An Essential Role for Lysophosphatidylcholine in the Inhibition of Platelet Aggregation by Secretory Phospholipase A₂

By Yuping Yuan, Shaun P. Jackson, Harvey H. Newnham, Christina A. Mitchell, and Hatem H. Salem

The release of secretory phospholipase A₂ (sPLA₂) into the mammalian circulation may contribute to the development of hemorrhagic and inflammatory diseases. sPLA₂ has previously been shown to alter the behavior of platelets, leukocytes, and endothelial cells, although the molecular basis for these cellular effects has not been established. Our studies indicate that the inhibition of platelet aggregation by snake, bee venom, and pancreatic sPLA₂ is dependent on a plasma cofactor. This cofactor resides within the lipoprotein fraction of plasma, with 54%, 31%, and 11% of the activity present in the high-density lipoprotein (HDL), low-density lipoprotein (LDL), and very low density lipoprotein (VLDL) fractions, respectively. Delipidation of HDL and LDL was associated with the complete loss of platelet-inhibitory activity. Incubation of purified sPLA₂ with the HDL fraction of plasma resulted in the time-dependent generation of lysophosphatidylcholine (lysoPC). The formation of lysoPC correlated with the inhibition of platelet aggregation. Purified lysoPC (10 to 100 μg/ml) inhibited platelet aggregation and dense granule release induced by thrombin (0.05 U/ml), collagen (1 μg/ml), ionophore A23187 (2 μmol/L), ADP (12.5 μmol/L), and adrenaline (3.2 μmol/L). The inhibition of platelet aggregation by lysoPC was dose-dependent and correlated with decreased fibrinogen binding to glycoprotein IIb-IIIa. Our studies indicate that the enzymatic generation of lysoPC from plasma lipoproteins is essential for the sPLA₂-mediated inhibition of platelet activation in the presence of albumin. These results raise the possibility that the toxic effects of circulating sPLA₂ may be due in part to the generation of the bioactive lysophospholipid, lysoPC.

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PHOSPHOLIPASES A₂ (PLA₂) are ubiquitous enzymes that specifically catalyze the hydrolysis of the 2-ester bond of sn-3-phosphoglycerides.¹ These enzymes participate in the metabolism of membrane phospholipids,¹ and are classified into secretory (sPLA₂) and cytosolic forms (cPLA₂).²,³ The secretory class is further divided into groups I, II and III, depending on their source of origin and amino acid sequence.⁴,⁵ The secretory enzymes generally have lower molecular weights (14 kD) than their cytosolic counterparts⁶ and do not show any fatty acid specificity in the sn-2 position of phospholipids.⁷

Mammalian sPLA₂ have been implicated in a broad range of physiologic functions, including digestion, fertilization,⁸ cell proliferation,⁹ and vascular and bronchial smooth muscle contraction.¹⁰ There is growing evidence that the release of sPLA₂ into the circulation may play an important role in the pathogenesis of a variety of inflammatory disorders, including acute pancreatitis,¹¹,¹² arthritis,¹³ endotoxic shock,¹⁴ and adult respiratory distress syndrome.¹⁵ In addition, snake venoms are an abundant source of sPLA₂ and the envenomation by a variety of snakes leads to disorders of blood coagulation and platelet aggregation, resulting in a hemorrhagic diathesis.¹⁶ A number of these enzymes have been isolated and shown to be potent inhibitors of platelet aggregation.¹⁷-²² However, unlike other snake venom proteins, such as the disintegrins, ADPases, and fibrinogenases, which inhibit platelet aggregation via well-characterized mechanisms, the molecular basis for the platelet inhibitory effects of a subset of sPLA₂ has remained ill-defined.

Recent studies have shown the existence of high-affinity receptors for sPLA₂ on the cell surface.²³-²⁵ It is unclear whether the toxic effects of sPLA₂ are mediated by these receptors or are due to the enzymatic production of several potent inflammatory mediators.²⁶ The hydrolysis of phospholipids by PLA₂ results in the production of free fatty acids and lysophospholipids. A broad range of biologic activities have been attributed to the natural phospholipid, lysophosphatidylcholine (lysoPC). LysoPC stimulates the transcription of adhesion molecules and growth factors in endothelial cells,²⁶-²⁷ induces chemotaxis in T lymphocytes²⁸ and monocytes,²⁹ enhances diacylglycerol-dependent activation of T lymphocytes,³⁰,³¹ and relaxes vascular smooth muscle cells.³² Although the physiologic function of lysoPC remains unclear, its ability to induce chemotaxis of leukocytes and upregulate adhesion receptors on endothelial cells has lead to the speculation that it may play an important role in the pathogenesis of atherosclerosis and inflammation.²⁶,²⁷,²⁹,³²

Although it has generally been assumed that the surface membranes of cells are the likely source of phospholipid substrate for extracellular PLA₂, studies in vitro suggest that only limited quantities of lysophospholipids are released from the membranes of resting cells by sPLA₂.²³,²⁵ In this report, we have examined the mechanism of platelet inhibition by various sPLA₂. Our studies indicate that, in the presence of serum albumin, the hydrolysis of membrane phospholipids has minimal effect on platelet activation and that the platelet inhibitory effects of sPLA₂ are dependent on the enzymatic generation of lysoPC from plasma lipoproteins.

MATERIALS AND METHODS

sPLA₂ was purified from the venom of Austrelaps superba (Australian Reptile Park, New South Wales, Australia), as previously described.²³ Pancreatic and bee venom sPLA₂, all lipids, all chemical reagents, and all chromatography media were purchased from Sigma Chemical Co (St Louis, MO), except for collagen (Chrono-Log Corp, Haventown, PA), adrenaline and bovine thrombin (Parke-Davis Co,
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Morrison Plains, NJ), ionophore A23187 (Calbiochem Corp, La Jolla, CA), and 32P-NaI and 3H-serotonin (New England Nuclear, Dupont, Wilmington, DE). Fibrinogen was purified to homogeneity and labeled with 125I-NaI according to the method of Marguerie et al. Thin-layer chromatography (TLC) plates (silica gel 60) were purchased from Merck (Darmstadt, Germany). Human plasma was donated by the Red Cross Blood Bank (Melbourne, Australia).

Preparation of washed platelets. Blood was drawn from healthy volunteers who had not received antiplatelet medication for 2 weeks. Whole blood was collected in acid-citrate-dextrose (6:1; vol/vol) containing 90 mmol/L sodium citrate, 7 mmol/L citric acid, pH 4.6, and 140 mmol/L dextrose, supplemented with 70 mmol/L theophylline. Platelet-rich plasma (PRP) was obtained by centrifugation of whole blood at 180 g for 15 minutes. PRP was centrifuged at 2,000 g for 5 minutes and the platelets were washed twice with platelet resuspension buffer containing 4.3 mmol/L NaH₂PO₄, 4.3 mmol/L KH₂PO₄, 24.3 mmol/L NaCl, pH 6.5, 113 mmol/L NaCl, 5.5 mmol/L glucose, 0.5% bovine serum albumin (BSA), and 10 mmol/L theophylline. The final platelet preparation was resuspended in a modified Tyrode's buffer, 12 mmol/L NaHCO₃, 0.32 mmol/L NaH₂PO₄, 10 mmol/L HEPES, pH 7.5, 137 mmol/L NaCl, 2.7 mmol/L KCl, 0.5 mmol/L MgCl₂, and 5.5 mmol/L glucose.

Platelet aggregation studies. Platelet aggregation was performed using a four-channel automated platelet analyzer (Kyoto Daichi, Japan) set to 950 rpm at 37°C. Each reaction mixture (400 μL) contained fibrinogen (1 mg/mL), BSA (5 mg/mL), and washed platelets (3 × 10⁸/mL). sPLA₂ was preincubated with plasma lipoproteins, or phospholipids for 10 minutes at 37°C, before the addition of the platelet agonist. Inhibition of platelet aggregation was expressed as the percentage difference in the initial rate of platelet aggregation in the presence versus absence of sPLA₂.

125I-Fibrinogen binding to platelets. Fibrinogen binding to washed platelets was performed according to the method of Marguerie et al. with slight modifications. Each platelet reaction mixture contained fibrinogen (1 mg/mL), BSA (5 mg/mL), and washed platelets (3 × 10⁸/mL). sPLA₂ was preincubated with plasma lipoproteins, or phospholipids for 10 minutes at 37°C, before the addition of the platelet agonist. Inhibition of platelet aggregation was expressed as the percentage difference in the initial rate of platelet aggregation in the presence versus absence of sPLA₂.

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purified enzyme did not inhibit aggregation in response to a wide variety of agonists, including thrombin (0.05 U/mL), collagen (1 μg/mL), ADP (12.5 μmol/L), and ionophore A23187 (2 μmol/L). The addition of normal plasma (10 to 20 μL) to purified sPLA2 restored the platelet-inhibitory effects of sPLA2 (Fig 1A). Inhibition of collagen-induced platelet aggregation was dose-dependent (Fig 1A) and required preincubation of sPLA2 with plasma (Fig 1B). The plasma dependence for sPLA2-mediated inhibition of platelet aggregation was also observed when thrombin (0.05 U/mL), ADP (12.5 μmol/L), or ionophore A23187 (2 μmol/L) were used as agonists (data not shown).

Identification and characterization of the sPLA2 plasma cofactor. Initial purification of the sPLA2 cofactor in human plasma was performed with hydrophobic interaction resins. Normal human plasma was chromatographed on Phenyl-Sepharose at neutral pH. Although under these conditions only 5% of the loaded protein bound, all sPLA2 cofactor activity was retained. Ninety percent of the sPLA2 cofactor activity was eluted with 75% ethylene glycol (data not shown). These preliminary studies suggested that the sPLA2 cofactor in human plasma was strongly hydrophobic and likely to represent a plasma lipoprotein.

Plasma lipoproteins were isolated by ultracentrifugation as described in the Materials and Methods. Ninety-six percent of the sPLA2 cofactor activity was recovered in the lipoprotein-rich fraction of plasma (d 1.211 which contains HDL, LDL, VLDL, and CM; Fig 2). Differential ultracentrifugation was used to separate HDL from LDL and VLDL. Forty-two percent of the cofactor activity was present in the lipoprotein fraction containing LDL, VLDL, and CM (d 1.063), whereas only 11% of the cofactor activity was present in the lipoprotein fraction containing VLDL and CM (d 1.006). Based on these results, the majority of sPLA2 cofactor activity (54%) resides within the HDL fraction of plasma, with 31% in the LDL fraction and 11% scattered between VLDL and CM fractions. The identity of each fraction was confirmed by SDS-PAGE analysis of the lipoprotein-specific apoproteins.

Delipidation of lipoproteins. To determine whether the lipid moiety or apoprotein component of the lipoprotein was essential for the sPLA2 cofactor activity, delipidation experiments were performed. Complete separation of the lipid from protein was confirmed by SDS-PAGE and TLC analysis (data not shown). All of the sPLA2 cofactor activity of HDL and LDL was recovered in the lipid extract from these lipoproteins (Fig 3). These results suggest that a lipid moiety common to HDL and LDL is essential for sPLA2-mediated platelet inhibition.

Phospholipid analysis of plasma lipoproteins. Analysis of phospholipids contained within the purified HDL fraction of plasma by TLC showed a predominance of phosphatidylcholine (PC; Fig 4). Treatment of HDL with purified sPLA2 resulted in the time-dependent generation of lysoPC and a lipid product that migrated directly below PC (Fig 4). To identify the lipid product responsible for the inhibition of platelet aggregation, each of the separated lipids was extracted from the TLC plate and assessed for their ability to inhibit aggregation.
not identified or investigated further. In contrast, lysoPC extracted from the TLC plate caused immediate inhibition of aggregation of washed platelets and PRP (Fig 5). Repeat TLC of the extracted lysoPC confirmed the presence of a single lipid species that comigrated identically with a commercial lysoPC standard. The platelet-inhibitory activity of extracted lysoPC was reproduced using commercial lysoPC. Concentrations of lysoPC greater than 10 µg/mL resulted in a dose-dependent inhibition of platelet aggregation stimulated by a variety of agonists (Table I). In control experiments, commercial PC, lysophosphatidic acid (lysoPA), and a range of free fatty acids at the same concentrations had no inhibitory effect on platelet aggregation.

In further experiments, we correlated the inhibitory effects inhibit collagen-induced platelet aggregation. Extracted PC did not inhibit platelet aggregation in the absence of sPLA₂ (Fig 5). The lipid migrating directly below PC was also incapable of inhibiting platelet aggregation in the presence or absence of sPLA₂ (results not shown). This lipid was

![Diagram](image_url)

Fig 2. sPLA₂ cofactor activity in lipoprotein fractions. sPLA₂ (5 µg/mL) was preincubated with plasma or the indicated lipoprotein fractions for 10 minutes at 37°C before being added to the washed platelet reaction mixture. Aggregation of washed platelets was induced with collagen (1.0 µg/mL). Quantitation of sPLA₂ cofactor in each lipoprotein fraction was calculated as described in the Materials and Methods. The data represent the mean ± SD from four separate experiments.

![Diagram](image_url)

Fig 3. sPLA₂ cofactor activity of delipidated HDL. Delipidation of HDL was performed according to the method of Reardon. The separated lipid and apoprotein components of HDL (10 mg/mL) were resuspended in the same volume as the original HDL sample. sPLA₂ (5.0 µg/mL) was incubated for 10 minutes with intact HDL (■), lipid extracted from HDL (○), or apoproteins derived from HDL (●) before addition to the washed platelet reaction mixture. Lipid extracted from HDL was incubated with control buffer for 10 minutes in the absence of sPLA₂ (□). Platelet aggregation was induced with collagen (1.0 µg/mL). These results are from one experiment and are representative of four.

![Diagram](image_url)

Fig 4. Generation of lysoPC from purified HDL by sPLA₂. sPLA₂ (10 µg/mL) was incubated with HDL (10 mg/mL) over a 60-minute time course at 37°C. The lipids were extracted before TLC analysis, as described in the Materials and Methods. Lane 1, untreated HDL; lanes 2 through 7, the incubation time of sPLA₂ with HDL: (2) 1 minute, (3) 2.5 minutes, (4) 5.0 minutes, (5) 10 minutes, (6) 20 minutes, and (7) 60 minutes. Arrows indicate the PC and lysoPC standards. These results are from one experiment and are representative of two separate experiments.
and assessed for platelet inhibitory activity. Aggregation was induced with collagen (1.0 pg/mL) after the addition of thrombin (0.01 U/mL) in modified Tyrode's buffer in the same volume was applied to the TLC plate, whereas the other 50% was referred to as nonchromatographed lipid. Separated lysoPC and phosphatidylcholine were extracted from the TLC plate as described in the Materials and Methods. Each extracted lipid was resuspended in Tyrode's buffer in the same volume and are representative of three experiments performed in duplicate.

Table 1. LysoPC-Mediated Inhibition of Platelet Aggregation by a Variety of Agonists

<table>
<thead>
<tr>
<th>Agonist</th>
<th>LysoPC Concentration (µg/mL)</th>
<th>% Inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Collagen</td>
<td>12.5</td>
<td>10 ± 10</td>
</tr>
<tr>
<td></td>
<td>25</td>
<td>50 ± 11</td>
</tr>
<tr>
<td></td>
<td>37.5</td>
<td>80 ± 5</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>93 ± 2.5</td>
</tr>
<tr>
<td>Thrombin</td>
<td>37.5</td>
<td>82 ± 4.6</td>
</tr>
<tr>
<td></td>
<td>62.25</td>
<td>93 ± 4.7</td>
</tr>
<tr>
<td>Adrenaline</td>
<td>37.5</td>
<td>76 ± 9.8</td>
</tr>
<tr>
<td></td>
<td>62.5</td>
<td>85 ± 3.5</td>
</tr>
<tr>
<td></td>
<td>112.5</td>
<td>90 ± 2.3</td>
</tr>
<tr>
<td>Ionophore</td>
<td>37.5</td>
<td>19 ± 10.7</td>
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<td></td>
<td>50</td>
<td>38 ± 1.4</td>
</tr>
<tr>
<td></td>
<td>75</td>
<td>58 ± 5</td>
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<tr>
<td></td>
<td>100</td>
<td>93 ± 2.1</td>
</tr>
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Washed platelets (3 x 10^8/mL) were stimulated by the indicated agonists in the presence of various concentrations of lysoPC. The percentage of inhibition of platelet aggregation was calculated as described in the Materials and Methods. The data represent the mean ± SD from two separate experiments performed in duplicate.

Figure 5. Inhibition of platelet aggregation by lysoPC extracted from TLC plate. HDL (10 mg/mL) was incubated in the presence or absence of sPLA2 for 10 minutes at 37°C. Fifty percent of each reaction mixture was applied to the TLC plate, whereas the other 50% was referred to as nonchromatographed lipid. Separated lysoPC and phosphatidylcholine were extracted from the TLC plate as described in the Materials and Methods. Each extracted lipid was resuspended in modified Tyrode's buffer in the same volume as the original sample and assessed for platelet inhibitory activity. Aggregation was induced with collagen (1.0 pg/mL) after the addition of nonchromatographed lipid from sPLA2-treated HDL (O), lysoPC extracted from TLC plate of sPLA2-treated HDL (■), or phosphatidylcholine extracted from TLC plate of untreated HDL (●). Results are from one experiment and are representative of three.

Figure 6. LysoPC inhibits thrombin-induced 125I-fibrinogen binding to platelets. Washed platelets (3 x 10^8/mL) were stimulated with 0.05 U/mL thrombin at 37°C for 10 minutes in the presence of the indicated concentrations of lysoPC, 1 µg/mL 125I-fibrinogen, and 5 mg/mL BSA. The platelets were then pelletted and specific fibrinogen binding was determined as described in the Materials and Methods. The results represent the mean ± SD from three separate experiments performed in duplicate.

LysoPC mediates its platelet-inhibitory effects without inducing platelet membrane lysis. LysoPC has well-documented detergent properties, causing cell membrane lysis at sufficiently high concentrations. Several lines of evidence suggest that the inhibition of platelet aggregation by lysoPC was not the result of cell membrane lysis. First, concentrations of lysoPC that completely inhibited platelet aggregation (75 to 100 µg/mL) did not increase the extracellular concentration of the cytosolic marker, LDH. Second, the morphology of lysoPC-treated platelets appeared to be normal when examined by phase-contrast microscopy. Finally, increasing the concentrations of collagen (20 µg/mL; Fig 7) or thrombin (0.15 U/mL) overcame the platelet-inhibitory effects of lysoPC (100 µg/mL), confirming the functional integrity of these cells.

Inhibition of other platelet responses by lysoPC. To further delineate the effects of lysoPC on platelet function, we assessed its ability to inhibit platelet shape change and dense granule release induced by collagen (1 µg/mL), thrombin (0.05 U/mL), ionophore A23187 (2 µmol/L), and ADP (12.5 µmol/L). Concentrations of lysoPC (100 µg/mL) that inhibited platelet aggregation abolished the change in platelet morphology induced by each of these agonists, as assessed by phase contrast microscopy. Similar concentrations of lysoPC (100 µg/mL) also inhibited agonist-induced serotonin release (Fig 8). These experiments indicate that the platelet-inhibitory effects of lysoPC are not confined to platelet aggregation but appear to involve multiple platelet responses induced by a variety of agonists.

Platelet inhibition by various sPLA2. To determine whether the generation of lysoPC from lipoproteins is spe-
specific to snake venom sPLA₂, we screened other sPLA₂ from bovine pancreas and bee venom for lipoprotein-dependent inhibition of platelet aggregation. Using HDL as a source of phospholipid, we found that both partially purified bee venom sPLA₂ and bovine pancreas sPLA₂ were capable of inhibiting the aggregation response of washed platelets to collagen (Table 2). The greater concentrations of pancreatic sPLA₂ required to inhibit platelet aggregation reflect the purity of the enzyme and the lower specific activity of pancreatic sPLA₂ compared with bee and snake venom sPLA₂.

Dose-response studies confirmed a close correlation between lecithin hydrolytic activity and the inhibition of platelet aggregation by each of the partially purified enzymes (data not shown). The inhibition of platelet aggregation by these non-snake venom sPLA₂ was time-dependent and required the enzymatic generation of lysoPC from HDL (data not shown).

Protective effect of albumin on lysoPC-mediated inhibition of platelet aggregation. We observed that, in the absence of albumin in the washed platelet reaction mixture, purified snake venom sPLA₂ was capable of inhibiting platelet aggregation induced by a variety of agonists, without the addition of exogenous lipoproteins. However, in the presence of 5 mg/mL of albumin in the reaction mixture, sPLA₂ was unable to inhibit platelet aggregation without the addition of plasma or purified lipoprotein. It has previously been shown that albumin binds avidly to free fatty acids and lysophospholipids, thereby sequestering these lipids away from the cell surface. We therefore examined the effect of increasing concentrations of albumin on the platelet inhibitory effects of lysoPC. In the absence of BSA, small quantities of lysoPC (5 pg/mL) resulted in complete inhibition of collagen-induced platelet aggregation. At concentrations of BSA approaching physiologic levels (20 mg/mL), much higher concentrations of lysoPC were needed to inhibit platelet aggregation (100 to 150 pg/mL; Fig 9). These observations suggest that, in the absence of albumin, sPLA₂ is able to generate sufficient quantities of lysoPC from the platelet membrane to inhibit platelet activation. However, in a concentrated albumin environment, the majority of lysoPC would be sequestered from the platelet surface. These results provide a plausible explanation for the lipoprotein-dependence for sPLA₂-mediated inhibition of platelet activation. According to this model, the presence of lipoprotein provides an additional source of phospholipid required for the gener-
The presence of plasma lipoproteins. (2) The inhibition of platelet aggregation in the presence of albumin. (3) The phospholipids is essential for sPLA2-mediated inhibition of platelet aggregation. (4) The enzymatic generation of lysoPC from lipoprotein plasma lipoproteins generated a lysophospholipid product capable of inhibiting platelet aggregation in an sPLA2-independent manner. (5) Time course experiments for the generation of lysoPC correlated closely with the time-dependence for platelet inhibition by sPLA2. The distribution of the sPLA2 cofactor activity in the lipoprotein subclasses was consistent with the phospholipid content in each of the individual lipoproteins. Finally, the addition of albumin to the washed platelet reaction mixture antagonized the platelet-inhibitory effects of both sPLA2 and lysoPC.

The observed protective effect of albumin on sPLA2-mediated inhibition of platelet aggregation is in keeping with its ability to bind lysophospholipids. Previous studies have shown that only small quantities of lysoPC are generated from the surface of resting cells by sPLA2, presumably because of limited substrate access to extracellular PLAs. The presence of albumin in the washed platelet assay system would therefore be expected to sequester the majority of lysoPC generated from the cell surface. This is consistent with our results showing the need for increasing doses of lysoPC to inhibit platelet aggregation in the presence of high concentrations of albumin. In a more concentrated albumin environment in vivo (35 to 50 mg/mL), the ability of sPLA2 to inhibit platelet activation would require the generation of large amounts of lysoPC, thereby explaining the requirement for plasma lipoproteins as an alternative source of phospholipid substrate.

These observations have important implications for the toxic effects of circulating sPLA2. Elevated serum levels of sPLA2 have been shown in patients with a variety of inflammatory diseases, including pancreatitis, rheumatoid arthritis, septic shock, and adult respiratory distress syndrome (ARDS). The level of sPLA2 correlates with the severity and duration of these disorders, and inhibitors of sPLA2 have been shown to decrease the serum PLA2 activity and improve clinical outcomes. Furthermore, the experimental administration of exogenous sPLA2 has reproduced characteristic features of these diseases. Whether sPLA2 can contribute to hemorrhage in other clinical settings has yet to be established. There is evidence in pancreatitis that the hemorrhagic complications of this disease are more common when the serum concentrations of sPLA2 are at their highest. Furthermore, the administration of large quantities of sPLA2 into mice leads to lethal hemorrhagic complications. It is therefore possible that hemorrhage is clinically evident only when large quantities of sPLA2 are released into the circulation, as is observed with snake envenomation and severe pancreatitis. Our in vitro studies support this possibility, as the amount of sPLA2 (~35 U/L) required to inhibit platelet function in vitro has lead to the suggestion that this enzyme may also contribute to the bleeding complications observed in snake bite victims. Whether sPLA2 can contribute to hemorrhage in in vivo. in a more concentrated albumin environment, the cellular effects of these enzymes may be mediated, at least in part, by the generation of lysoPC from plasma lipoproteins. The enzymatic production of lysoPC from LDL has previously been postulated to play a pathogenic role in the development of atherosclerosis. LysoPC induces endothelial cell expression of adhesion molecules important in monocellular leukocyte recruitment into the arterial intima. Furthermore, lysoPC is chemotactic for atherogenic monocytes and can upregulate growth factor gene expression in these cells. The ability of lysoPC to inhibit platelet aggregation and to induce vascular smooth muscle relaxation (thus potentially antagonizing the vasoconstrictor response to vascular damage) is consistent with a role for this bioactive lipid in the development of hemostatic disturbances.

The potential pathophysiologic effects of lysoPC may be particularly relevant in patients with a low level of serum albumin, as is frequently observed in patients with inflammatory diseases. Under these conditions, higher concentrations of unbound lysoPC may considerably enhance disease
progression. Although lysoPC has been postulated to play a role in human disease, it has not been easy to prove that increased levels of extracellular lysoPC occur in vivo. Unlike sPLA₂, which is not rapidly proteolyzed and has no natural inhibitors in serum, lysoPC undergoes rapid metabolism by circulating lysophospholipase and lysoPC transacylase, resulting in a short serum half-life. Studies showing increased levels of plasma lysoPC in the venous or lymphatic effluents of ischemic tissue have not detected increases in the systemic venous concentration of lysoPC. These observations suggest that the cellular effects of lysoPC may be largely confined to the local environment in which it is produced, rather than having general systemic effects. Based on our in vitro data with PRP, this increase in the local concentration of lysoPC would be sufficient to induce alterations in platelet function, lending support to our hypothesis that the conditions of our assays have relevance to the in vivo situation.

Although a broad range of biologic activities have been attributed to lysoPC, there is currently limited information on the mechanism of its cellular effects. Other bioactive phospholipids, such as lysoPA and platelet-activating factor (PAF), have well-characterized effects on cell behavior that are mediated through specific receptor-coupled signaling systems. LysoPC has been shown to regulate the activity of a number of intracellular signaling enzymes in vitro, including the activation of both protein kinase C and guanylate cyclase and the inhibition of adenylate cyclase. Although these studies suggest that lysoPC can directly regulate the activity of a variety of signaling enzymes, they provide limited information on the effects of extracellular lysoPC on signaling events in intact cells. Recent studies from our laboratory indicate that lysoPC treatment of platelets or monocyte-like cells stimulates the production of cAMP (unpublished manuscript in preparation). This effect of lysoPC is abolished by the G-protein inhibitor, GDP/B₅S, raising the interesting possibility that the cellular effects of lysoPC, like those of lysoPA and PAF, are mediated through specific G-protein-coupled cell surface receptors.

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