoplasm. Figure 4, D and E, shows that cPLA₂ expression was reduced in the asthmatic, untreated rats, but treatment with HyPE reversed this process and enhanced the cPLA₂ level even beyond that of naïve rats. Comparison of Fig. 3 with Fig. 4 shows that the changes in cPLA₂ paralleled those in PGE₂ production. In the same experiment,

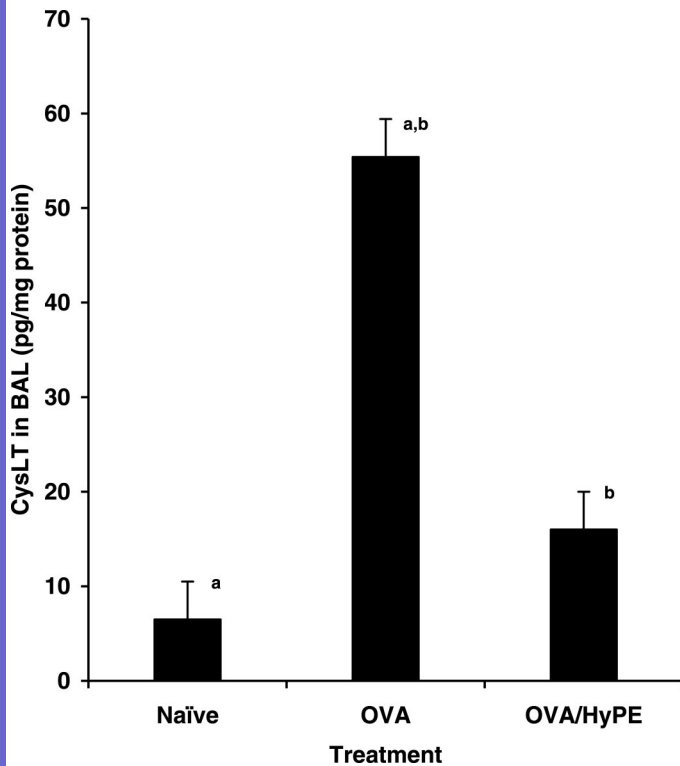


Fig. 2. Effect of HyPE on cysteinyl leukotriene (CysLT; LTC₄, LTD₄, and LTE₄) level in the bronchoalveolar lavage (BAL) of OVA-induced asthmatic rats. BAL was collected upon death, and CysLT levels were determined by enzyme immunoassay, as described in MATERIALS AND METHODS. Data are means $\pm$ SE for 10 rats. ^a, ^b*P* < 0.01. There was no significant difference between HyPE-treated and naïve rats.

sPLA₂ could not be detected by immunochemical staining of lung tissues, and this could be either due to inaccessibility of sPLA₂ (which is membrane bound) to its antibody and/or due to the sPLA₂ secretion to the extracellular medium.

To further elaborate on the changes in cPLA₂ and sPLA₂, we determined the protein expression of these isoforms in the lung tissue homogenates following the different treatments. As shown in Fig. 5, using this method, sPLA₂ could also be detected in the lung tissue. This figure shows that, similar to the immunohistochemical staining (Fig. 4), cPLA₂ protein expression was lower in the asthmatic rats and was recovered by treatment with ExPLI. At the same time, sPLA₂ protein expression was elevated in the lung of asthmatic rats and was reduced by treatment with ExPLI.

PLA₂ activity in lung tissues. Subsequent to the changes in PLA₂ protein levels in lung, the related activities in lung tissues were measured by the hydrolysis of lipid membrane composed of AA-containing PL, which is a substrate for both isoforms. To differentiate the cPLA₂ activity from that of sPLA₂, the hydrolysis was determined in the absence of the reducing agent DTT (to obtain total activity) and in the presence of DTT, which abolishes sPLA₂ activity (14), thus enabling the determination of cPLA₂ activity alone (see MATERIALS AND METHODS). As shown in Fig. 6, cPLA₂ activity was sharply suppressed in lung tissues of the asthmatic rats and was augmented to that of the control healthy rats by treatment with HyPE. The corresponding changes in sPLA₂ enzymatic activity in lung tissues (calculated by deduction of the cPLA₂ from

total activity) were in the opposite direction to that of cPLA₂, as was expected, i.e., elevated in the lungs of asthmatic rats and reduced by treatment with HyPE (not shown). It should be noted that attempts to determine sPLA₂ activity in BAL were not successful, probably due to excessive dilution during the process of BAL collection.

DISCUSSION

The contributions of cPLA₂ and sPLA₂ to the development of inflammatory/allergic diseases have been the subject of disparate reports and sometimes of disagreement. As discussed above, since cPLA₂ is specific to AA-carrying PL, and its activation has been linked to production of PG, it has been suggested that this isoform is the important one in the production of proinflammatory eicosanoids. On the other hand, sPLA₂ activation and participation in pathophysiology have been observed in many inflammatory conditions (7, 25). In addition, it has been suggested that in cells that express sPLA₂, the bulk of AA is produced by sPLA₂ of different types, particularly under inflammatory conditions (2, 25, 34), and sPLA₂ enzymes have thus been considered the inflammatory enzymes (13, 18). As discussed in the Introduction, many studies suggested apposite feedback and a synergistic action between sPLA₂ and cPLA₂, but the relative contribution of the two isoenzymes to inflammatory conditions and the interrelation between them might differ between tissues and cell types.

With regard to asthma, previous studies suggested that cPLA₂ plays a role in its pathophysiology, and it has been reported that cPLA₂-deficient mice had decreased sensitivity to induction of asthma by OVA (35, 38). On the other hand, it has been reported that in ASM, cPLA₂ is responsible for the

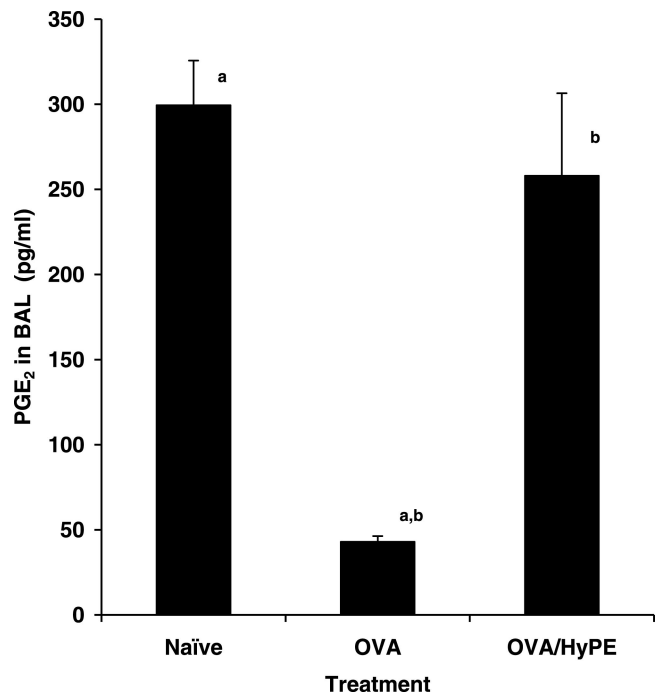


Fig. 3. Effect of HyPE on PGE₂ levels in BAL of rats with OVA-induced asthma. PGE₂ levels were determined in BAL fluid by RIA (see MATERIALS AND METHODS). Data are means $\pm$ SE for 10 rats. Bars that have a common letter are significantly different at *P* < 0.01.

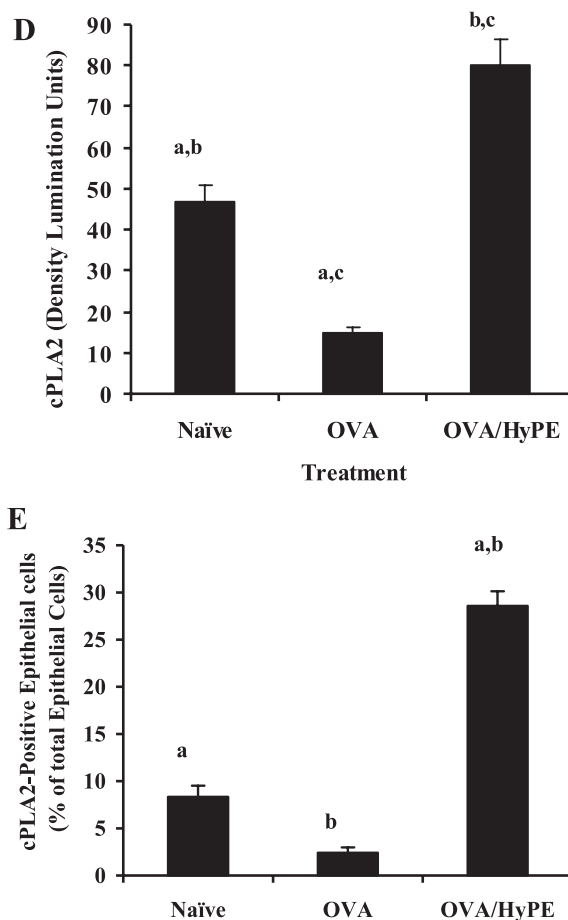
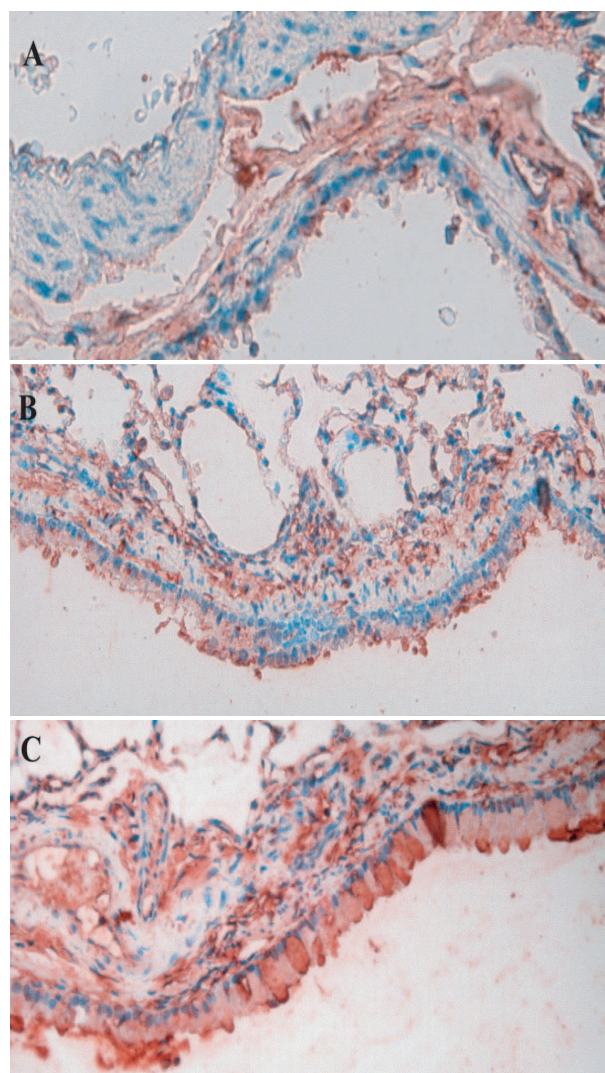


Fig. 4. Immunohistochemistry of cytosolic phospholipase A₂ (cPLA₂) in lung of rat with OVA-induced asthma. A: naïve, control rats. B: OVA-challenged rat. C: OVA-challenged rats treated with HyPE ($\times 40$ magnification). D: total staining density. Data are means $\pm$ SE for 50 arbitrarily chosen fields. ^a, ^b $P < 0.05$; ^c $P < 0.01$. E: point counting of cPLA₂ staining in epithelial cells (expressed as percentage of stained/total epithelial cells). Data are the average of points counted in 3 separate sections for each treatment. ^a, ^b $P < 0.01$.

release of PGE₂ (30), which acts as an antagonist to CysLT by inducing bronchodilation and inhibiting smooth muscle cell mitogenesis (17, 40), and that “the capacity of airway epithelium to CysLT synthesis is inversely related to its ability to make PGE₂” (17). In accordance with that, it has been reported that in cells that play a key role in asthma, namely human eosinophils and basophils, PGE₂ is produced from a cPLA₂-linked AA pool, whereas CysLT are produced from a different, sPLA₂-linked AA pool (7, 24). Indeed, sPLA₂ is found at high levels in BAL and bronchotracheal smooth muscle cells (32, 39). These reports thus indicate opposing roles for these enzymes in asthma pathophysiology. This hypothesis is clearly supported by the findings of the present study, summarized in Table 1. This table shows that in asthmatic rats, OVA-induced bronchoconstriction is accompanied by elevation of airway sPLA₂ protein expression and CysLT production, concomitantly with suppression of cPLA₂ protein expression and activity, and PGE₂ production. These processes are reversed when the rats are treated with cell-impermeable sPLA₂ inhib-

itor, resulting in amelioration of bronchoconstriction and decrease in sPLA₂ expression and CysLT production, concomitantly with enhancement of PGE₂ production and cPLA₂ expression. Hence, this study shows, for the first time in vivo, inverse modulation of sPLA₂ and cPLA₂ in asthma pathophysiology and further suggests that their activities are interdependent.

Although the concomitant elevation of sPLA₂ and suppression of cPLA₂ suggests a linkage between the enzymes, simultaneous appearance of two phenomena does not necessarily suggest a cause-and-effect relationship between them, and the two effects might be independent results of a common effector. In accordance with that is the aforementioned report that in human eosinophils, CysLT production results from sPLA₂ activity independent of cPLA₂ (24). However, the present study shows that treatment of the asthmatic rats with the sPLA₂ inhibitor not only suppressed this sPLA₂ expression (and corresponding CysLT production) but also upregulated cPLA₂ expression (and corresponding PGE₂ production). This

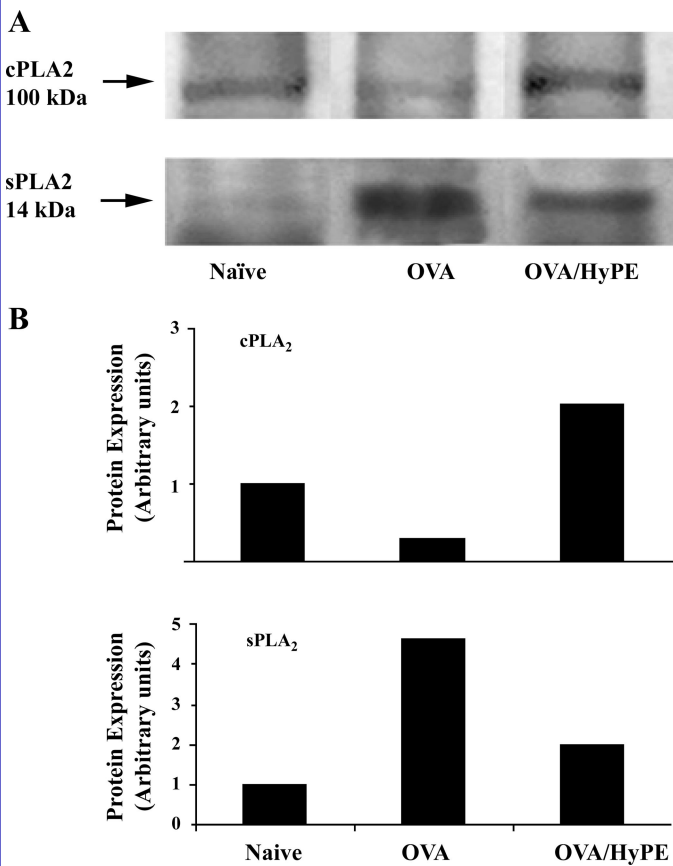


Fig. 5. Effect of HyPE on cPLA₂ and secretory phospholipase A₂ (sPLA₂) expression in lung of rats with OVA-induced asthma. Shown are Western blot (A) and corresponding densitometry (B) of cPLA₂ and sPLA₂ in lung homogenates of rats with OVA-induced asthma, treated as indicated. In B, for each enzyme, the density values were normalized to corresponding naïve rats.

strongly suggests a negative feedback between sPLA₂ and cPLA₂ types.

A possible explanation for this phenomenon can be that a product of PL hydrolysis by sPLA₂ downregulates cPLA₂ expression and activity. For example, sPLA₂, which is enhanced in airways of allergen-stimulated asthmatic patients, produces excessive amounts of LysoPL (5, 8), which in turn induces cPLA₂ phosphorylation (18, 19, 26). Although cPLA₂ phosphorylation is generally assumed to induce the enzyme's activation, the opposite was found (in vascular smooth muscle cells) when cPLA₂ was phosphorylated by cGMP-dependent kinases, which inhibited the enzyme's activity as expressed by AA production (26). It is likely that a similar process is responsible for the sPLA₂-associated suppression of cPLA₂ activity and PGE₂ production and its reversal by the sPLA₂ inhibitor. Yet, in the present study, we have found that sPLA₂ activation is associated not only with suppression of cPLA₂ activity (Fig. 6) but also with its protein expression (Figs. 4 and 5), and this is reversed by the ExPLI. sPLA₂ is able to affect cPLA₂ expression and activity by induction of cell signaling via a receptor-mediated process, independently of its lipolytic activity (1). Although this usually leads to increased cPLA₂ synthesis, on the grounds of the present study we cannot rule out an opposite effect in OVA-induced bronchoconstriction, as suggested by the negative feedback modulation of sPLA₂ and cPLA₂. It is therefore possible that

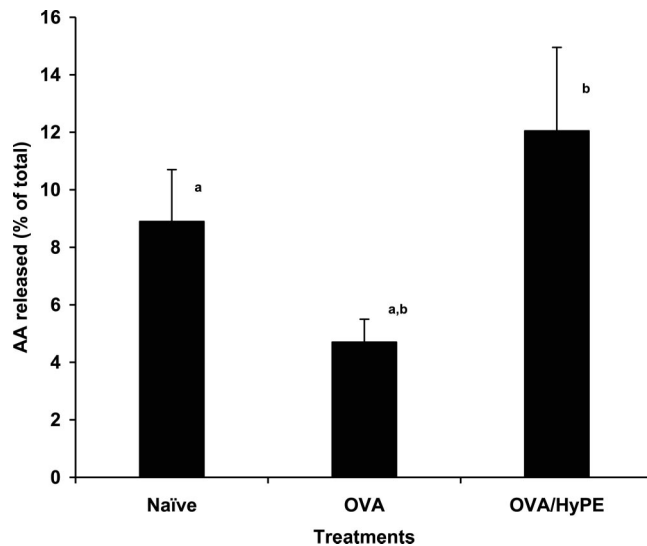


Fig. 6. Effect of HyPE on cPLA₂ activity in homogenates of lung tissue of rats with OVA-induced asthma. cPLA₂ activity was determined by the capacity of homogenates of lung tissue of rats, treated as indicated, to hydrolyze 2-arachidonic acid (AA)-phosphatidylcholine containing liposomes in the presence of DTT, as described in MATERIALS AND METHODS. Data are means $\pm$ SE for 8 rats. ^a*P* < 0.05; ^b*P* < 0.01.

sPLA₂ modulates cPLA₂ in more than one way, and the exact mechanism(s) of this interrelationship in OVA-induced bronchoconstriction has yet to be explored.

All in all, the present study, which shows for the first time in vivo that OVA-induced bronchoconstriction is controlled by inverse modulation of sPLA₂ and cPLA₂, supports the hypothesis that these enzymes have opposing roles in the pathophysiology of asthma. These findings also support the previous reports that in airway epithelial cells, CysLT, the major bronchoconstricting eicosanoids, are produced subsequent to sPLA₂ action, whereas the bronchodilator PGE₂ is produced from a separate, cPLA₂-linked AA pool. It is plausible to conclude that in OVA-induced asthma in rats, sPLA₂ activity contributes to the disease development while cPLA₂ activity contributes to its amelioration, and the control of the negative feedback between these isoenzymes by inhibition of sPLA₂ might introduce a potentially novel approach in the treatment of asthma.

GRANTS

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Table 1. Effect of HyPE on inflammatory markers in Brown Norway rat with OVA-induced asthma

	Penh	sPLA ₂ expression	CysLT	cPLA ₂ expression	PGE ₂
Naïve	↓	↓	↓	↑	↑
OVA/OVA	↑	↑	↑	↓	↓
ExPLI/OVA	↓	↓	↓	↑	↑

Naïve, normal untreated rats; OVA/OVA, OVA-sensitized and -challenged rats; OVA/ExPLI, OVA-sensitized and -challenged rats treated with phosphatidylethanolamine linked to hyaluronic acid (HyPE); OVA, ovalbumin; ExPLI, extracellular inhibitor of secretory phospholipase A₂ (sPLA₂); Penh, enhanced pause; CysLT, cysteinyl leukotrienes; cPLA₂, cytosolic phospholipase A₂.

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